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ICAR Technical Series no. 28

SUSTAINABLE LIVESTOCK PRODUCTION SYSTEMS IN A NEW ERA

Proceedings of the 47th ICAR Annual Conference
held in Bled, Slovenia
19 -24 May 2024

Editors: M. Klopcic, S. Orlandini and C. Mosconi

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SiallSCM: a nation-wide tool for milking monitoring to enhance efficiency and welfare of Italian dairy animals

L. Pascarella[1,2], C. Melilli [1], M.P.G. Rezende[1], M. Fioretti[1] and R. Negrini[1,2]

[1]*Associazione Italiana Allevatori, via XXIV Maggio 44, Rome, Italy*
[2]*Dipartimento di Scienze animali, della nutrizione e degli alimenti, Università Cattolica del Sacro Cuore, Via E. Parmense, 29122 Piacenza, Italy*
Corresponding Author: pascarella.l@aia.it

Introduction

The development of milking machines began in the late 19th century. Over the decades, the field of milking technology experienced significant improvements in performance, culminating in the complete automation of the milking process whit the advent of Automatic Milking Systems (AMS). Still nowadays, despite these advancements, the well-documented consequences of inconsistent milking due to improper functioning of milking machine continue to impact the udder health of dairy cows and hinder the competitiveness of farm. For example, poorly adjusted machine milking can contribute to the incidence of mastitis acting as vector of bacteria or causing traumas. Milking machine processes, including the equipment washing and cleaning can also influence milk quality diminishing its cheese yield potential. In conclusion, monitoring the milking process through data helps optimize labour time, which typically accounts for over 50% of the total labour input in the milking parlour.

Since 1970, the Italian DHI (AIA) has established and operated a national service called Milking Control Service (SCM). More than 100 highly skilled technicians serve within the SCM framework, with their distribution strategically aligned to the regional concentration of dairy farms. They are equipped with instruments to carry-out the dry test (flowmeter, pulsameter, vacuum gauge, etc). Equipment also includes VaDia® kit (Biocontrol, Rakkestad, Norway) and Lactocorders® (WMB AG, Balgach, CH). The SCM service operate in compliance with ISO standards 3918:2007, 5707:2007, 6690:2007 and 11008:2002.

SCM technicians assess the efficiency of milking systems through both dry tests (conducted without animals) and wet tests (performed with animals). Based on the test results, they calibrate the milking equipment, establish an effective milking routine, optimize the cleaning process and ensure the efficiency of milk cooling tank. Finally, in line with ICAR guidelines for DHI, the technicians ensure that the devices used for milk performance recording are functioning correctly. Those services will be further enhanced leveraging all herd available data and employing cutting-edge milking sensors. While milking technologies and devices efficiently generate massive and accurate information both on milking systems and animals, the real challenge lies in developing standardized methods for recording, organizing, and normalising this data and extract meaningful and ready-to-use information for farmers and technicians. To address this challenge, AIA developed a comprehensive procedure for monitoring the milking process and has engineered a software solution called SiallSCM. This system is designed to collect data, assist SCM technicians, and provide farmers with valuable tools to improve the efficiency and effectiveness of their milking operations. By integrating this solution with herd data and advanced milking sensors, AIA ensures a seamless approach to optimizing performance.

The App SiallSCM

The SiallSCM app is developed using Python 3.11.7, with the code written in the Spyder IDE. Spyder is an integrated development environment tailored for Python programming. In the development process, we utilized the CustomTkinter package, specifically its CTk() function, to create the main application window that gives users access to all available tools. Upon selecting a tool, corresponding child windows are displayed. The app incorporates various widgets and classes from the CustomTkinter package, including CTkButton, CTkLabel, CTkFrame, CTkToplevel, CTkRadiobutton, CTkCheckBox, CTkTextBox, and CTkEntry, to build the interface.

Each tool in the app is nested within its own class, which contains the functionality specific to that tool. Additionally, we create a main class that imports all the tool classes and assembles the complete GUI application. The project's file structure is neatly organized into three primary sections: data, GUI, and testing. The testing process was divided into several key steps: network testing (synchronization with the API), authentication, file sorting, data compilation and transmission to the central database, clearing the bin, and locating or extracting information from the database. The evaluation metrics used to assess performance included CPU utilization, memory utilization, and response time.

The Python script was then converted into an executable file. PyInstaller was used to bundle the Python application along with all its dependencies into a single package, allowing users to run the application without needing to install a Python interpreter or any additional modules. This packaged app can be run on both Windows and Linux operating systems, and it does not require an Internet connection for use.

The communication and data synchronization between the SiallSCM graphical interface and the AIA central database (SQL) is trough APIs. The communication framework is organized into six key API sets: user management, authentication, farm data extraction (retrieving general farm information from AIA's database), catalogue data extraction (including lists of milking machine types, models, and equipment), milking system configuration, and types of milking control.

When SCM technician logs-in with a personal password, a user token is generated, validating all synchronization activities (Figure 1), and identifying the technician's operational area, granting access to all farms within that area. Exceptions can be configured as needed. After successful logging in, the specific API allows the local storage of all machine systems and equipment catalogues in a JSON file.

The next step involves planning configuration and milking control activities. In this phase, the farm API is used to retrieve information on previously completed and synchronized activities, as well as the forecast for adding a new service.

At this stage, the user can enter all data and information related to the scheduled tests into the APP tables, with the flexibility to work both online and offline. Once data entry is completed, the APIs synchronize the data with the central database.

The AIA' R&D department conducted a comprehensive analysis to anticipate potential areas of information and data acquisition related to milking machines configuration, setting parameters and milking tests.

The procedure follows a farm-specific approach, allowing for the collection of all tests and information at farm level. After selecting a farm, the first step in data entry involves configuring the complete milking system and its equipment (Figure 2) filling specific masks on:

- Farm general information (species, type of milking machine, etc).
- Milking system general information (number of claws, dimensions, etc).

- Vacuum system (milking pump type, model and characteristics, vacuum pipelines dimensions, etc).
- Milk system (milk pipeline dimensions and characteristics, milk receiver, etc).
- Cleaning and washing system.
- Milking units (type of tubes, material, claws model, liners, etc).
- Pulsation systems (type, model and settings of pulsators).
- Recording device (type, model and number, if present).

Specific input forms are developed for entering data based on the specific milking test selected (Figure 3)

SiallSCM facilitate the data entry and analysis of the following tests. Data entry is controlled by the input field, restriction which is configured to accept strings of number of specific length and composition according with the parameter being entered.

- Dry test (not under milking conditions): this test includes a physical assessment of the milking machine and the measurement of vacuum capacity at appropriate points, vacuum gauge accuracy, milk system leakage;
- Wet test (under milking conditions): the test evaluates the performances of the milking machine and the interaction between cow, milker and the machine. It includes precise measurements of vacuum levels at the claw, the liners, and the accurate monitoring of milk flows during different phases of milking.

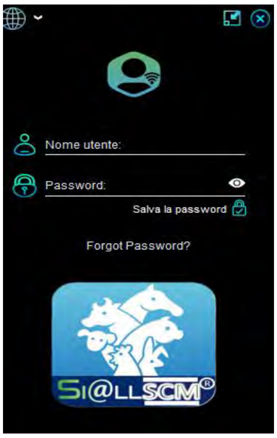


Figure 1. Authentication of SiallSCM.

Figure 2. Milking system configuration.

Figure 3. SiallSCM panel to initialize the selected test (left). Data entry mask for pulsation parameters and pump (right).

Figure 4. Data and information flow of SiallSCM procedure.

- Fall-of test: it assesses whether the system has enough airflow capacity to cope with a unit fall-off.
- Pulsation test: this test check whether the pulsator is correctly calibrated and ensure it remain consistent by monitoring various pulsation parameters and phases.

Table 1 shows the main parameters that can be collected for each test.

When used on-line, all tests' data and milking systems information are synchronized in real-time with the central AIA database and integrated with the DHI data, following two-tier normalization process.

The first tier of normalization ensures that data gathering complies with ISO standards by following official operative protocols. Additionally, the data gathering is validated using SCM' instruments and milking devices, which are officially calibrated at least once a year. The second tier applies a set of thresholds, from UNI-ISO standards, and algorithms to identify and flag any aberrant or outlier data entries.

Once the data has been processed and merged in the central database, it can be retrieved by the front-end application to generate dynamic, easy-to-read farm reports.

Table 1. Main parameters recorded per SCM test.

Test	Parameters
Dry test	working vacuum (kpa), effective reserve of milking (l/min), regulation sensitivity (kpa), vacuum pump airflow (l/min), airflow at vacuum and/or milk pipelines (l/m).
Wet test	claw vacuum during peak flow (kpa), bimodality, claw vacuum drop (kpa), milk flow during peak (kg/min), overmilking (min), milk yield overmilking (kg), overmilking (min), milking flows (kg/min).
Fall-off test	avg vacuum phase 1 (kpa), undershoot(kpa), vacuum drop(kpa), overshoot(kpa), avg vacuum phase 2(kpa), avg vacuum phase 4 (kpa).
Pulsation test	frequency (bpm), ratio (%), limping (%), dipping (kpa), a, b, c, d phases of pulsator (% and ms).

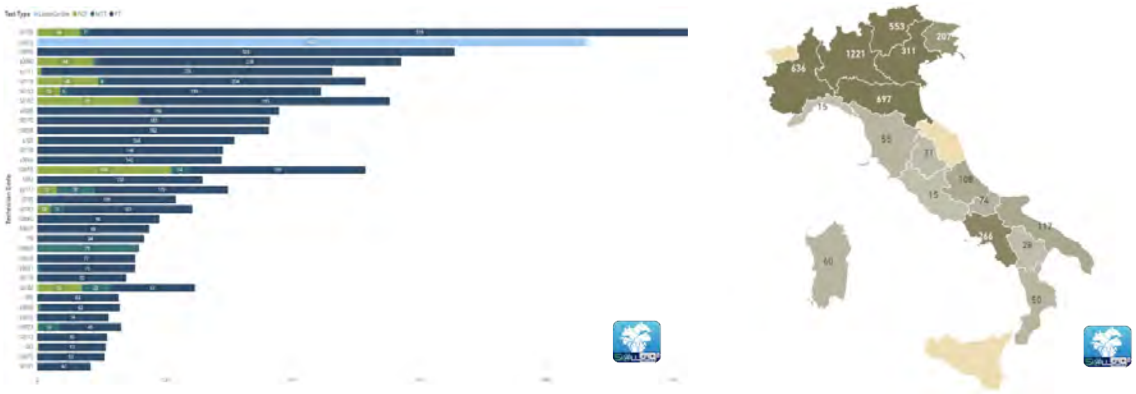


Figure 5. SiallSCM Power BI dashboards. Type of test per technician code (left). Farm tested per region (right).

Results and discussion

Data collected through the app

During the inaugural campaign of SiallSCM, held between September 2022 and April 2024, more than 500.000 milking machine milking machines configuration and setting parameters were acquired and about 25.000 pulsation tests were conducted on 1.255 farms by 56 SCM technicians. In addition, in the same timeframe, 4.348 milking wet test were performed involving 160.000 lactating animals and fall-of tests were carried in 398 farms. Finally, 2.374 data streams were uploaded from Lactocorder and Vadia direct link.

The tool and services potential spans the whole set of farms subscribed to DHI encompassing more than 15 thousand dairy farms and 1,5 million of heads (Table 2)

Data visualization and reporting is performed using Microsoft Power BI, a business intelligence tool developed by Microsoft, which enables interactive data dashboards and reporting. The SCM central office conduct a weekly review these statistics weekly to monitor the SiallSCM data flow by farm, type of milking test, and SCM technician (Figure 5).

Preliminary results on Buffaloes and Holstein

The limited understanding of buffalo udder physiology and optimal milking parameters prompted to utilize the SiallSCM dataset. The study aimed to investigate the influence of different milking conditions on milk quality traits and define the most appropriate milking machine parameters.

Milking machine configuration information and Dry test parameters (working vacuum, pulsation rate, effective vacuum reserve) were modelized together with official DHI data through a mixed linear model.

The preliminary analysis showed a direct relation between LS and the working vacuum level (Table 3). A higher pulsation rate (70:30) was responsible for significantly ($P < 0.001$) lower LS and higher FP compared to a 60:40 pulsation rate (Table 4). The presence of automatic cluster removal showed a significant effect ($P < 0.001$) on fat and milk yield and a slight ($P < 0.05$) reduction in LS.

Furthermore, a multivariate analysis revealed that different types of systems had different effect on milk quality and quantity traits. Notably, somatic cell counts (LS) was lower when a herringbone system was used compared to tandem system.

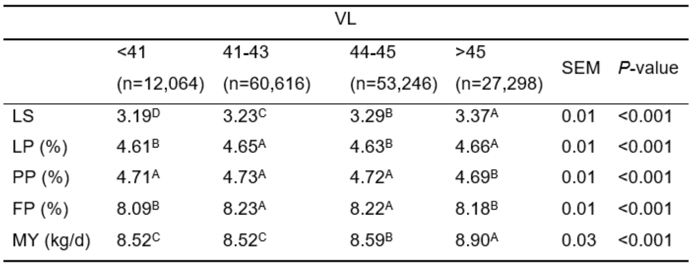
Results emphasized that buffaloes have specific requirements for milking parameters and incorrect setting can lead to an increase in somatic cell count and a reduction in lactose levels. In this study, we evaluate 10 different types of milking systems.

We also gathered preliminary finding using data from Lactocorders connected to the SiallSCM system. This analysis involved a sample of approximately 3,000 Italian Holstein cows and focused on three key parameters: vacuum level at the milking cluster, grouped into four classes; peak milk flow sustained over one minute; and total

Table 2. Numbers of farms, milking systems and lactating animals involved in Milking Control Service of AIA Source: A.I.A. (http://bollettino.aia.it)

Species	n° of farms	n° of milking systems	n° of lactating heads	Average heads per farm
Bovine	14,007	23,812	1,407,368	100.5
Ovine	833	953	127,427	152.9
Goat	395	512	36,498	92.4
Buffaloes	346	588	74,299	214.7
Total	**15.581**	**25.865**	**1.645.592**	

Table 3. Least square means ± standard error of milk characteristics for working vacuum classes.



	VL					
	<41 (n=12,064)	41-43 (n=60,616)	44-45 (n=53,246)	>45 (n=27,298)	SEM	*P*-value
LS	3.19^{D}	3.23^{C}	3.29^{B}	3.37^{A}	0.01	<0.001
LP (%)	4.61^{B}	4.65^{A}	4.63^{B}	4.66^{A}	0.01	<0.001
PP (%)	4.71^{A}	4.73^{A}	4.72^{A}	4.69^{B}	0.01	<0.001
FP (%)	8.09^{B}	8.23^{A}	8.22^{A}	8.18^{B}	0.01	<0.001
MY (kg/d)	8.52^{C}	8.52^{C}	8.59^{B}	8.90^{A}	0.03	<0.001

Table 4. Least square means ± standard error of milk characteristics for working vacuum classes.

	PR			
	60:40 (n=81 925)	70:30 (n=71 309)	SEM	*P*-value
LS	3.55	3.44	0.01	<0.001
LP (%)	4.65	4.63	0.01	0.330
PP (%)	4.71	4.71	0.01	0.300
FP (%)	8.17	8.22	0.01	<0.001
MY (kg/d)	8.68	8.66	0.02	0.440

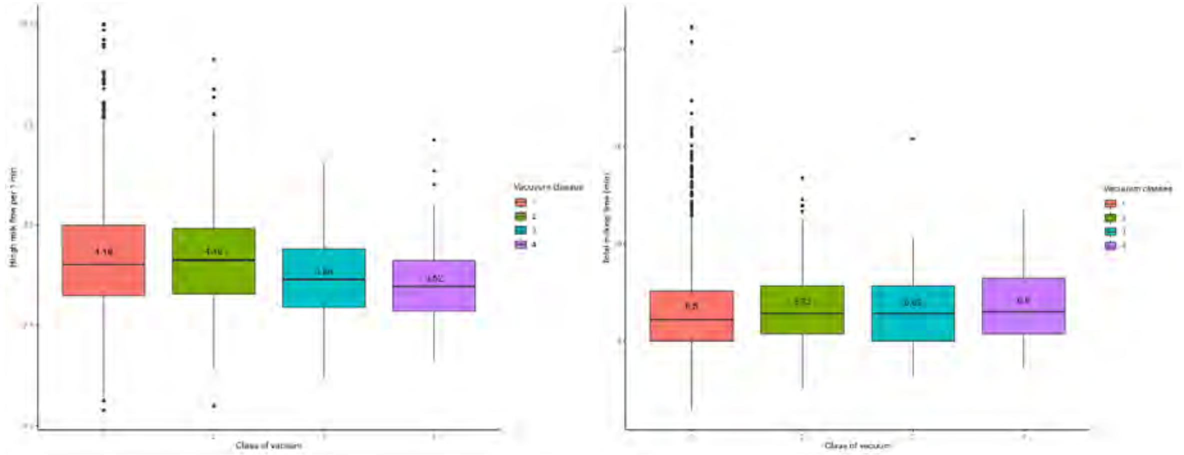


Figure 6. Max milk flow in one minute per class of vacuum (left). Milking time per class of vacuum (right).

milking duration. The data analysis indicates an inverse relationship between vacuum class and average milk flow (Figure 6, right). However, the total milking duration remains consistent across all vacuum classes ranging from 6.5 minutes for class 1 to 6.8 minutes for class 4 (Figure 6, left). These results suggest that the common practice of increasing vacuum levels to speed up milking may not be efficient. Lower vacuum levels likely reduce stress on the animals, creating optimal physiological conditions that allow for greater milk release within the same time frame.

Conclusions

The SiallSCM tool, with its capacity to collect and structure vast amounts of data, provides valuable insights for improving milking management across a wide range of farm types.

The design of the SiallSCM app facilitates farm-specific data management, offering key information for improving milking management, milk quality, and overall economic competitiveness. Additionally, the app unlocks broader possibilities, as the study and identification of negative impacts associated with milking processes. In-depth analysis of the large dataset can uncover critical insight on animal susceptibility to specific equipment settings or management practices, supporting farms and their advisors in optimizing both performance and welfare.

Further implementation of SiallSCM will expand its capabilities, including data and information collection related to milking routines and management, and the development of meaningful reports.

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The Commercial Beef Value (CBV) encourages the adoption of sustainable and profitable practices in beef production

M.M. Kelleher, C. Daly, K. Downing, R. Evans and J. McCarthy

ICBF, Link Road, Ballincollig, Co. Cork, Ireland
Corresponding Author: mmkelleher@icbf.com

Abstract

The calving season is a compact period concentrated in the first 3 months of the year for three quarters of the 1.6 million national dairy cows in Ireland. Many of the resulting dairy-beef sired calves are sold off farm before the calf reaches 6-weeks of age whereby after that age a Tuberculosis test is necessary for sale. The abundance of these animals frequently results in market saturation, presenting challenges for sellers. Conversely, determining which animals will yield the greatest profit for subsequent buyers poses its own difficulties, as buyers face a gamble due to the absence of distinct visual disparities between young animals. The introduction of the Commercial Beef Value (CBV) by the Irish Cattle Breeding Federation (ICBF) marks a significant advancement in the dairy-beef industry in Ireland. This index addresses the challenge of assessing the profit potential of non-breeding beef animals, particularly calves sold off-farm before six weeks of age. The CBV contributes to;;

1. Genetic potential assessment: The CBV incorporates genetic factors related to intake, growth, docility, and carcass traits. This allows purchasers to make more informed decisions regarding the profit potential of individual calves.
2. 2. Decision support tool: The CBV serves as a decision support tool for farmers, helping them evaluate the performance and value of calves beyond what is visually apparent at a young age.
3. 3. Improved predictability: By providing more reliable information and predictability, the CBV enhances the purchasing process and encourages breeders to focus on producing higher-quality beef stock.
4. 4. Integration with existing systems: The CBV complements the existing Dairy-Beef Index (DBI), providing a comprehensive set of tools for both breeders and purchasers to evaluate animals' genetic potential.
5. 5. Environmental impact: Finishing animals at a younger age not only increases efficiency but also reduces environmental impact by consuming less feed and emitting fewer greenhouse gases over their lifetime.
6. 6. Genotype verification: Animals eligible for the CBV must undergo parentage verification through genotyping, ensuring the accuracy and reliability of the index.
7. 7. Availability in auction houses: The CBV is accessible through digital screens in auction houses, making it readily available to purchasers.

Overall, the introduction of the CBV represents a significant step forward in the dairy-beef industry, providing tangible benefits to both purchasers and breeders. By leveraging genetic information and technology, stakeholders can make more

informed decisions, ultimately driving improvements in animal quality, profitability, and environmental sustainability.

Keywords: dairy-beef, genetics, carcass.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 1a: Decision Support Tools of the Future – Promoting Sustainability Farm Management

Introduction

In Ireland, three quarters of the 1.6 million dairy cows calve within the first three months of the year. In recent years, there has been a notable rise in the numbers of dairy-beef animals, yet simultaneously, a concerning decline in their carcass conformation scores has emerged. Historically, beef farmers lacked important information regarding the genetic quality of these progenies from dairy origins. However, the introduction of the CBV (Commercial Beef Value) has addressed this gap, empowering beef farmers with crucial insights for informed purchasing decisions, regardless of the animal's age. This tool holds significant potential to instigate tangible transformations within the industry, provided that beef farmers leverage this information in their procurement processes and that breeders of such animals respond effectively to industry demands.

Trends in the dairy-beef industry

Since 2017, beef calvings have accounted for approximately 40% of the overall dairy calvings. Nevertheless, this pattern has shifted in the past two years, witnessing a noticeable increase in the birth of calves sired by beef bulls within the dairy herd (Figure 1). Remarkably, this year marks the first instance where the number of beef calves born from dairy cows has surpassed the number of dairy calves born from dairy cows after the peak spring calving season. This trend also indicates that dairy farmers are increasingly using beef sires earlier in the breeding season.

The rise in dairy-beef offspring has led to a decline in dairy male calves, largely due to the use of sexed semen, which reduces both male births and the need for dairy females. Figure 2 compares this trend between 2020 and 2024 (up to April 20th). As this shift continues, following global patterns, the dairy male segment is expected to keep shrinking, with beef-on-dairy offspring filling the gap. This increase in dairy-beef calves offers beef rearers more choices, potentially leading to more profitable animals. The key question is whether these calves are improving in quality and becoming more cost-effective to rear.

The Commercial Beef Value

The CBV, or Commercial Beef Value, is a tool for gauging the quality and anticipated profitability of non-breeding animals.

The CBV offers farmers valuable insights into the genetic worth of their animals, encompassing traits important only for non-breeding farming, such as carcass weight, conformation, and feed intake (Figure 3). Similar to the EBI and Euro-Star Indexes, CBV is denoted as a €uro value. A higher €uro value signifies superior genetic merit across the included traits. Having the CBV available will allow farmers to make more informed decisions when purchasing or selling animals. Genotyped animals being traded through auction houses will have their CBV displayed on digital boards. When

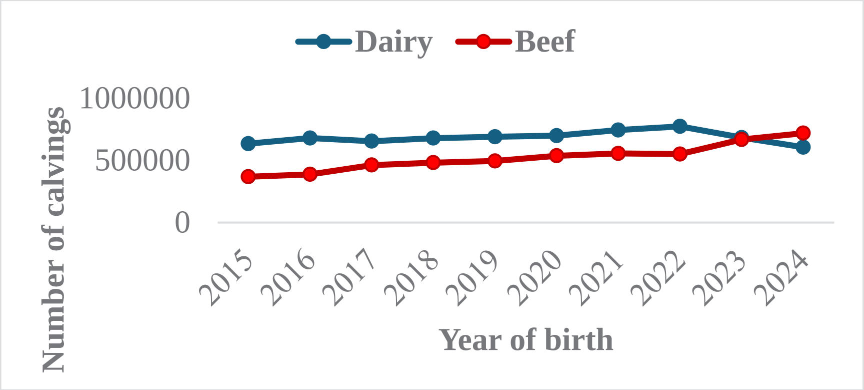


Figure 1. Number of calvings by April 20th each year for dairy and beef sired progeny in the dairy herd.

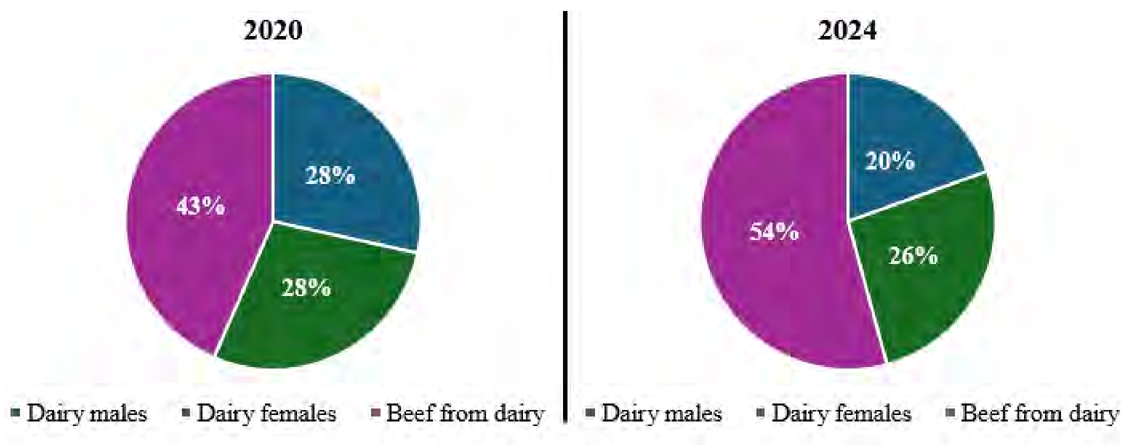


Figure 2. Proportion of dairy male, dairy female and beef from dairy offspring for spring calving up to April 20th for each year, 2020 and 2024.

Figure 3. The Commercial Beef Value.

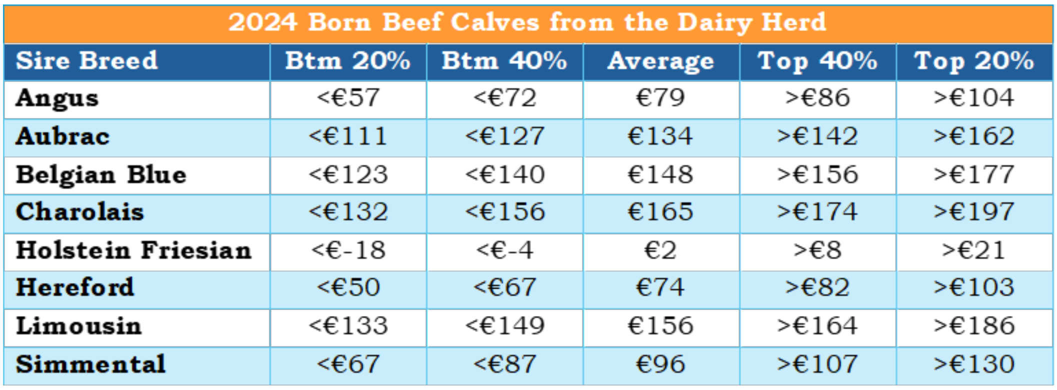
engaging in farm-to-farm sales, purchasers can request the seller's CBV profile which can be obtained via their ICBF Herdplus account. The beef merit of calves can vary significantly even within the same breeds (Table 1).

Validation

Analysis using the data from the ICBF national database shows that calves sired by beef bulls with higher genetic merit achieve better carcass weights, conformation, and are more likely to meet factory specifications than those sired by lower-merit bulls (Table 2).

Dairy steers showed only a €43 difference in calf purchase price between the bottom and top 10%. However, top CBV steers finished 16 days earlier and earned €275 more on finishing price. Angus crosses had even greater differences, with high CBV animals finishing 54 days sooner. Since CBV impacts the animal's entire life, beef finishers can use this tool to evaluate quality and efficiency when purchasing calves, weanlings, or store cattle.

Table 1. CBV values by breed for 2024 born dairy-beef calves (source www.icbf.com).



2024 Born Beef Calves from the Dairy Herd

Sire Breed	Btm 20%	Btm 40%	Average	Top 40%	Top 20%
Angus	<€57	<€72	€79	>€86	>€104
Aubrac	<€111	<€127	€134	>€142	>€162
Belgian Blue	<€123	<€140	€148	>€156	>€177
Charolais	<€132	<€156	€165	>€174	>€197
Holstein Friesian	<€-18	<€-4	€2	>€8	>€21
Hereford	<€50	<€67	€74	>€82	>€103
Limousin	<€133	<€149	€156	>€164	>€186
Simmental	<€67	<€87	€96	>€107	>€130

Table 2. Calf price, finishing price and finishing age for A) dairy × dairy steers and B) Angus × dairy steers finished in 2023 by CBV decile.

A: Dairy steers finished in 2023

CBV Rank	Calf Price	Finishing Price	Finishing Age
Top 10%	**€99**	**€1,538**	**817**
2	€85	€1,471	823
3	€79	€1,455	826
4	€79	€1,439	826
5	€78	€1,415	824
6	€71	€1,409	826
7	€72	€1,392	827
8	€67	€1,373	829
9	€65	€1,338	832
Btm 10%	**€56**	**€1,263**	**833**
Difference Top & Bottom 10%	€43	€275	-16

B: AA X FR steers finished in 2023

CBV Rank	Calf Price	Finishing Price	Finishing Age
Top 10%	**€249**	**€1,763**	**778**
2	€235	€1,689	787
3	€230	€1,684	789
4	€231	€1,653	790
5	€229	€1,632	788
6	€223	€1,608	792
7	€218	€1,589	802
8	€211	€1,569	805
9	€208	€1,541	817
Btm 10%	**€191**	**€1,490**	**831**
Difference Top & Bottom 10%	€58	€273	-53

The National Genotyping Programme

The National Genotyping Programme (NGP) was launched in Ireland in 2024 with the aim of establishing a fully genotyped national dairy and beef herd. This initiative involves the genotyping of all calves born in the participating herds at birth. The genotype results are integrated into the national calf registration system through the DNA calf registration process. There is a significant benefit to farmers and the industry as inaccuracies in the recorded dam, sire and sex of each calf can be corrected before the bovine passport has been issued. In NGP herds this year, over 93% of all calves born have been verified to a sire. This provides more accurate CBV values and buyers can buy with confidence that the animal has been registered to the correct parents and their genetic merit potential has increased in accuracy. However, the overall quality of the population is one that needs some further scrutiny.

The Dairy Beef Index

To improve the CBV quality of beef progeny from the dairy herd, dairy farmers can use the Dairy Beef Index (DBI) breeding index to select beef bulls that will produce calves suitable for beef production while also maintaining desirable calving traits. The index consists of three sub-indices; Calving, Beef and Carbon (Figure 4).

Traits such as gestation, calving difficulty and mortality contribute to the Calving sub-index. Trends indicate that dairy farmers are increasingly prioritizing favourable calving traits and are making consistent advancements in this aspect annually. However, in the Beef sub-index, which encompasses traits like carcass weight, conformation, and feed intake, progress appears to be less pronounced (Figure 5).

Quality of dairy-beef progeny

The quality of dairy-beef animals, as measured by the CBV, has slightly declined since 2015. Between 2017 and 2022, the trend remained relatively stagnant, however, the last two years have seen a decline in CBV scores (Figure 6). This period also saw more dairy herds using beef sires and more dairy cows bred to beef sires. Trying to disentangle why the CBV values have declined is difficult and not all herds have witnessed a decline. For example, dairy herds consistently using beef sires have

Figure 4. Dairy Beef Index (DBI) composition.

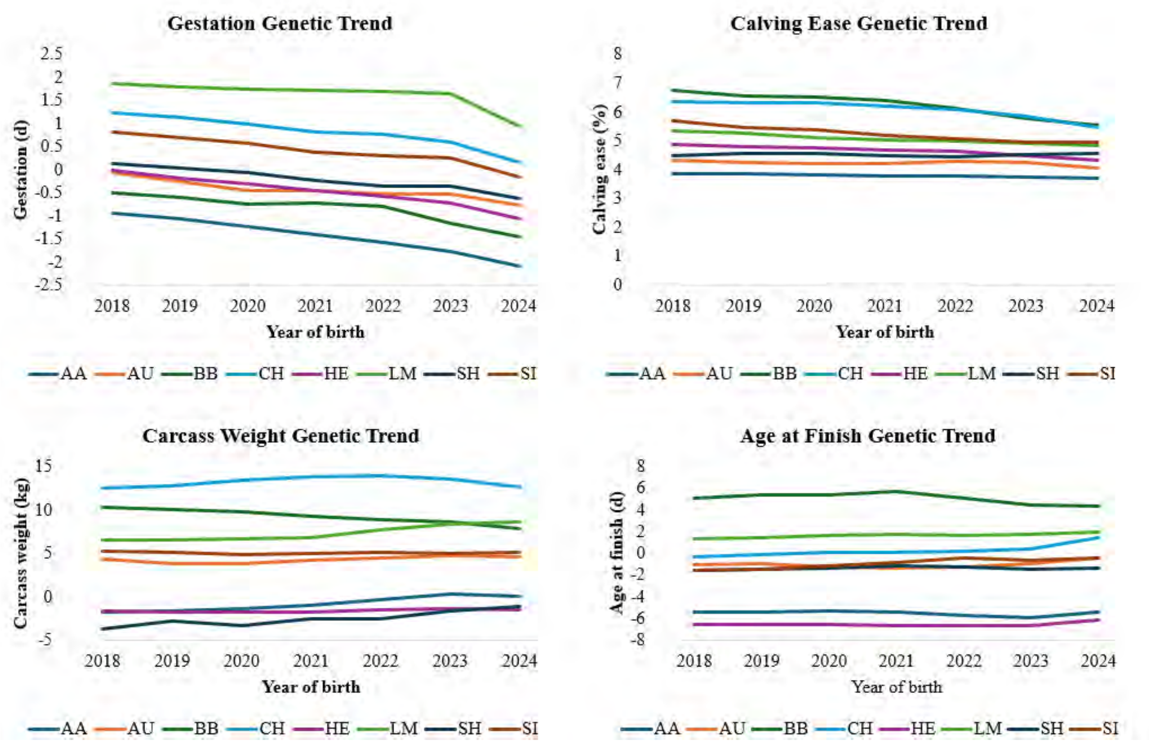


Figure 5. Genetic trend for calving traits (gestation and calving ease) and beef traits (carcass weight and age at finish) by year of birth and by breed.

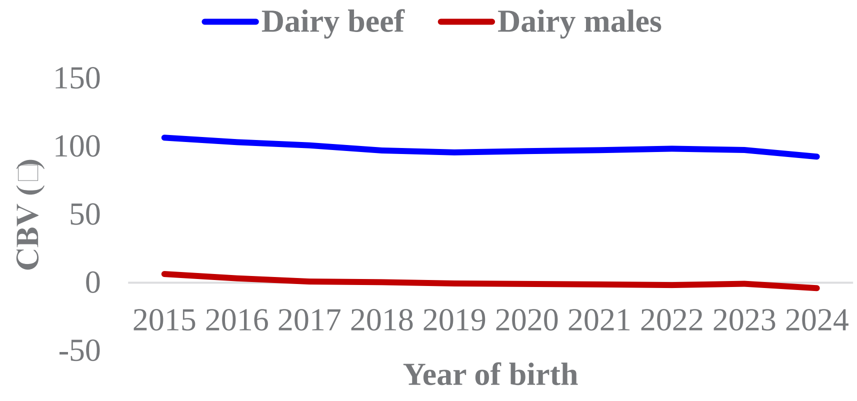


Figure 6. Trend in CBV for dairy beef and dairy male animals

generally seen annual improvements in dairy-beef calf quality, except for this year, although data is incomplete (Figure 7).

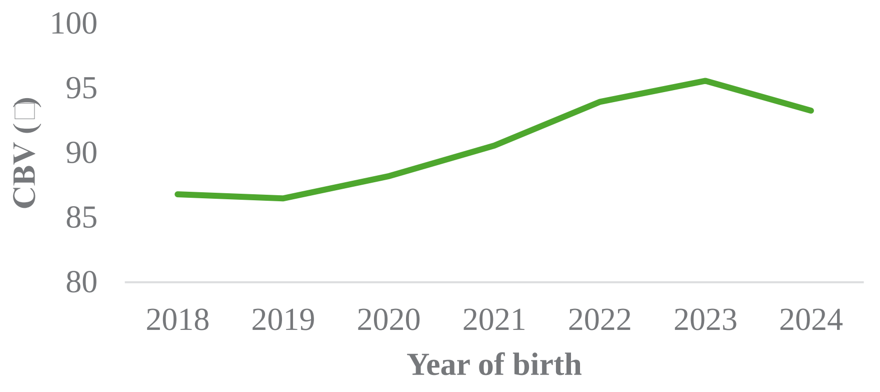


Figure 7. CBV by year of birth for dairy herds that consistently used beef on dairy (>25% calves dairy-beef in 2018 and remain so in 2024).

Conclusion

Breeding for higher beef quality traits is possible through careful selection of dairy-beef bulls on their DBI. Dairy farmers should opt for bulls with high beef sub-index values in the DBI as this is necessary for fostering improvements in calf quality. Beef producers play a pivotal role in expediting this progress by leveraging the CBV to inform their purchasing choices. The CBV stands as a catalyst for driving essential improvements in dairy-beef animal quality. Every industry stakeholder – from bull breeders to AI companies, farmers selecting bulls and those that raise the subsequent progeny, advisors, researchers and ICBF – all play a vital role in driving this change.

HeiferHub – A decision support tool to forecast sales of beef calves and future heifer replacements

R.H. Fourdraine and J.S. Clay

Dairy Records Management Systems, 313 Chapanoke Road,
Suite 100, Raleigh, NC 27603, USA

Abstract

Utilizing the combined breeding, calving, culling and calf data, DRMS build a web based tool called HeiferHub. HeiferHub uses the various input variables necessary to forecast the number of available dairy replacement animals approximately 34 months from breeding. Expected number of dairy replacement animals are compared against the anticipated need of replacements and informs the producer if there is a shortage. In addition, it provides an economic analysis that projects the estimated costs in semen and revenue generated from selling excess female dairy calves, dairy bull calves and dairy x beef cross calves.

Users can compare different breeding strategies but also analyze the impact on number of future replacements based on making management improvements. One example of this would be the anticipated extra revenue from selling dairy x beef cross calves if the farm builds a new calf facility and lowers calf losses.

HeiferHub provides a valuable tool that takes the guesswork out of making breeding decisions and makes a complex process much easier to manage.

Keywords: Fourdraine, breeding, forecast, beef, heifer, replacements
Presented at the ICAR Anual Conference 2024 in Bled at the Session Session 1a: Decision Support Tools of the Future – Promoting Sustainability Farm Management

Introduction

Historically, the process of maintaining the preferred milking and replacement herd size was relatively simple. Cows were bred exclusively to dairy semen and typically the number of female calves generated would be adequate to ensure that enough animals would be available to replace those that left the herd for either voluntary or involuntary reasons. Excess heifer inventories and dairy bull calves would be sold at market prices.

Since the introduction of breeding dairy cows with AI beef bulls and the availability of sexed semen to create more dairy replacements, the decisions surrounding the number of cows to breed to the various types of semen has become more complex. U.S. dairy farmers can sell a dairy x beef cross calf for a significantly higher price than a dairy bull calf. Producers have reported revenues for a single cross bred calf in the range of 200 to 800 dollars while dairy bull calves would typically generate less than 100 dollars. This provides a significant additional source of revenue for dairy producers, especially in times when milk prices are low. To seize on this opportunity, dairy farmers have increased the number of animals bred to AI beef sires. In addition, they have reduced the number of animals bred to conventional dairy semen and increased the number of animals bred to dairy sexed semen to ensure enough replacements are available in the future. The math can be complicated because there are many factors to consider post breeding – to include conception rates, pregnancy losses, stillbirths, heifer losses and level of involuntary culling. Combining the impact of all these factors can dramatically impact the final number of replacements available 34 months from when the cows are bred. Dairy farmers typically track conception and pregnancy losses. But it is more complicated to obtain information about calf losses and stillbirths, for example, from on farm management software and it requires access and summarization of historic records that typically are not retained on farm. Therefore, dairy farmers make their best guess which in some cases has led to either an excess or shortage of dairy replacements. When a shortage occurs, the dairy has to find suitable replacements typically at a higher price and genetically lower quality and assume the risk of disease exposure. Current heifer purchase prices range between $2,400 and $3,500 so there is a significant economic incentive not to be short on replacements.

The financial incentive is clear, however the problem at hand for many producers is: "How do I maximize my returns while breeding the maximum number of cows to AI beef bulls while also ensuring enough replacement animals?" This paper will provide an overview of a new and exciting web based decision support tool named HeiferHub offered by DRMS that takes the guesswork out of making breeding decisions, maximizes future returns and ensure the farm has enough replacement animals.

A new reality

Using DRMS breeding data, the annual trend for number of breedings by semen type continues to be more breedings to beef and sexed dairy semen and less breedings with conventional dairy semen. Figure 1 shows March 2024 DRMS breeding data separated by semen type. When compared to the same month in 2023, conventional dairy semen usage dropped from 45% to 40%, sexed dairy semen is up 1% and beef semen is up 4% from the prior year.

The data shown in Table 1 represents the 2023 overall breeding trend based on data from more than 9200 dairy farms. However there are significant differences between herds. Evaluating a single farm's data can provide valuable insights about the current breeding program and adjustments that have been made over time. Figures 2 and 3

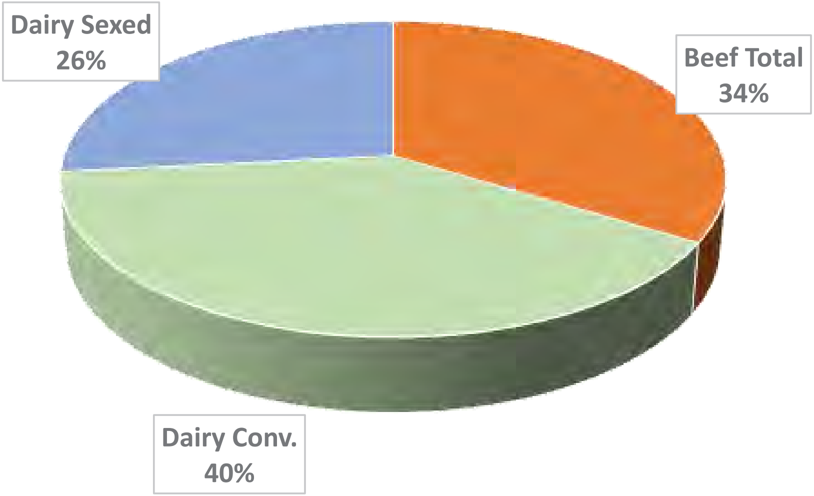
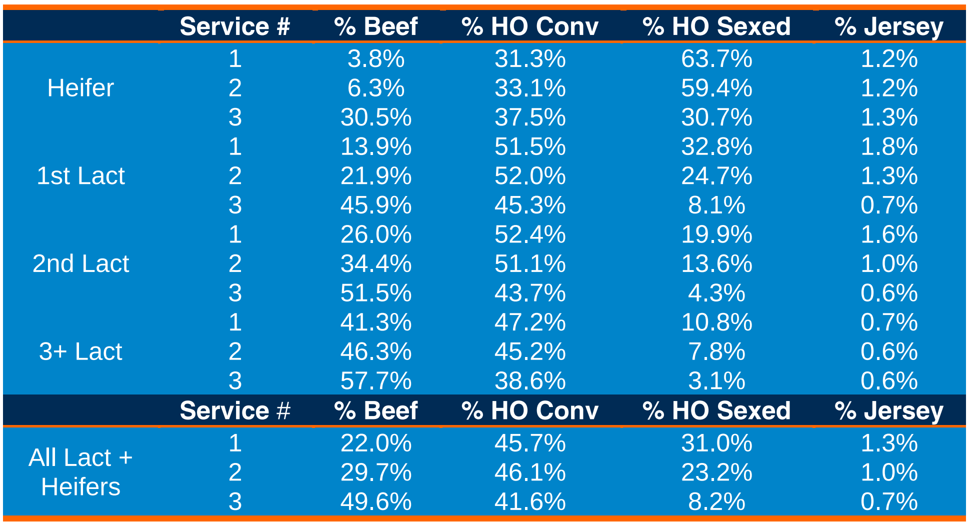


Figure 1. DRMS March 2024 semen distribution by semen type (Source: DRMS)

Figure 1. DRMS March 2024 semen distribution by semen type (Source: DRMS).



	Service #	% Beef	% HO Conv	% HO Sexed	% Jersey
Heifer	1	3.8%	31.3%	63.7%	1.2%
	2	6.3%	33.1%	59.4%	1.2%
	3	30.5%	37.5%	30.7%	1.3%
1st Lact	1	13.9%	51.5%	32.8%	1.8%
	2	21.9%	52.0%	24.7%	1.3%
	3	45.9%	45.3%	8.1%	0.7%
2nd Lact	1	26.0%	52.4%	19.9%	1.6%
	2	34.4%	51.1%	13.6%	1.0%
	3	51.5%	43.7%	4.3%	0.6%
3+ Lact	1	41.3%	47.2%	10.8%	0.7%
	2	46.3%	45.2%	7.8%	0.6%
	3	57.7%	38.6%	3.1%	0.6%
	Service #	**% Beef**	**% HO Conv**	**% HO Sexed**	**% Jersey**
All Lact + Heifers	1	22.0%	45.7%	31.0%	1.3%
	2	29.7%	46.1%	23.2%	1.0%
	3	49.6%	41.6%	8.2%	0.7%

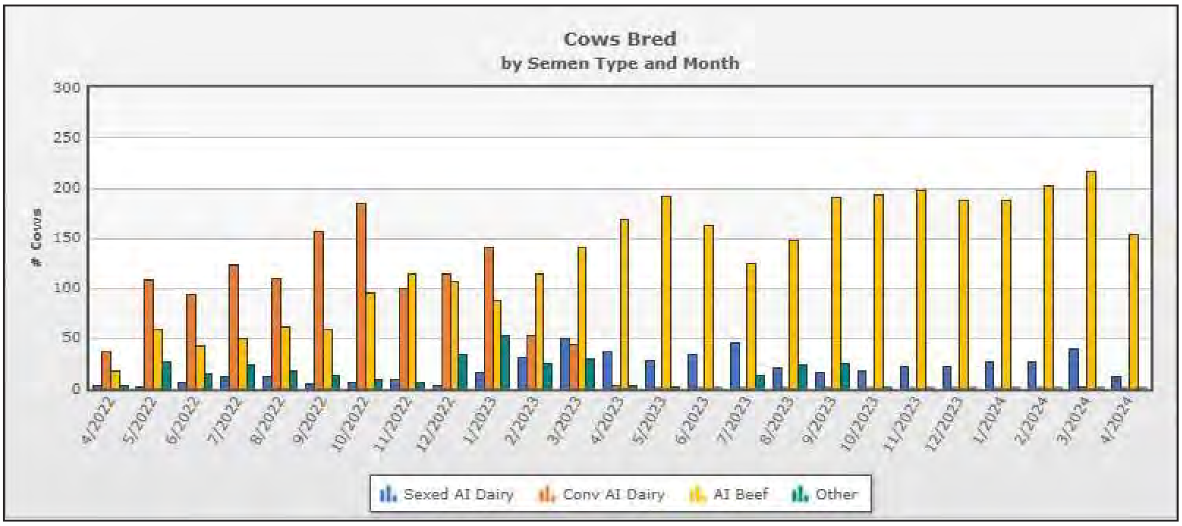


Figure 2. Percent of cows bred by semen type and month (Source: DRMS HerdHQ)

show a single herd's breeding trend based on semen type for the milking cows and heifers respectively.

Based on the data shown in Figures 2 and 3, the farm made a significant change in March 2023. The staff stopped breeding cows to conventional dairy semen while dramatically increasing the use of beef semen to breed cows. To offset the reduction in dairy replacement animals, they increased the use of sexed dairy semen on some of the cows but more so on the dairy heifers. The number of replacement animals resulting from these decisions will not be known until calves are born and reach breeding age and subsequent calving.

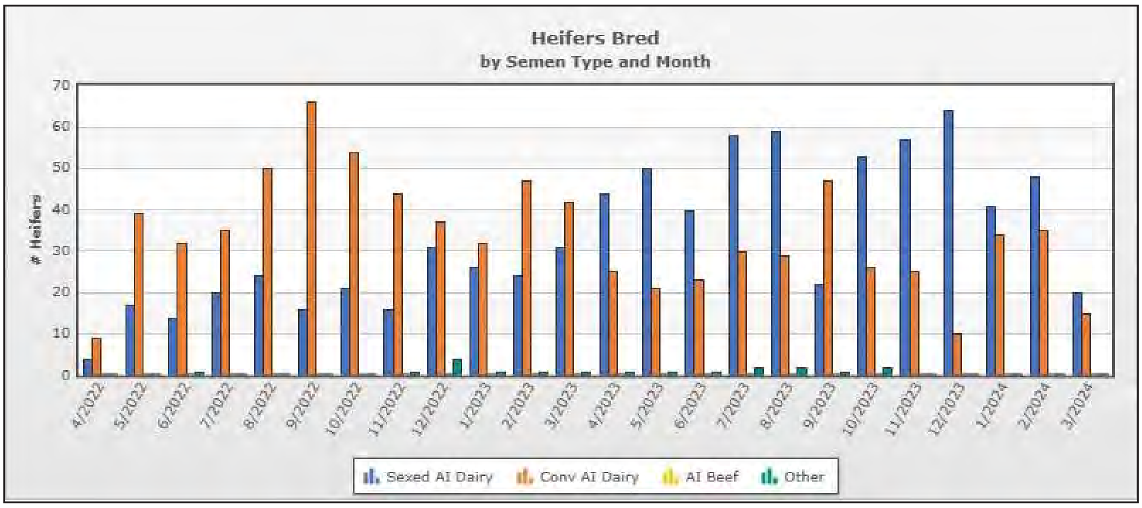


Figure 3. Percent of heifers bred by semen type and month (Source: DRMS HerdHQ)

In addition to the change in breeding strategy, one can also determine the variation in number of animals bred each month which in turn adds more variability to the number of available replacements in the future.

Consistency in producing the right number of replacements each month is an important consideration as well. Factors that will determine how many replacement animals are available in the future are:

- Conception Rate.
- Pregnancy loss.
- Stillbirth.
- Calf loss.
- Heifer losses (between birth to calving).
- Age at Calving.

These numbers can vary throughout the year. For example, summer heat can negatively impact conception rates and pregnancy losses while winter cold and humidity can negatively impact stillbirths and calf losses. Figure 4 shows projected calvings for the same herd over the next 8 months. Quite a lot of variation is expected in the projected number of replacement dairy calves. In May 2024, the projected number of dairy replacement calves born is more than twice as the prediction for June 2024. Typically, farmers will evaluate the projected number of replacement calves born from month to month and subsequently adjust the percentages of animals bred to each semen type. Using historic data allows the farmer to determine patterns in effects such as conception rates and pregnancy losses to enable him to proactively select the correct number of animals to breed to the various semen types.

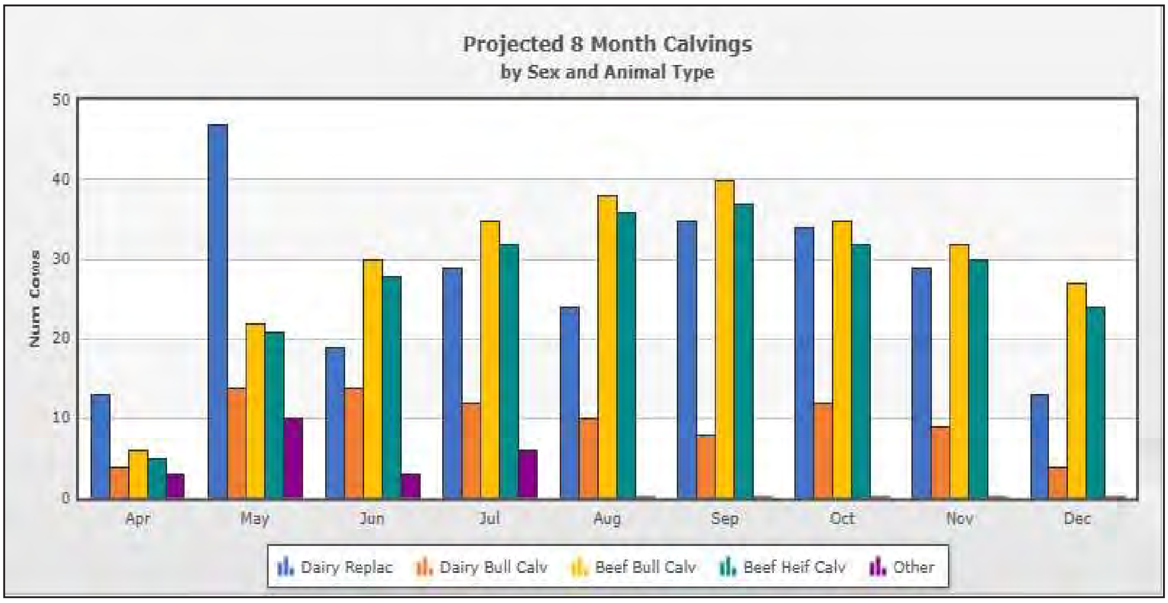


Figure 4. Projected 8 month calvings by sex and animal type (Source: DRMS HerdHQ).

How can HeiferHub improve the breeding decision making process?

The primary concept of HeiferHub is to use common understandings about biological facts and apply simple math to create predictions. The starting points are

1. required number of replacements as affected by yearly voluntary and involuntary culling rates,
2. number of animals to be bred in the upcoming month or week and
3. percent of animals bred to each semen type.

The next step in the process is to project the number of pregnancies, live calves born, and calves that will become pregnant and survive to calve themselves. This step takes into account conception rates, pregnancy losses, and other factors.

The challenge for most farmers is to obtain the input parameters. When farmers are asked for their herds' stillbirth rate or percent of heifers that become pregnant and survive to calving, most will make a rough guess. Determining these numbers requires investigation using current and historic records. DRMS simplified this process by using data collected on DHIA testday or daily via Dart herd management software. Automating the process drops the guesswork of determining the input parameters used in computations. HeiferHub flexibly allows input parameters to be changed from default yearly numbers derived from data files and allows the user to adjust for variation throughout the year. For example, stillbirth rates in Midwest U.S. are usually higher in January and February. Additionally calf losses are typically higher in the winter months.

HeiferHub provides default input parameters based on the herd's history. However the user can also adjust these numbers to accommodate expected changes in management practices and to evaluate the impact on projected number of replacements.

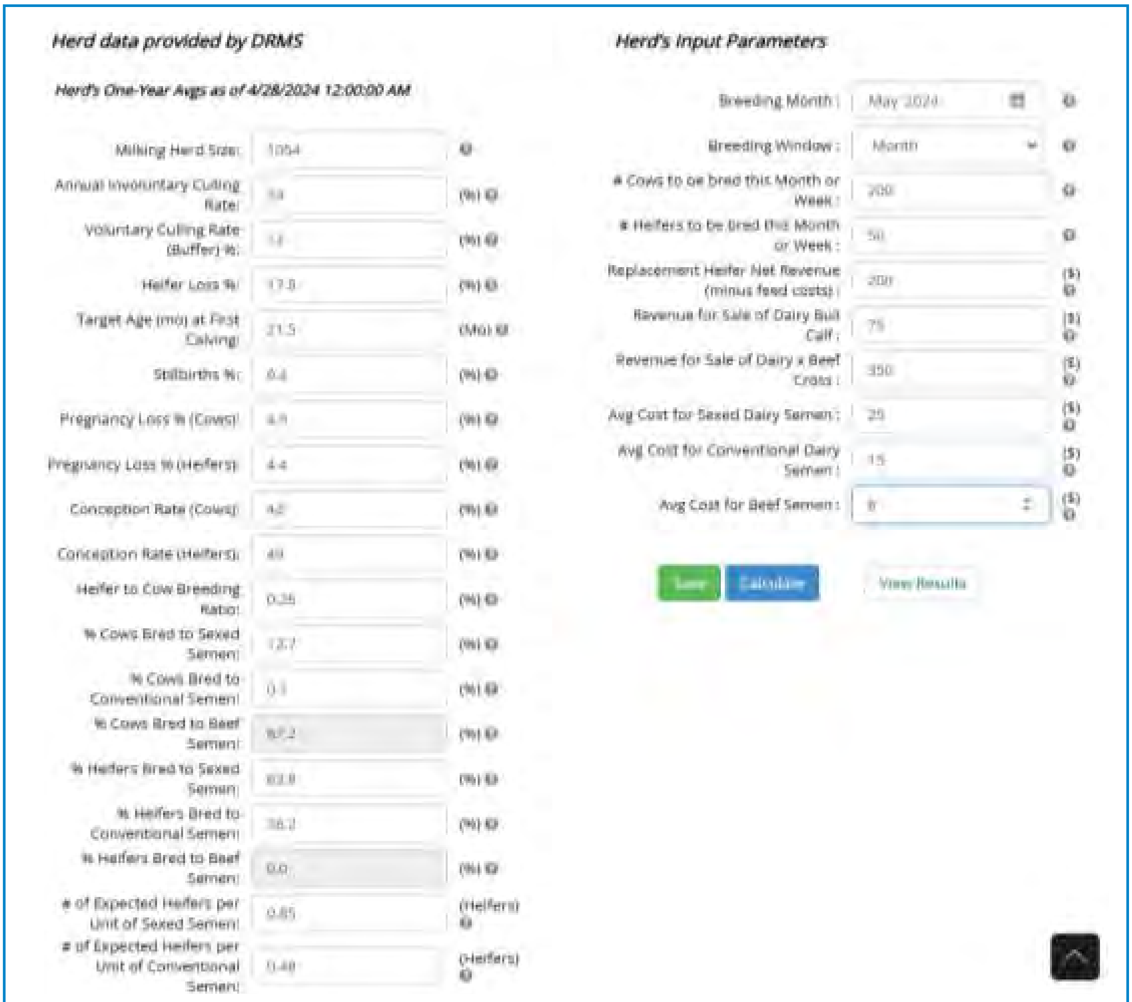
Figure 5 shows the HeiferHub input screen. Most values are prefilled based on the data collected by DRMS. However numbers such as semen price and revenue from selling calves need to be provided by the user. HeiferHub will calculate the number of anticipated calves based on number of animals to be bred by month or week. Setup screens can be saved so the user can easily return and make new projections.

As shown in Figure 6, results are presented in a stepwise process starting with:

1. The number of cows and heifers being bred to each semen type.
2. The number of anticipated pregnancies by type of offspring.
3. Finally, the projected number of replacements.

HeiferHub will calculate the anticipated number of replacement animals needed based on the herd's involuntary culling rates and heifer losses, and, it will show either the number of deficit replacements or the number of extra heifers that may be sold.

An additional feature of HeiferHub is that it will calculate the anticipated revenues from selling dairy replacement calves, dairy bull calves and Dairy x Beef crossbred calves



Herd data provided by DRMS

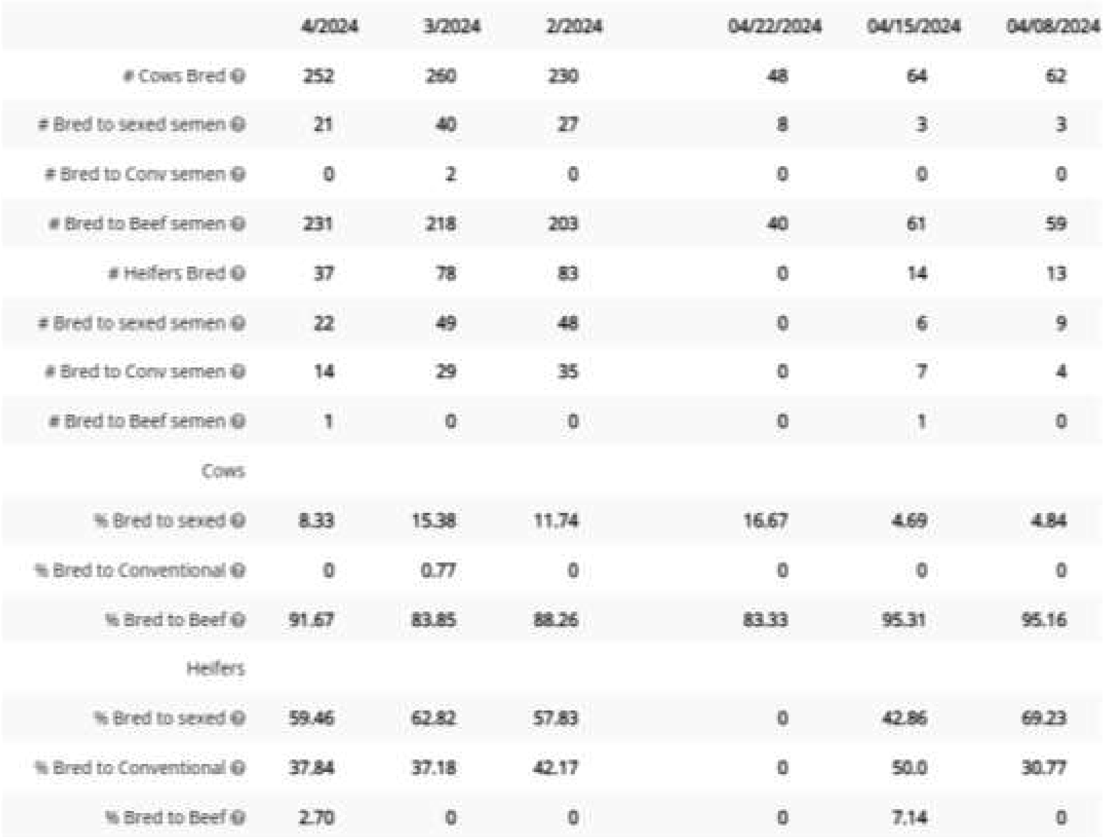
Herd's One-Year Avgs as of 4/28/2024 12:00:00 AM

Milking Herd Size:	1054	
Annual Involuntary Culling Rate:	[illegible]	(%)
Voluntary Culling Rate (Buffer) %:	[illegible]	(%)
Heifer Loss %:	17.0	(%)
Target Age (mo) at First Calving:	21.5	(Mo)
Stillbirths %:	6.4	(%)
Pregnancy Loss % (Cows):	8.9	(%)
Pregnancy Loss % (Heifers):	4.4	(%)
Conception Rate (Cows):	42	(%)
Conception Rate (Heifers):	49	(%)
Heifer to Cow Breeding Ratio:	0.26	(%)
% Cows Bred to Sexed Semen:	12.7	(%)
% Cows Bred to Conventional Semen:	0.1	(%)
% Cows Bred to Beef Semen:	87.2	(%)
% Heifers Bred to Sexed Semen:	63.8	(%)
% Heifers Bred to Conventional Semen:	36.2	(%)
% Heifers Bred to Beef Semen:	0.0	(%)
# of Expected Heifers per Unit of Sexed Semen:	0.85	(Heifers)
# of Expected Heifers per Unit of Conventional Semen:	0.48	(Heifers)

Herd's Input Parameters

Breeding Month :	May 2024	
Breeding Window :	Month	
# Cows to be bred this Month or Week :	200	
# Heifers to be bred this Month or Week :	50	
Replacement Heifer Net Revenue (minus feed costs) :	200	($)
Revenue for Sale of Dairy Bull Calf :	75	($)
Revenue for Sale of Dairy x Beef Cross :	350	($)
Avg Cost for Sexed Dairy Semen :	25	($)
Avg Cost for Conventional Dairy Semen :	15	($)
Avg Cost for Beef Semen :	8	($)

Figure 5. HeiferHub herd input parameter screen



	4/2024	3/2024	2/2024	04/22/2024	04/15/2024	04/08/2024
# Cows Bred ❓	252	260	230	48	64	62
# Bred to sexed semen ❓	21	40	27	8	3	3
# Bred to Conv semen ❓	0	2	0	0	0	0
# Bred to Beef semen ❓	231	218	203	40	61	59
# Heifers Bred ❓	37	78	83	0	14	13
# Bred to sexed semen ❓	22	49	48	0	6	9
# Bred to Conv semen ❓	14	29	35	0	7	4
# Bred to Beef semen ❓	1	0	0	0	1	0
Cows						
% Bred to sexed ❓	8.33	15.38	11.74	16.67	4.69	4.84
% Bred to Conventional ❓	0	0.77	0	0	0	0
% Bred to Beef ❓	91.67	83.85	88.26	83.33	95.31	95.16
Heifers						
% Bred to sexed ❓	59.46	62.82	57.83	0	42.86	69.23
% Bred to Conventional ❓	37.84	37.18	42.17	0	50.0	30.77
% Bred to Beef ❓	2.70	0	0	0	7.14	0

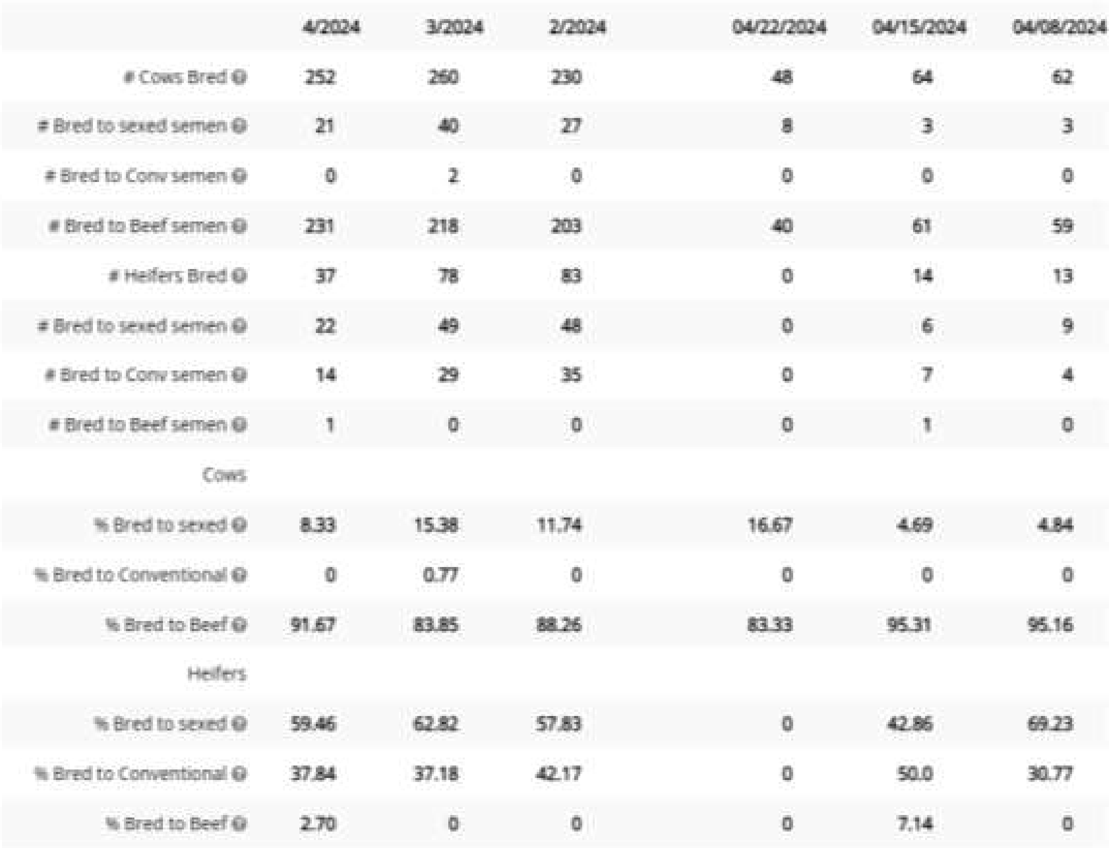
Figure 6. Results Screen for HeiferHub.

minus semen costs. The user can adjust the numbers and determine which scenario provides the greatest return from selling animals while still meeting required numbers of replacement animals.

Conclusions

HeiferHub uses the past year’s breeding events to determine the percent of animals that were bred to each semen type. However, often producers will adjust their actions from month to month. Therefore, it became valuable to add a summary for the both the recent three months and recent three weeks - showing number of animals bred to each semen type. Figure 7 shows an example of this summary.

Understanding the number of animals bred to each semen type for the past months (or weeks) will provide a more recent perspective of the breeding decisions made. These numbers can be used to adjust the annual percentages on the input parameter screen.



	4/2024	3/2024	2/2024	04/22/2024	04/15/2024	04/08/2024
# Cows Bred	252	260	230	48	64	62
# Bred to sexed semen	21	40	27	8	3	3
# Bred to Conv semen	0	2	0	0	0	0
# Bred to Beef semen	231	218	203	40	61	59
# Heifers Bred	37	78	83	0	14	13
# Bred to sexed semen	22	49	48	0	6	9
# Bred to Conv semen	14	29	35	0	7	4
# Bred to Beef semen	1	0	0	0	1	0
Cows						
% Bred to sexed	8.33	15.38	11.74	16.67	4.69	4.84
% Bred to Conventional	0	0.77	0	0	0	0
% Bred to Beef	91.67	83.85	88.26	83.33	95.31	95.16
Heifers						
% Bred to sexed	59.46	62.82	57.83	0	42.86	69.23
% Bred to Conventional	37.84	37.18	42.17	0	50.0	30.77
% Bred to Beef	2.70	0	0	0	7.14	0

Figure 7. Breeding History from HeiferHub.

HeiferHub is an easy-to-use web tool that allows producers to make quick and-well informed breeding decisions to ensure maintenance of replacement needs while also capitalizing on potential revenue from the sale of crossbred calves. HeiferHub can be used by both producers and consultants that have permission to access herd data. HeiferHub can use either DHIA testday data or up-to-the-minute data from herds enrolled on the Dart herd management software and the DRMS on-farm and web synchrony system DartSync.

HeiferHub does not select individual service sires to use in a mating program. Nor does it provide mating recommendations for individual cows. Producers can work with their AI company to match their cows with the appropriate service sire.

Optimization of dairy herd replacements combining conventional, sexed and beef semen in mating programs

V. Ferrari[1,2], M. Marusi[1], M. Penasa[2], J.B.C.H.M. van Kaam[1], R. Finocchiaro[1] and M. Cassandro[1,2]

[1]Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ), Via Bergamo 292, 26100 Cremona (CR), Italy
[2]Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
Corresponding Author: valentinaferrari@anafi.it

Abstract

Farmers have often an abundance of replacement heifers in their herds due to increasing use of sex-sorted semen and genomic tools. Given the current Italian market conditions, rearing more heifers than needed is not a profitable strategy. On the other hand, the higher market value of crossbred dairy calves is an attractive strategy for dairy farmers. The aim of this study was to develop a tool to help Italian dairy farmers identify the annual female replacement needs to optimise economic outcome of the dairy herd. The approach is based on herd performance and combination of different semen types (conventional, sex-sorted, and beef semen), with the ultimate goal of enhancing farm profit. A case study based on a 350-cow Holstein herd was used and 3 levels of herd fertility (high, medium, and low) were simulated to define the required yearly number of dairy female replacements and the number of females yielded under different scenarios of semen utilization. The number of annual dairy replacements was obtained as the number of cows multiplied by the replacement rate, adjusted by the age at first calving, and the number of animals yielded was derived by semen type utilization, calf and heifer mortality, pregnancy losses, and calving interval, and it was used to evaluate the replacement cost per 100 L of milk. The latter was calculated from all costs incurred from birth to first calving of all females yielded minus revenues from selling cull cows, heifers, dairy male calves, and calves from beef when beef semen was used, and dividing the result by income from 100 L of milk sold. Then, four strategies of sexed semen utilization were combined with five strategies of beef semen use. Animals that were not inseminated with sexed or beef semen were bred with conventional semen. Regardless of fertility level, the required number of dairy female replacement heifers were 110. Increasing beef semen use allowed to yield less replacement heifers. Furthermore, as beef semen use increased and the number of replacement heifers decreased, replacement cost per 100 L of milk reduced. Our results suggested that replacement costs increase with increasing number of yielded heifers. Hence, combining beef and sexed semen to reach heifer balance close to zero, decreased the replacement cost. Farmers should choose the strategy that allows them to reach the annual heifer replacement needs, considering the effects of fertility. Once obtained, they should select the scheme that decreases the replacement cost. The tool will be implemented into ANAFIBJ online mating program and used prior to select which heifers or cows to mate with a given bull to enhance herd genetic potential, decrease inbreeding, lower GHG emissions and to provide farmers an approach to identify the best replacement strategy.

Keywords: heifer, management, tool, crossbreeding, sexed semen, mating program. Presented at the ICAR Anual Conference 2024 in Bled at the Session 1a: Decision Support Tools of the Future – Promoting Sustainability Farm Management"

Introduction

When farmers devise their breeding plans, they must weigh numerous factors such as semen type semen destination use (e.g., dairy or beef), and semen price (De Vries *et al.*, 2022). Additionally, integrating sex-sorted semen with genomic tools can accelerate genetic advancements and increase the availability of young females for future herd replacements (Hjortø *et al.*, 2015). As a result, some farmers display surplus replacement heifers and it has been shown that culling cows to leave space to replacement heifers is not necessarily the most profitable strategy for a herd (DeVries, 2020). In the current Italian market, it's generally unprofitable to breed excess heifers for sale to other farmers. However, there's a growing interest among dairy farmers in the higher market value of crossbred dairy calves (Cabrera, 2022).

The choice to breed high-genetic-merit animals with sexed semen to meet replacement needs, while using beef semen for the rest, presents an opportunity to simultaneously enhance herd genetics and profitability. Various studies have explored different breeding strategies to optimize herd performance, highlighting the advantages of using sex-sorted semen, particularly on genetically superior and fertile animals (Ettema *et al.*, 2017; Holden and Butler, 2018; Clasen *et al.*, 2021). Despite its lower conception rate compared to conventional semen, sexed semen is preferred for virgin heifers and first-lactation cows due to their better fertility performance. Moreover, it's been observed a risk reduction of dystocia and stillbirth with the use of sex-sorted semen, as female calves are typically smaller and easier to deliver (Holden and Butler, 2018; Pahmeyer and Britz, 2020).

Determining the optimal number of replacement heifers to keep and selecting the best strategy are crucial aspects of herd management. Currently, there's a lack of a specific tool for Italian dairy farmers to aid in selecting the most suitable replacement strategy based on their herd's productivity and reproductive data. Therefore, the aim of this study was to develop a replacement tool to help Italian dairy farmers identify the annual female replacement needs by varying use of sexed and beef semen on herd costs and stability under Italian conditions.

Material and methods

The method is based on the approach proposed by Genex Cooperative (Ontario, CA) and adjusted to the Italian herd and market conditions. A practical tool, housed in an Excel spreadsheet, has been devised to allow users to customize it according to their own situations. To illustrate its functionality, a hypothetical scenario was constructed around a 350-cow Holstein herd (250 cows and 100 heifers entering per year) located in the Po Valley (Northern Italy), targeting 40% replacement rate, 7% stillbirth rate, 5% calves and heifers rearing loss, and 8% pregnancy loss, which represents averages extrapolated by the Italian Holstein, Brown Swiss and Jersey Association (Cremona, Italy). Additionally, to account for unexpected issues or to allow for more "voluntary" culling, an additional 10% of heifers has been considered.

The tool simulates different fertility scenarios: high (HFL), medium (MFL), and low fertility (LFL). Age at first calving was set at 24 mo (regardless of the fertility level of the herd), conception rate (CR) at 50%, 43%, and 32% for HFL, MFL, and LFL, respectively, and calving interval at 13, 14, and 14.5 mo for HFL, MFL, and LFL, respectively. It assumes equal fertility rates for conventional beef and dairy semen, with reduced fertility for sexed dairy semen. Percentage of female calves from conventional and beef semen was set at 47%, and from sexed semen at 90%. Farmers can adjust input data to match their specific herd characteristics and objectives. Input variables are displayed in Table 1.

The tool initially calculates the number of dairy female replacements needed annually, assuming all inseminations are done with conventional semen and herd size remaining stable. The number of annual dairy replacements was obtained as the number of cows multiplied by the replacement rate and adjusted by the age at first calving, in order to account only for heifers that are going to calve during the considered year. It then explores various combinations of sexed and beef semen utilization.

The sexed semen scenarios were:

1. No use of sexed semen (NOSS).
2. 100% of heifers inseminated with sexed semen (H100).
3. 100% of heifers and 20% of top cows inseminated with sexed semen (H100C20), and 4) 80% of heifers and 20% of top cows inseminated with sexed semen (H80C20).

Beef semen utilization was allocated to cows that were not inseminated with sexed semen, according to farm management decisions, at the following percentages: 1) 0%, 2) 25%, 3) 50%, 4) 75%, and 5) 100%. All remaining eligible animals that were

Table 1. Input variables of the heifer management tool. All input data can be changed by the farmer or technician according to specific herd situation.

Variable	Input value
Cows (lactating and dry) (n)	250
Breeding heifers entering the herd (n/yr)	100
Annual replacement rate (%)	40
Annual herd growth rate target (%)	0
Heifers' safety percentage (%)	10
Sex ratio (females/males) by semen type (%)	47/53 (conventional and beef) 90/10 (sexed)
Calving interval according to the fertility level[1] (mo)	13 (high), 14 (medium), 14.5 (low)
Animals not inseminated (%)	2
Pregnancy loss (%)	8
Stillbirth rate (%)	7
Mortality from weaning to first calving (%)	5
Age at first calving (mo)	24
Average heifer rearing cost (€/d)	4.29
Average heifer market value (€)	1800
Average cost for disposal of dead-on-farm cow (€)	300
Average cull cow market value (€)	800
Average purebred male dairy calf market value (€)	51.60
Average crossbred calf market value (€)	245
Milk production (L/d)	31
Total milk sold per year (L)	2,828,750

[1] high = high herd fertility level (50% conception rate and 13 mo calving interval); medium = medium herd fertility level (43% conception rate and 14 mo calving interval); low = low herd fertility level (32% conception rate and 14.5 mo calving interval).

not inseminated with sexed or beef semen were bred with conventional semen. The method calculates heifer balance between the number of heifers yielded and the annual dairy replacement needs. The number of animals yielded was used to evaluate the replacement cost per 100 L of milk. This information helps evaluate the cost-effectiveness of different semen utilization protocols, taking into account feed costs and market values. Replacement cost (RC) is the cost to maintain a herd at the same size per 100 L of milk sold and is generally used to compare different breeding strategies. It depends on some economic factors such as annual replacement rate, heifer rearing cost, and revenue from selling milk (Bethard and Nunes 2011).

Results and discussion

The method presented in the paper is a valuable instrument to help farmers identify the correct number of dairy heifers to be inseminated to maintain constant the herd size (or to set an annual growth rate) and to minimize rearing costs. Table 2 reported the annual number of heifers and cows eligible to be mated, the number of services per conception needed to maintain a constant adult herd size, the conception rate under the 3 fertility levels (HFL, MFL, and LFL), the number of the annual dairy female replacement cows, and the number of heifers yielded. The number of dairy female replacement heifers that the farm needs is 110, for HFL, MFL, and LFL.

Table 3 summarizes the possible pairwise solutions of the tool (replacement costs per 100 L of milk, and heifer balance) that result from the different strategies of beef and sexed semen use under the 3 different herd fertility levels. Larger use of beef semen allows farmers to yield less heifers, on a yearly basis; indeed, when heifer balance is negative, farmers are breeding less heifers than needed, whereas positive values means that farmers are breeding more than needed heifers. Accordingly, as beef semen use increases and reared heifers reduces, replacement cost per 100 L of milk decreases regardless of reproductive performance. When heifer balance is below zero, replacement cost is reported, but it should be noted that this is not a replacement strategy that should be pursued by farmers, as it means that, if followed, herd size will decrease, or farmers have to buy heifers to maintain their herd size.

Furthermore, increasing the use of dairy sexed-sorted semen within the four dairy sexed semen utilization strategies (NO_{SS}, H_{100}, $H_{100}C_{20}$, $H_{80}C_{20}$) leads to an increase

Table 2. Number of heifers and cows to breed, number of dairy replacements needed per year, number of dairy heifers yielded, number of services per conception, and average conception rate (%) needed to maintain a constant herd size under 3 fertility levels1, assuming 100% use of conventional semen.

		Services/conception, n			Conception rate, %		
Animals	**Eligible animals, n**	**High**	**Medium**	**Low**	**High**	**Medium**	**Low**
Heifers	100	1.8	2.0	2.5	55	50	40
Cows	250	2.2	2.9	4.3	45	35	23
Annual replacements needed	110						
Number of dairy heifers yielded	90 (low) 94 (medium) 98 (high)						

[1] High = high herd fertility level (50% conception rate and 13 mo calving interval); medium = medium herd fertility level (43% conception rate and 14 mo calving interval); low = low herd fertility level (32% conception rate and 14.5 mo calving interval).

Table 3. Replacement costs per 100 L of milk (€) and heifer balance1 (in parentheses) for different strategies of beef and sexed semen use under different herd fertility levels. Missing values refer to breeding strategies that cannot be pursued.

	Dairy sexed semen use^3			
Beef semen use, %	**NO_{SS}**	**H_{100}**	**$H_{100}C_{20}$**	**$H_{80}C_{20}$**
Low fertility level^2				
0	9.02 (-20)	9.73 (4)	10.00 (16)	9.87 (11)
25	8.52 (-36)	9.18 (-12)	9.50 (0)	9.37 (-5)
50	8.03 (-52)	8.68 (-28)	9.00 (-16)	8.87 (-21)
75	7.53 (-68)	8.18 (-44)	8.51 (-32)	8.37 (-37)
100	7.03 (-84)	7.69 (-60)	- (-)	- (-)
Medium fertility level^2				
0	9.11 (-16)	9.79 (8)	10.12 (20)	9.98 (16)
25	8.59 (-33)	9.27 (-8)	9.61 (4)	9.47 (-1)
50	8.08 (-50)	8.76 (-25)	9.09 (-13)	8.95 (-18)
75	7.56 (-66)	8.24 (-41)	8.57 (-29)	8.44 (-34)
100	7.05 (-83)	7.73 (-58)	- (-)	- (-)
High fertility level^2				
0	9.22 (-12)	10.01 (17)	10.4 (31)	10.24 (25)
25	8.70 (-29)	9.50 (0)	9.88 (14)	9.73 (8)
50	8.19 (-45)	8.98 (-16)	9.37 (-2)	9.21 (-8)
75	7.67 (-62)	8.46 (-33)	8.85 (-19)	8.69 (-25)
100	7.15 (-78)	7.94 (-49)	- (-)	- (-)

[1] Heifer balance was calculated as annual dairy replacements needed minus annual dairy heifers yielded.

[2] High = high herd fertility level (50% conception rate and 13 mo calving interval); medium = medium herd fertility level (43% conception rate and 14 mo calving interval); low = low herd fertility level (32% conception rate and 14.5 mo calving interval).

[3] NO_{SS} = no use of sexed semen; H_{100} = 100% of heifers inseminated with sexed semen; $H_{100}C_{20}$ = 100% of heifers and 20% of top cows inseminated with sexed semen; $H_{80}C_{20}$ = 80% of heifers and 20% of top cows inseminated with sexed semen. All remaining eligible animals that were not inseminated with sexed or beef semen were bred with conventional semen.

of replacement cost (and higher number of reared heifers), regardless of beef semen use. Better fertility level leads to higher number of heifers reared, at the same level of beef and sexed semen use. Looking at these results, it is clear the positive relationship between replacement cost and heifer balance as greater replacement costs were obtained with higher number of heifers yielded, which also corresponds to lower use of beef semen.

The highest replacement cost has been obtained with 0% beef semen and $H_{100}C_{20}$ (rearing from 20 to 31 heifers more than needed, for MFL and HFL, respectively), whereas the lowest with 100% use of beef semen and NO_{SS} (but rearing from -84 to -83 heifers than needed, for LFL and MFL, respectively, to maintain constant the herd size). Ideal situations can be reached adjusting beef and sexed semen, to reach heifer balance close to zero (Table 3), indeed, the combination of beef semen and sexed semen, within strategies and reproductive performances, decreased the replacement cost. Within their reproductive performance, farmers should choose the strategy that allow them to reach their annual heifer replacement needs; once obtained, they should select the scheme that decreases the replacement cost.

The tool provides dairy farmers with a method to determine the optimal replacement strategy, taking into account the impact of fertility by varying the use of sexed and beef semen on herd costs and stability. This tool will be integrated into the ANAFIBJ online mating program and used beforehand to decide which heifers or cows to mate with a specific bull, aiming to improve the herd's genetic potential and reduce inbreeding.

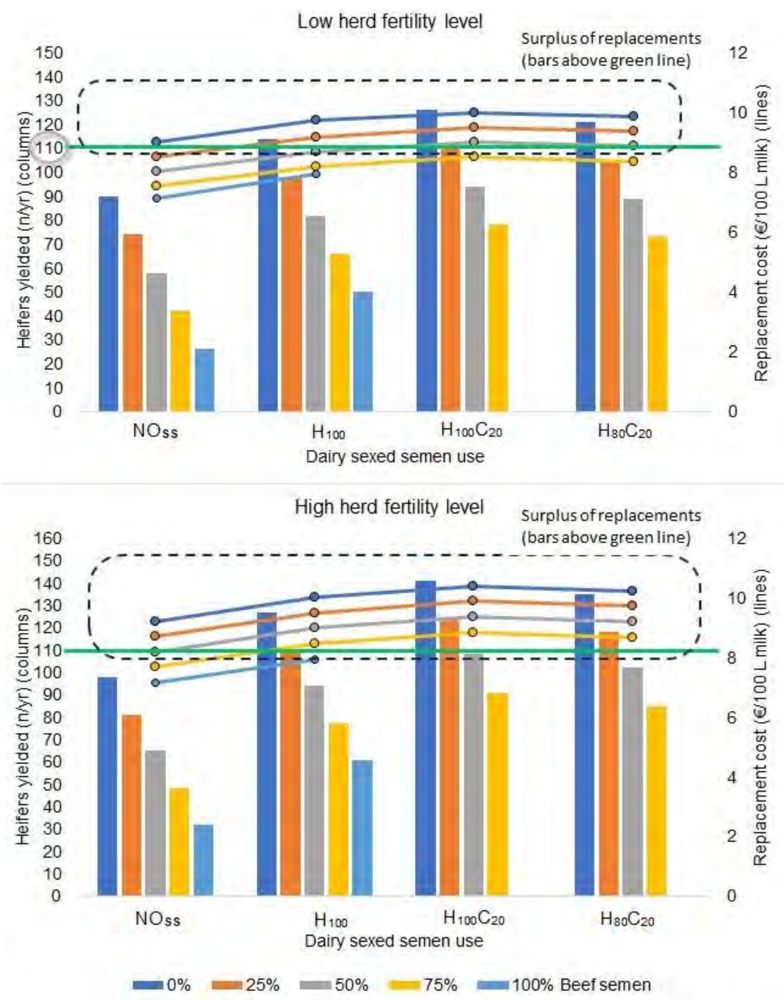


Figure 1. Annual dairy heifers yielded for the different sex semen utilization strategies by different dairy sexed semen use (NOSS = no use of sexed semen; H100 = 100% of heifers inseminated with sexed semen; H100C20 = 100% of heifers and 20% of top cows inseminated with sexed semen; H80C20 = 80% of heifers and 20% of top cows inseminated with sexed semen) (bars) and replacement costs (lines, second axis) at different beef semen use for a) low herd fertility and b) high herd fertility level. Green line represents the number of annual dairy female replacements needed.

Replacement cost:
RC = [cost of rearing replacements – (cull cow income + income from male calves sold)] / income from 100 L of milk sold (1)

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Slovenian dairy farmers' view on breeding goals

A. Ule[1], K. Erjavec[2] and M. Klopčič[1]

[1]Department of Animal Science, Biotechnical Faculty, University of Ljubljana,
Groblje 3, 1230 Domžale, Slovenia
[2]Faculty of Economics and Informatics, University of Novo mesto,
Na Loko 1, 8000 Novo mesto, Slovenia
Corresponding Author: anita.ule@bf.uni-lj.si

Abstract

This study examines the influence of socio-demographic factors on the breeding goals of dairy farmers and their clustering in Slovenia. It is important to understand farmers' perspectives on breeding objectives, as they may differ from those of other stakeholders involved in breeding. Involving farmers in the process of setting breeding goals can improve the use of selection tools and confidence in the selection process. A mixed methods approach was used. As focus groups are a useful qualitative method to quickly obtain in-depth information on participants' attitudes and opinions on the topic under study, the aim of using focus groups was to find out farmers' views on breeding target traits. To obtain views that are representative of the population, we conducted a quantitative survey to determine how preferences regarding breeding objectives vary across the cattle breeding community, focusing on the role of farmers' socio-demographic factors. Three focus groups with 30 participants explored farmers' needs, attitudes towards genomic selection, barriers and benefits to adoption, the structure of the Total Merit Index and preferences in breeding objectives. An online questionnaire distributed to Slovenian dairy farmers received 212 responses. A cluster analysis based on the distribution of the weights of the trait categories in the Total Merit Index identified three different groups of farmers. Despite the differences, animal health, animal welfare and reproduction traits dominated across the sample, while environmental and meat traits were considered less important. The quantitative analysis revealed that new environmental traits are less important, which is attributed to societal pressure and negative perceptions of the environmental impact of dairy farming. In the focus group discussions, reservations were expressed about traits such as greenhouse gas emissions, reflecting societal sentiment and the constraints of farming. This study demonstrates the importance of combining qualitative and quantitative methods to gain a comprehensive understanding. The results show that farmers aspire to a new structure of Total Merit Index that includes several trait categories, with milk production traits being the most important. Three distinct groups of farmers emerged, each with their own focus. Animal health and welfare were seen as the most important traits, while new traits such as environmental traits were viewed less positively. The results of the study can help to develop new breeding goals and increase the confidence of breeders in the selection process through active engagement.

Key words: breeding goals, traits, total merit index, dairy cattle breeders.
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Introduction

Genomic selection has transformed dairy cattle breeding, enabling farmers to achieve higher annual rates of genetic gain by using genomically tested animals in their herds. This approach also makes it possible to select for traits that are difficult to measure, such as feed efficiency, methane emissions, and energy balance. By optimizing mating plans, genomic selection helps to maximize genetic gain while controlling inbreeding, ensuring accurate pedigrees and avoiding genetic defects (Pryce and Hayes, 2012; Schefers and Weigel, 2012; Schöpke and Swalve, 2016; Seidel *et al*., 2020; Gutierrez-Reinoso *et al*., 2021). Despite these advances, farmers' preferences regarding key traits in their herds are often overlooked when developing breeding goals (Nielsen and Amer, 2007; Ahlman *et al*., 2014). Recognizing the importance of understanding these preferences, Martin-Collado *et al*. (2021) introduced a reference measure to assess farmers' attitudes towards breeding tools. This emphasizes the need for farmers to actively participate in the design of breeding objectives together with stakeholders, as such participation increases the acceptance of the resulting tools and objectives (Hill, 2016). Breeders, who play a crucial role in determining the genetic direction of future generations, have a major influence on the future of the breed through their selection decisions.

In past centuries, the focus of dairy cattle breeding programs has been predominantly on milk production and composition, which has led to unfavourable genetic consequences for traits such as fertility, health, longevity and environmental sensitivity (Nielsen and Amer, 2007; Nielsen *et al*., 2014; Miglior *et al*., 2017; Brito *et al*., 2021; Gutierrez-Reinoso *et al*., 2021). In addition, reliance on a limited number of dairy breeds and a small number of sires within breeds has contributed to a decline in genetic diversity, leading to problems such as inbreeding depression and an increasing incidence of recessive genetic diseases. By de-emphasizing milk yield and focusing on a broader range of traits, long-term genetic variability can be improved (Brito *et al*., 2021). The dairy industry therefore needs to refine its selection indices to place more emphasis on traits related to animal welfare, health, longevity, environmental efficiency (e.g. lower methane emissions) and resilience (de Hass *et al*., 2021). Some countries, particularly in Western Europe, North America, Australia and New Zealand, have already started to implement these broader breeding objectives (Miglior *et al*., 2017; Cole and VanRaden, 2018). A review of various studies on breeding challenges highlights the following key traits in dairy farming: Production (milk yield, fat and protein yield, somatic cell count, longevity); Reproduction (fertility, calving interval, ease of calving, perinatal mortality); Health and welfare (disease resistance, immune response, adaptability, survival); Environmental traits (climate adaptation, feed efficiency, methane emissions) and conformation traits (udder traits, feet and leg traits, locomotion) (Meijer *et al*., 2015).

Research shows that farmers' openness to innovation is influenced by personal factors such as age, education and income, as well as farm characteristics such as size, production systems and conditions (Padel *et al*., 2015; Roussy *et al*., 2017; Läpple and Thorne, 2019). Younger, wealthier and better educated farmers, especially those with larger farms, are more open to innovation (Naspetti *et al*., 2017; Skjerve *et al*., 2018). Fertility is often prioritized by farmers (Byrne *et al*., 2016; Slagboom *et al*., 2016), with older farmers focusing on production traits and younger ones on functional traits (Martin-Collado *et al*., 2015). Most research has focused on farmers' views on bioeconomic models of animal traits (Byrne *et al*., 2016; Fuerst-Waltl *et al*., 2016; Paakala *et al*., 2018; Martin-Collado *et al*., 2015) and theoretical identification of traits (Gutierrez-Reinoso *et al*., 2021), while socio-demographic influences on the development of breeding tools have been overlooked, especially in countries with small herds (Skjerve *et al*., 2018). Therefore, a more in-depth study of these factors is crucial, as underscored in the research by Skjerve *et al*. (2018).

The aim of this study is to investigate the influence of socio-demographic factors on dairy farmers' preferences for breeding traits, with a particular focus on new environmental traits. Furthermore, it will be investigated how these preferences differ within dairy farmers in Slovenia.

Material and methods

A mixed methods approach was used. Focus groups to gather in-depth opinions on selection tools, genomic selection and breeding traits. The focus groups were led by a social scientist experienced in qualitative analysis and moderated by a researcher unknown to the participants to ensure an unbiased discussion. Twenty-seven farmers took part in the focus groups, which were conducted online in the context of COVID-19. Participants discussed selection needs, genomic selection knowledge and preferences for breeding goals. The data was evaluated using thematic analysis, identifying key topics such as productivity, resistance and functionality. For the quantitative approach, an online survey was distributed via email and social media in August and December 2021. The survey, in which 212 people participated, assessed farmers' preferences for various breeding traits using a seven-point Likert scale. The traits included production, reproduction, health, environment and functional traits. Respondents also indicated their desired weighting of traits in an overall merit index. Socio-demographic and farm characteristics were collected to investigate how these factors influence characteristic preferences.

The statistical analyses were carried out using SAS (Version 9.4, SAS Institute, Cary, NC, USA) and IBM SPSS Statistics (Version 25). Using data from 212 respondents, farmer groups were identified based on their preferred composition of a Total Merit Index. A two-stage cluster analysis was performed in SPSS. First, a hierarchical technique (Ward method with squared Euclidean distances) was used to determine the number of clusters and their centers. Then a non-hierarchical *k*-means method was applied using the identified cluster centers as starting points. The differences between the groups of farmers were analysed using the non-parametric Kruskal-Wallis test, as the proportions of characteristics in the Total Merit Index were not normally distributed.

Basic statistics were calculated for individual traits and trait groups such as milk production, reproduction and health. General linear models (SAS, GLM procedure) were used to investigate the relationship between farmer/farm characteristics and trait preferences. Mean differences were tested using the "pdiff" option of the "LS means" statement and adjusted using the Tukey-Kramer method. We used the following model:

$$Y_{ijklm} = \mu + A_i + E_j + M_k + H_l + e_{ijklm}$$

where Y_{ijklm} is the trait of interest; μ s the overall mean; A_i is the fixed effect of a farmer's age (i=2 classes; <40, $\geq$40); E_j is the fixed effect of the jth class of education (j=3 classes; primary and vocational school, secondary education, higher education); M_k is the fixed effect of milk production level (k=4 classes; <8000 kg, 8000–9000 kg, 9001–10000 kg, >10000); H_l is the fixed effect of herd size (l=4 classes: <24 dairy cows, 24–42, 43–60, >60); and e_{ijklm} is the random residual. The residuals were assumed to be normally distributed with a mean of zero and variances of σ_e.

Results

The study investigated the preferences of dairy farmers in Slovenia with regard to target breeding goals and breeding traits, using both quantitative and qualitative methods. Table 1 shows the socio-economic characteristics of the farmers in the sample together with the characteristics of their farms. Of the 212 farmers interviewed, the majority were conventional producers, with only one certified for organic farming. In addition, 62.3% of

Table 1. Farmer and farm characteristics of the sample (n = 212).

Characteristics	n	%	Characteristics	Mean	SD	Median	CV
Gender of respondents			Land owned and rented (ha)	48.2	47.6	36.0	99.1
Female	45	21.2					
Male	167	78.8					
Age of respondents			No. of livestock	102.6	59.1	97.0	57.5
< 40	96	45.3					
> 40	116	54.7	No. of cattle	51.3	27.1	50.0	52.7
Education of respondents							
Primary and vocational school	20	9.4					
Secondary education[1]	107	50.5	Milk yield (kg of milk in standard lactation)	8768.0	1691.0	9000.0	20.4
Higher education[2]	85	40.1					
Production system							
Conventional	211	99.5					
Organic	1	0.5					
Farm with limited environmental factors?			Milk production per cow in 2020 (kg of milk per year)	8844.0	1807.0	9000.0	19.3
Yes	132	62.3					
No	80	37.7					

[1] Vocational secondary education, Technical and vocational secondary education, General secondary education

[2] Vocational college, Bachelor's degree, Master's degree, PhD degree

respondents farmed in less-favoured areas. The average farm size was 48.2 hectares of owned and leased agricultural land, with an average of 97 cattle (including cows, heifers, breeding bulls, fattening bulls and calves) and 50 dairy cows. In 2020, the average milk production per cow was 8,844.5 kg.

The composition of the current Total Merit Index (TMI) for the Holstein breed and the changes proposed by farmers are shown in Figure 1. The current TMI includes milk

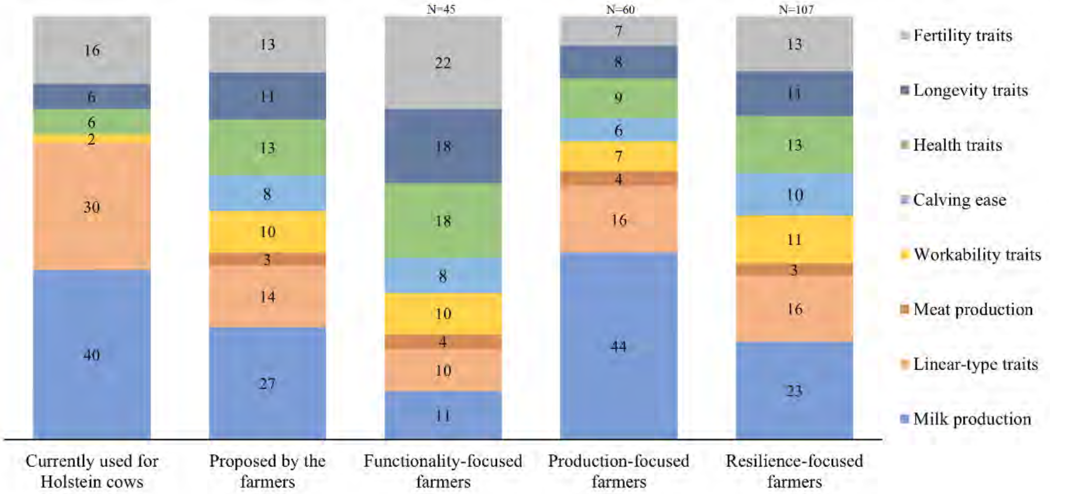

Figure 1. Composition of the Total Merit Index currently used for the Holstein breed and the share of a group of traits in the Total Merit Index proposed by farmers.

traits (40%), linear-type traits (30%), health traits (6%), longevity traits (6%), workability traits (2%) and fertility traits (16%). On average, farmers were in favour of decreasing the emphasis on milk production (27%), linear-type traits (14%) and fertility traits (13%), while they wanted to increase the proportion of traits for health (13%), longevity (11%) and workability traits (10%). They were also in favour of including incorporating new traits such as meat production (3%) and calving ease (8%).

The cluster analysis identified three groups of respondents (Figure 1): Functionality-oriented farmers who prioritised fertility (22%), longevity (18%) and health (18%). This group consisted of 45 respondents (21%), mainly middle-aged, with secondary education (44.0%) or higher education (46.6%). In 2020, they kept an average of 50 dairy cows with a milk yield of 8,306 kg per cow. The production-oriented farmers focused more on milk production (44%) and linear traits (16%). This group included 60 respondents (28.3%), who were predominantly older and had a secondary (43.3%) or higher education (53.3%). In 2020, they kept an average of 46 Holsteins with a milk yield of 8,671 kg per cow. Resilience-oriented farmers include breeders who have reduced the proportion of milk production (23%) in favour of traits such as fertility (13%), health (13%), longevity (11%) and workability (11%). This group accounted for 50.4% of respondents, most of whom were younger and had a secondary (57.0%) or higher education (29.1%). In 2020, they kept 54 dairy cows with a milk yield of 8,995 kg per cow.

The discussions in the focus groups reflected these findings. Functionality-oriented farmers advocated placing less emphasis on milk production and instead promoting traits such as fertility, longevity and health to ensure stable production, with a typical comment being: *“The cows are in the barn to give milk. As cessation of production due to health problems or death is a major problem, the proportion of longevity and fertility should be increased”* (farmer 2, male, 57 years old, secondary school). Production-oriented farmers, often referred to as traditionalists, were more inclined to increase milk production, as one participant explained: *“The cow is there to be milked. The share of production should not be reduced, but increased a little, and the share for conformation traits should be added. Fertility is irrelevant because we have no data, we should put it in the frame.”* (farmer 3, male, 31 years old, bachelor degree). However, the majority of focus group participants were resilience-oriented farmers who advocated a balanced approach to breeding goals. One farmer emphasized the importance of long-term profitability: *“We should find the golden mean between the different traits. We need to include everything from health, fertility, temperament, milk flow to physical traits so that we do not over-exploit cows in the long run. What good is it if, like me, you have extreme milkers and then health problems arise? It is important to make a profit in the long term.”* (farmer 1, male, 38 years old, Master degree).

Figure 2 shows that respondents ranked animal health and welfare as the most important traits (M = 6.32, SD = 0.71), followed by reproduction (M = 6.16, SD = 0.78). Meat production traits received the lowest scores (M = 4.14; SD = 1.63). The environmental traits were rated lower, with methane emissions (M = 4.62) and energy metabolism (M = 5.43) being rated particularly negatively. Some farmers dismissed these traits as “media agitation” or “environmental extremist mania,” as one participant commented: “The issue of methane emissions and greenhouse gases is complete nonsense, because agriculture is not to blame” (farmer 6, male, 38 years old, Master degree). Farmers with a lower level of education rated traits such as climate adaptation and methane emissions higher, while larger herd owners and those with higher milk yields attached greater importance to traits such as consumption capacity and feed efficiency.

In this study, the preferences of Slovenian dairy farmers regarding breeding goals and breeding traits were investigated using a mixed methods approach. Focus

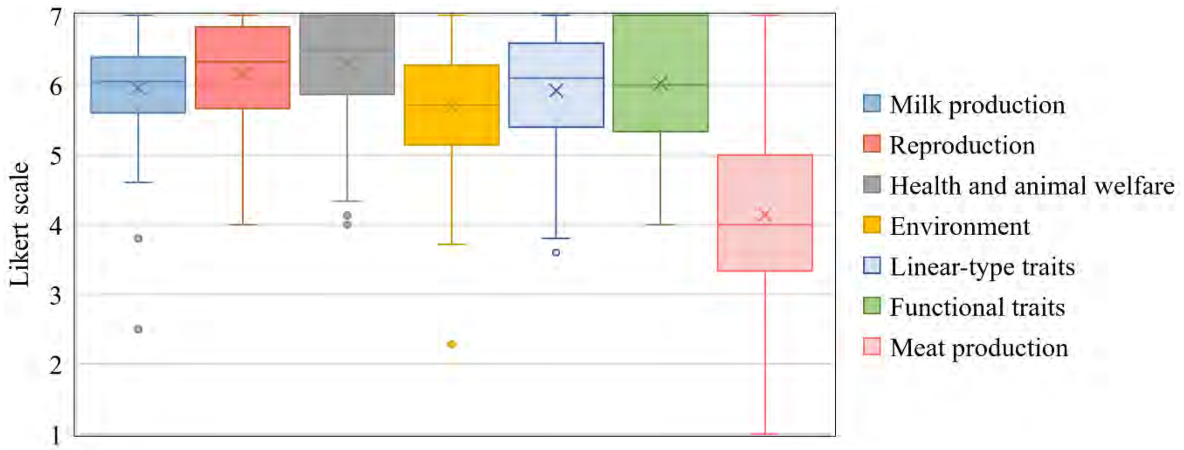

Figure 2. Farmers' preferences for a group of traits. Boxplots show the mean (solid lines), median (x), first and third quartiles (in the boxes), dispersion and outliers (dot) for farmers' preferences for a group of traits (N=212).

Discussion

groups provided an in-depth understanding of farmers' views, while the quantitative survey revealed the differences in these preferences, particularly in relation to socio-demographic factors. The results show that the majority of dairy farmers want to develop a new Total Merit Index, placing the greatest weight on milk production traits. However, there is a clear shift away from the German structure of the index, which emphasizes milk production (36%), to the Dutch approach, which gives less weight to production traits (28%; EuroGenomics, 2022). The differences between the selection indices of the individual countries are due to different economic conditions, traits recorded, and breeds used (Miglior *et al*., 2017; Cole and VanRaden, 2018; EuroGenomics, 2022).

Relying only on average preferences does not adequately capture the diversity of farmers (Martin-Collado *et al*., 2015). In this study, three different types of farmers were identified, although no significant differences in socio-demographic characteristics were found. While previous studies have emphasized farmers' strong preference for milk production traits (Skjerve *et al*., 2018; Martin-Collado *et al*., 2015), this study found a shift among Slovenian dairy farmers, who now place more emphasis on functional traits. Milk production and linear-type traits, which currently dominate in the Total Merit Index, are becoming less important.

The analysis of the focus groups revealed that farmers have a more negative attitude towards environmental traits than indicated in the survey. This discrepancy mirrors the findings of other Slovenian studies in which negative attitudes are attributed to ignorance of environmental issues and fear of increased regulatory pressure (Benedičič *et al*., 2022; Purcell *et al*., 2023). Less educated, older farmers with smaller herds who are concerned about the environmental impact of their practises were particularly worried about potential EU regulations on methane emissions. Dealing with environmental features remains a challenge as many farmers view them with scepticism due to public pressure on agriculture (Erjavec and Erjavec, 2020; van der Ploeg, 2020). Effective communication tailored to different groups of farmers can help to promote acceptance of environmental breeding goals and facilitate positive changes in dairy cow breeding. New traits need to be introduced for direct selection of environmental traits, such as methane emissions (Klopčič and Kuipers, 2009).

Overall, farmers consider all breeding traits to be important, although they attach less importance to environmental traits. This is consistent with the findings of Wallenbeck *et al*. (2013), who showed that farmers prioritize traits directly related to profitability, such as feet and legs, health and longevity, while they place less importance on traits such as methane production. Understanding these preferences allows farmers to adapt to new trends, improve animal welfare and effectively manage market volatility (Benedičič *et al*., 2022). Animal health, welfare and reproductive traits were most important, while meat production was least important, as dairy farmers focus mainly on milk. These results reflect previous research highlighting reproductive traits as crucial for profitability (Martin-Collado *et al*., 2015; Skjerve *et al*., 2018). Slovenian farmers, especially those in less-favoured areas with limited expansion opportunities, consider animal welfare as crucial for maximizing production (Benedičič *et al*., 2022). The relatively low importance given to environmental breeding traits could be due to the fact that they are new and farmers are not familiar with them. However, as the focus group results show, farmers are increasingly aware of the need to strike a balance between environmental sustainability and profitability to ensure the long-term viability of their farming practices.

Conclusions

This study on the breeding goal preferences of Slovenian dairy farmers emphasizes the value of integrating qualitative and quantitative methods to gain a comprehensive understanding of the topic. The results show that farmers aspire to a redesigned Total Merit Index that includes several trait categories, with milk production traits being the most important. However, farmers' preferences for the proposed Total Merit Index varied, leading to the identification of three distinct groups: those who prioritize production traits, those who emphasize functional traits, and those who focus on resilience. Above all, animal health and welfare proved to be the most important traits, while new traits, especially environmental ones, were met with less enthusiasm. The study also found that certain traits, such as greenhouse gas emissions, were perceived negatively, highlighting the need for targeted communication strategies to promote their acceptance. These findings can inform the development of new breeding goals and programs and increase breeders' confidence in their selection processes by actively involving them in decision-making.

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How data can have a big impact on small farms

T. Ammerlaan – Sieperda

UNIFORM-Agri, Stationsplein 14 9401 LB Assen, The Netherlands
Corresponding Author: t.sieperda@uniform-agri.com

Whereas the dairy industry often speaks about improving sustainability of the dairy industry by herd management changes on large dairy farms, statistics actually show us that most dairy cows on this planet are kept in herds of small holders in developing countries. So when we want to have a global impact we should also take a look at these countries.

An example of such a developing country is Ethiopia, where you can find more than 15 million dairy cows. This is more than the number of dairy cows in the US and Canada together. While so many of us are focussing on improving results of these large farms that are already producing lots of high quality milk, with a low footprint per kg of product. Small adaptations in dairy farms in developing countries will let them take huge leaps in milk quality results and productivity, having major impact on the income of the farmer, food supply, carbon footprint and the local economy.

If we take a deeper look at the example of Ethiopia we can see that their domestic milk production is increasing, from 3 bilion liters in 2016 to 4.96 bilion liters in 2021. However, this growth has been insufficient and, at times, inefficient, because while some larger, commercial farms exist, almost 95% of dairy cows are kept by rural, smallholder farmers with fewer than five head of cattle per household. A typical cow produces just 1-2 liters of milk a day, which are either consumed at home or sold through informal market systems with little or no quality control. These farmers struggle to access inputs and services needed to improve their herds - such as feed, veterinary care, and artificial insemination - -and there is little incentive for them to sell to the formal market. (Hughes, 2023)

Having more tools to manage data can be an essential part of these leaps in development. However, it is obvious that the tool used by a farmer with 1000 cows has no use for a farmer with 25 cows. With simple decision support tools a farmer can take better care of the cows health, fertility and productivity and with that increase their income and decrease the environmental impact of 1 Kg of Milk. Besides, the consultants that are advising these farms will be able to work much more efficient when data is available and bring more added value. Although this all sounds simple there are big challenges in countries where internet, technical devices and agricultural knowledge is not always at hand. As UNIFORM-Agri we have seen that it is possible to improve herd management on these farms by providing a simple registration application for smartphones that connects what is happening on the farm to the consultant at the dairy factory.

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Comparing the milking behaviour of primiparous vs. multiparous Holstein and Jersey cows in an automatic batch milking system

P. Munoz-Boettcher[1], N. Rodriguez[2], I. Klaas[3], J. Velez[2] and P Pinedo[1]

[1]Colorado State University, 350 W. Pitkin St. Fort Collins, CO 80523, USA
[2]Aurora Organic Dairy, 560 Co Rd 347, Dublin, TX 76446, USA
[3]DeLaval International AB, Gustaf De Lavals väg 15, 147 41 Tumba, Sweden
Corresponding Author: pinedop@colostate.edu

Abstract

The onset of lactation and the subsequent period of habituation to the milking routine is a stressful process for dairy cows, where new social groups and novel stimuli converge. This period seems to be particularly challenging for primiparous cows. The objective of this study was to compare the dynamics of milking behaviour during the early lactation of primiparous vs. multiparous Holstein (HO) and Jersey (JE) cows, under an automatic milking system with a semi-voluntary batch milking design. This retrospective observational study included information from milking events in 2,138 cows from May to December 2023 in an organic certified herd in Texas, USA. Milking behaviour information for the first four weeks of lactation included % of incomplete milkings (INC), % of kick-offs (KO), and % of teat cleaning failure (TCF), collected from DelPro software (DeLaval, Sweden) and used as a proxy for habituation to the milking routine and system. Cows were moved to the milking barn twice per day, where they could select their milking visits among 22 robots (DeLaval, Sweden). Parity [primiparous (PRIM) and multiparous (MULT)] and calving data were extracted from PCDART software (DRMS, NC, USA). Data were analysed by logistic regression to assess the differences in milking behaviour between PRIM and MULT cows within two breed groups (HO and JE) in weekly intervals following calving (W1; W2; W3; and W4). After edits, 28,165 milking records were analysed in 2,138 cows (27% primiparous; 73% multiparous). The frequencies of undesirable milking behaviours (INC; KO; and TCF) were greatest in PRI cows in both HO and JE during most of the weekly periods. The greatest frequencies of INC per milking event were in PRI cows during W2 (HO = 8.6% and JE = 12.0%). The greatest frequencies of KO were also in PRI during W1 (HO = 10.2%; JE = 17.2%), while the greatest TCF for HO and JE were 8.38% (W1) and 4.98% (W2), respectively. In HO, the odds (95% confidence interval) of INC were greater for PRIM compared with MULT cows during W2 [2.39 (1.72-3.31)], W3 [1.63 (1.21-2.18)] and W4 [1.65 (1.20-2.25)]. Similarly, in JE the odds of INC were greater for PRIM for all the weekly periods: W1 = 77.6 (15.1-1,419), W2 = 7.54 (5.28-10.9), W3 = 3.06 (2.19-4.27), and W4 = 2.75 (1.94-3.87). The odds of KO were greater in PRIM HO during W1 [3.42 (1.74-6.62)], W2 [2.28 (1.66-3.12)], and W3 [1.83 (1.35-2.48)]. In JE, the odds of KO were greater in PRIM during W1 [8.33 (4.53-15.48)], W2 [3.61 (2.76-4.70)], W3 [2.43 (1.90-3.08)], and W4 [1.69 (1.30-2.18)]. Finally, the odds of TCF were greater in PRIM HO during W1 [2.05 (1.02-3.92)] and W2 [1.98 (1.39-2.80)]. In JE, the odds of TCF were greater in PRIM during W1 [4.09 (1.35-11.5)], W2 [5.10 (3.08-8.44], and W3 [2.71 (1.39-5.15)]. These results highlight the differences in milking behaviour during the early lactation of primiparous vs. multiparous cows in both Holstein and Jersey cows. As anticipated, the magnitude of these differences decreased during weeks 3 and 4, which may be associated with the process of habituation to milking during the first lactation. The greater disparity between primiparous and multiparous reported in Jersey compared with Holsten cows may relate to differences in udder conformation, incidence of udder oedema, body size, or temperament.

Keywords: primiparous, habituation, automatic milking, behaviour:
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Introduction

Acute stress in dairy cows, characterized by negative affective states, is widely acknowledged to have detrimental effects on both welfare and productivity. Moreover, it can significantly impact cattle handling practices and compromise worker safety (Grandin, 1993; 1999), especially during the milking process (Grandin, 1998; Douphrate *et al*., 2013; Edwards and Kuhn-Sherlock, 2021).

The onset of a dairy cow's first lactation and the subsequent habituation period to the milking routine constitute a particularly stressful phase in their lives. This period involves increased interaction with human caretakers, integration into new social groups, and exposure to various novel stimuli during milking. Handling primiparous heifers during this transition can also pose challenges to human handlers, increasing the difficulty of milking tasks and the risk of cattle-related injuries (Sorge *et al*., 2014; Edwards and Kuhn-Sherlock, 2021; Phillips *et al*., 2021). Despite this, there remains a research gap regarding specific behavioural changes in cows throughout the first lactation.

Previous studies on heifer habituation to the milking routine have shown that primiparous cows tend to exhibit higher levels of excitability compared to multiparous cows at various stages of the milking process (Andrea *et al*., 2015). Most research efforts have primarily focused on expediting the habituation process through pre-lactation exposure to milking routines and/or early lactation interactions with caretakers (Bremner, 1997; Kutzer *et al*., 2015).

The behavioural responses exhibited by dairy cows to novel stimuli, such as kicking during the milking are a reflection of stress or discomfort. Consequently, a deeper understanding of this process is particularly relevant. Interestingly, in a recent study by Kness *et al* (2023) where milking unit kick-off was used as a proxy for habituation to the milking procedure, this behaviour was consistently greater in primiparous compared to multiparous cows. Furthermore, the relationship between days in milk and the proportion of cows displaying milking unit kick-off was not linear, but rather increased for the first several weeks before decreasing again.

In recent decades, automatic milking systems (AMS) have been steadily gaining in popularity. Among multiple advantages provided by AMS, improved cow comfort is considered central to these systems. However, heifers may exhibit stress reactions, such as kicking and stepping as well as vocalization and elimination, during their first visits to the milking robot (Jacobs and Siegford, 2012). Moreover, although in these systems the human-cow interaction component is significantly reduced, first parity cows, require some guidance and training during the beginning of their lactation (Jago *et al*., 2011; Tse *et al*., 2018).

Notably, studies documenting daily changes in stress behaviours during the habituation period or differences in these behaviours between primiparous and multiparous cows during the initial months of lactation in AMS are scarce (von Kuhlberg *et al*., 2020). Moreover, to the best of the authors' knowledge, studies analysing data originated from AMS with a semi-voluntary batch milking design are missing. In these systems, cows are moved to the milking barn at fixed times of the day, where they can select their milking visits among multiple robots.

We hypothesized that adverse behaviours [milking unit kick-off (KO)] and undesirable events [incomplete milking (INC), teat cleaning failure (TCF)] would be most frequent in primiparous cows that are starting their lactation, as compared to older cows. We also envisioned that there is variation in these behaviours associated with the cow's breed. In consequence, the objective of this study was to compare the dynamics of milking behaviour during the early lactation of Holstein (HO) and Jersey (JE) cows in an automatic milking system with a semi-voluntary batch milking design.

Material and methods

This retrospective observational study included information from milking events in 2,138 cows from May to December 2023 in a grass-fed organic certified herd in Texas, USA. Milking behaviour information for the first four weeks of lactation included incomplete milkings (%), milking unit kick-off (%), and teat cleaning failure (%), collected from DelPro software (DeLaval, Sweden) and used as a proxy for habituation to the milking routine and system.

Cows were moved to the milking barn by grazing group twice per day, where they could select their milking visits among 22 robots (DeLaval, Sweden). Parity [primiparous (PRIM) and multiparous (MULT)] and calving data were extracted from PCDART software (DRMS, NC, USA).

Data exploration and descriptive analyses for the variables in analysis were performed using R, version 4.2.2 (R Core Team, 2022) using the lmer4 (Bates *et al*., 2015), lmerTest (Kuznetsova *et al*., 2017), and emmeans (Lenth, 2022) packages. Data were analysed by logistic regression to assess the differences in milking behaviour between PRIM and MULT cows within two breed groups (HO and JE) in weekly intervals following calving (W1; W2; W3; and W4). Cow ID was considered as random effect for adjustment. Potential interactions were tested and removed from the models when not significant. Average milking trait values were compared using Tukey-adjusted pairwise comparisons. Statistical significance was assessed at $P < 0.05$ level using a likelihood ratio test.

Results and discussion

After edits, 28,165 milking records were analysed in 2,138 cows (27% primiparous; 73% multiparous). The frequencies of undesirable milking behaviours (INC; KO; and TCF) were greatest in PRI cows in both HO and JE during most of the weekly periods (Figure 1 and Figure 2). The greatest frequencies of INC per milking event were in PRI cows during W2 (HO = 8.6% and JE = 12.0%). The greatest frequencies of KO were also in PRI during W1 (HO = 10.2%; JE = 17.2%), while the greatest TCF for HO and JE were 8.38% (W1) and 4.98% (W2), respectively.

In HO, the odds (95% confidence interval) of INC were greater for PRIM compared with MULT cows during W2, W3 and W4 (Table 1). Similarly, in JE the odds of INC were greater for PRIM for all the weekly periods (Table 2). The odds of KO were greater in PRIM HO during W1, W2, and W3. In JE, the odds of KO were greater in PRIM during all the weekly periods (Table 4). Finally, the odds of TCF were greater in PRIM HO during W1 and W2. In JE, the odds of TCF were greater in PRIM during W1, W2, and W3.

Figure 1. Frequency (%) of incomplete milking (top panel), milking unit kick-off (middle panel), and teat cleaning failure (bottom panel) in primiparous versus multiparous Holstein cows by week postpartum.

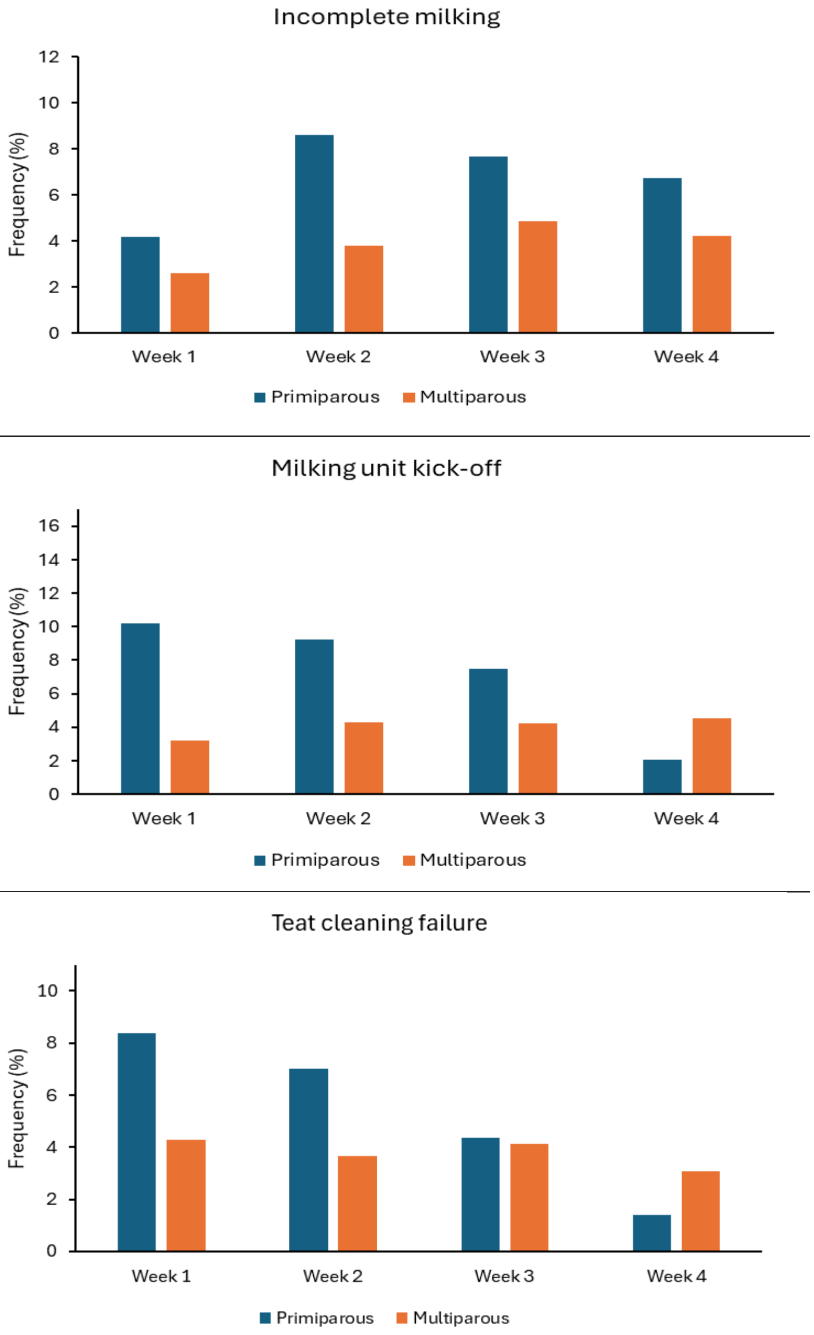
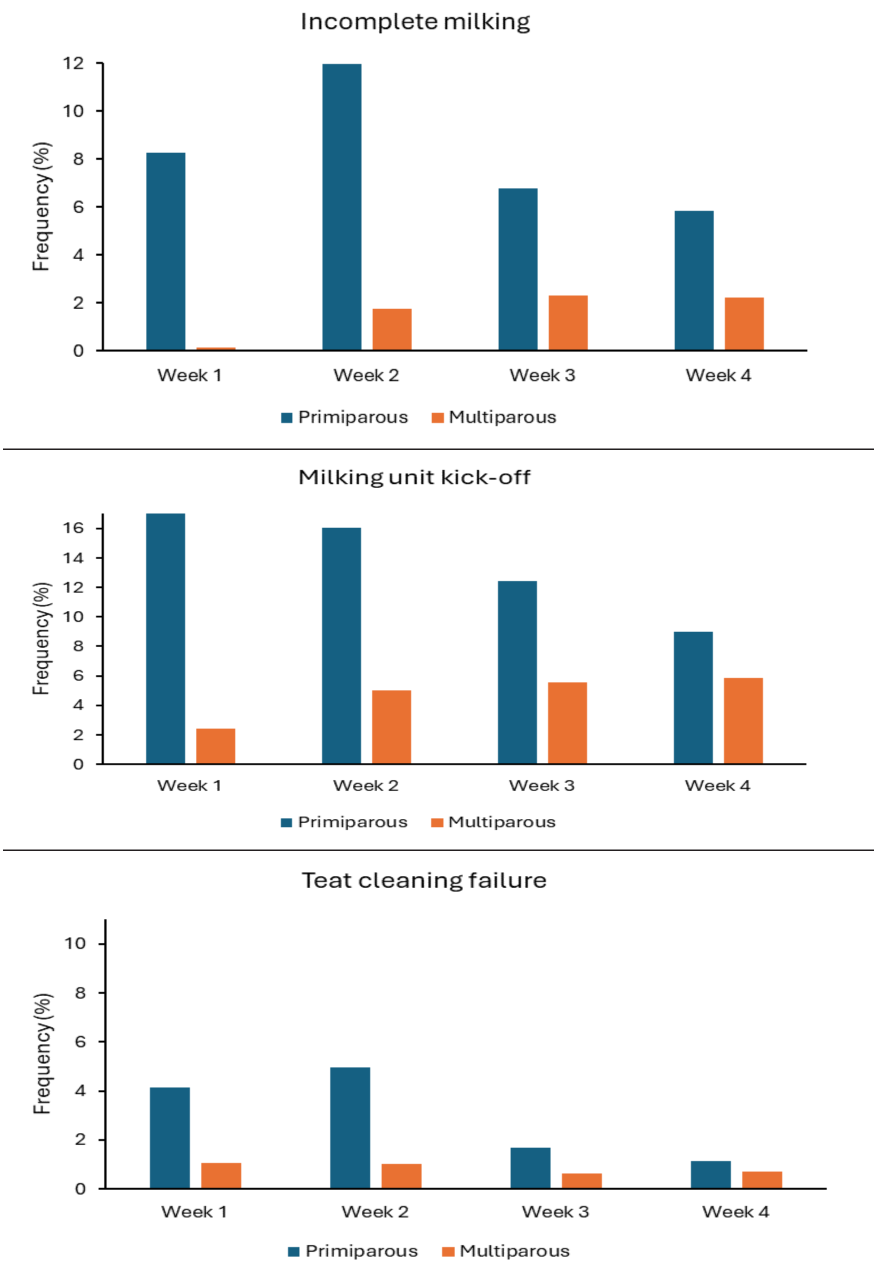


Figure 2. Frequency (%) of incomplete milking (top panel), milking unit kick-off (middle panel), and teat cleaning failure (bottom panel) in primiparous versus multiparous Jersey cows by week postpartum.



Earlier studies focused on cow behavior at the milking parlor are based on visual observation (Rousing *et al*., 2004; Cerqueira *et al*., 2017). However, the advent of precision technologies and in particular the precise information provided by robotic milking creates opportunities for the monitoring of multiple behaviors in large numbers of animals.

Supporting the concept of habituation to novel conditions, in a recent study, von Kuhlberg *et al*. (2020) reported that training of heifers on a phantom milking robot prepared the animals for being milked in the AMS, resulting in increased number of milking visits and a reduced proportion of animals that had to be fetched into the AMS for milking.

In agreement with Knees *et al*. (2023), in our study, occurrence of KO was greater in PRIM than in MULT during most of the monitoring period (up to 90 DIM). Moreover, the decreasing trend as primiparous cows advanced in their lactation agrees with Bremner (1997), which found that primiparous cows moved and kicked more frequently during the first 7 milkings than during subsequent milkings.

Overall, the results from the current study highlight the differences in milking behaviour during the early lactation of primiparous vs. multiparous cows in both Holstein and Jersey cows. As anticipated, the magnitude of these differences decreased as the lactation advanced, which may be associated with the process of habituation to milking during the first lactation. The greater disparity between primiparous and multiparous reported in Jersey compared with Holsten cows may relate to differences in udder conformation, incidence of udder oedema, body size, or temperament.

A better understanding of these undesirable behaviours using data originated from automatic milking systems, as well as research exploring strategies to reduce their incidences during early lactation, could result in improved transition of first parity cows into milking.

Table 1. Adjusted odds ratios (OR) and 95% CI for incomplete milking, milking unit kick-off, and teat cleaning failure in primiparous versus multiparous (reference) Holstein cows by week postpartum.

Week	Incompletes			Kick-offs			Teat cleaning failure		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
1	1.64	0.62-3.87	>0.05	3.42	1.74-6.62	<0.001	2.05	1.02-3.92	0.04
2	2.39	1.72-3.31	<0.001	2.28	1.66-3.12	<0.001	1.98	1.39-2.80	0.0002
3	1.63	1.21-2.18	0.002	1.83	1.35-2.48	0.001	1.05	0.73-1.50	>0.05
4	1.65	1.20-2.25	0.002	1.34	0.96-1.84	0.08	1.35	0.91-1.97	>0.05

Table 2. Adjusted odds ratios (OR) and 95% CI for incomplete milking, milking unit kick-off, and teat cleaning failure in primiparous versus multiparous (reference) Jersey cows by week postpartum.

Week	Incompletes			Kick-offs			Teat cleaning failure		
	OR	95% CI		OR	95% CI		OR	95% CI	
1	77.6	15.1-14.2	< 0.001	8.33	4.53-15.5	< 0.001	4.09	1.35-11.5	0.01
2	7.54	5.28-10.9	< 0.001	3.61	2.76-4.70	< 0.001	5.1	3.08-8.44	< 0.001
3	3.06	2.19-4.27	< 0.001	2.43	1.90-3.08	< 0.001	2.71	1.39-5.15	0.004
4	2.75	1.94-3.87	< 0.001	1.69	1.30-2.18	<0.001	1.75	0.84-3.47	> 0.05

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Stakeholder engagement to support the development of next generation decision support tools

J.E. Newton[1,2] and L.C. Monks[3,4]

[1]Agriculture Victoria, AgriBio, 5 Ring Road, Bundoora, Victoria 3083, Australia
[2]School of Applied Systems Biology, La Trobe University,
Bundoora, Victoria 3083, Australia
[3]DataGene Pty Ltd. 5 Ring Rd, Bundoora Vic 3083
[4]Monks Communication, 15/1 Vantage Dr Yaroomba Qld 4573
Corresponding Author: jo.newton@agriculture.vic.gov.au

Abstract

Making informed management decisions about mating and culling cows impacts farm financial performance and the health and welfare of cows. There is now an opportunity to develop next generation ('next-gen') decision support tools which combine genetic effects (e.g. breeding values), non-genetic effects and novel data sources to predict the future performance of cows. To ensure this next generation of decision support tools aligns to farmer needs, a series of focus groups and one-on-one interviews were held to seek feedback from 33 dairy farmers and industry stakeholders on tools being considered for development. A semi-structured facilitation approach was used to understand what information was currently being used to make decisions about culling and mating and gauge farmer interest in the next generation of management tools. Iterative thematic analysis of workshop and interviews transcripts and notes was then undertaken.

As anticipated, farmers are heterogeneous in their data recording and use of data in decision making. Differences in approach to data use could be broadly represented by two distinct data user groups, "data-driven" and "data-disconnected" and a third overlapping data user group, "data-dippers." Interest and demand for 'next-gen' tools varied – and appeared to be influenced by both individual farm factors and regional factors - though was generally positive. Individual factors also impacted whether farmers preferred a new culling or mating tool with no clear preference seen overall. A recurring theme in conversations was how interlinked mating and culling decisions are, with things like herd replacement rate being heavily influenced by both. All stakeholders identified features or data they viewed as important to include in 'next-gen' tools. However, less than half of these data sources are currently captured in the Australian dairy industry's central data repository. Whilst farmers were open to new tools – feedback was clear that such tools should not require duplication of data entry. Data access, availability and integration across systems at both individual farm and industry level is a key barrier to 'next-gen' tool development and adoption. The semi-structured facilitation style provided opportunity for diverse feedback and insights across a range of related topics to be captured. Feedback from stakeholders was that the opportunity to participate in workshops and engage directly with researchers was highly valued. As delivering a 'next-gen' tool accessible to most farmers is not yet possible, a decision has been made to not continue further tool development in the short term. The tool may be revisited in the future when data barriers are overcome. We will continue to explore other research that can answer some of the questions raised in this study and to ensure the findings of this study are disseminated to industry.

Keywords: sexed semen, beef on dairy, mating tool, culling tool, co-design.
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Introduction

Making informed management decisions about mating and culling cows impacts farm financial performance and the health and welfare of cows. Cow performance is influenced by many factors including genetic effects (e.g. breeding values), non-genetic effects (e.g. lactation number, calving date, illness) and farming system (e.g. feeding system, climate). Novel data sources (i.e. sensors), continued improvements to data pipelines, more frequent genetic evaluations and computing advances means an opportunity exists to develop new decision support tools which combine genetic effects, non-genetic information and novel data sources to predict the future performance of cows. Current management tools available in Australia do not jointly consider all these information sources. However, a limited number of these tools have been developed and implemented overseas, such as in Ireland (Kelleher *et al.* 2015) where farmer feedback and uptake has been very positive (Kelleher *et al.* 2018).

Adoption of decision support tools by dairy farmers is contingent on them been valued by farmers and industry stakeholders and meeting their needs. One approach for ensuring tools developed are relevant and meet the needs of the end-user is to use a co-design process, involving stakeholders throughout a project (Moser 2016). The benefits of involving farmers in dairy research and extension activities has been previously documented by (Crawford *et al.* 2007). An engaged stakeholder network is also helpful in the development and piloting of extension resources which in turn can help support adoption (Newton *et al.* 2021). To ensure the next generation of decision supports tools aligns to farmer needs, a series of focus groups and one-on-one interviews were held to seek dairy farmer and industry stakeholders’ feedback.

Material and methods

A semi-structured facilitation style was used in series of workshops and interviews to seek feedback from 33 dairy industry stakeholders to understand farmer interest in “next-gen” (next generation) management tools from December 2023 – April 2024.

Description of participants and workshops

Twenty-three dairy farmers and 10 service providers were interviewed via 5 workshops – 4 targeting farmer participation and 1 targeting service provider participation. A further 7 one-on-one interviews were conducted, primarily with participants who were unable to attend workshops. Several approaches were used to recruit participants. Targeted emails were sent to 2 mailing lists; a network of service providers providing reproduction advice to farmers, and farmers participating in the genomic information nucleus program in Australia (and therefore known to be actively engaged in good data recording practices). Dairy Australia regional extension staff based in major dairy regions were approached for support to hold regionally specific workshops. This supported including generalised workshop promotion and targeted invitations to encourage participation in workshops from stakeholders with diverse backgrounds. Two workshops in Northern Victoria and 1 interview (Melbourne) were conducted in person, with the remainder completed online.

Using a semi-structured facilitation style, these sessions first sought to understand what information farmers were currently using to make decisions on culling and mating. Participants were then introduced to the concept of ‘next-gen’ management tools, shown an overseas case study and asked questions like: “*Does a tool like this interest you?*”, “*What would you like to see in a tool?*” Participants were also shown some examples of different dairy breeding programs incorporating conventional dairy semen, female sex-sorted semen and beef semen and asked which best represented their business and how they allocated semen types.

Qualitative analysis

Iterative thematic analysis was conducted to code workshop and interviews transcripts and notes into categories, following Charmaz (2014). Five main categories were identified: current use of data; demand and interest in new tools; barriers to engagement; tool features and preferences and other insights. Additional analysis of each category sought to identify recurrent themes, differences within each category and possible reasons for differences. These insights are presented below. Early insights from the analysis were shared with key dairy stakeholders and a facilitated discussion held to seek their input on the implications of the findings on future project milestones including ‘next-gen’ tool development.

Results and discussion

As anticipated, farmers were heterogeneous in what data they record and how they use that information when making culling and mating decisions. Similarly, interest and demand for ‘next-gen’ tools varied – seemingly influenced by both individual farm factors and regional factors – though was generally positive. All stakeholders identified features or data they viewed as important to include in ‘next-gen’ tools. Data access, availability and integration across systems at both individual farm and industry level was identified as a key barrier to ‘next-gen’ tool development and adoption. The semi-structured facilitation style provided opportunity for diverse feedback and insights across a range of related topics to be captured. Feedback from stakeholders was that the opportunity to participate in workshops and share their insights was highly valued. Key research findings are highlighted in Table 1, and discussed in further detail in subsequent sections.

Farmer’s current use of data in decision making varied

On-farm approaches to decision making varied widely across workshop participants (and their clients). While every farmer is unique, we propose differences in approach to data can be broadly represented by two distinct data user groups and a third overlapping data user group. Key features of each proposed user group are outlined in Table 2. The ‘data-driven’ user group tended to take a systematic approach to culling and mating decisions, often using Microsoft Excel to bring together information from multiple on-farm software programs. In contrast, the ‘data-disconnected’ user group recorded limited data and appeared comfortable making decisions with incomplete information. The ‘data-dippers’ shared features of each group, generally recording some data but not necessarily using it to support decision making. This also means the benefits of investing time and money into collecting data are not being realised. ‘Data-dippers’ are the users who are most likely to move between user groups - being at risk of becoming ‘data-disconnected’ but also having the potential to be encouraged to become ‘data-driven’. Identification of user groups with differing needs that warrant consideration in tool design and development of research programs has been documented previously (Monks *et al.* 2021). Interestingly, we found limited external advice was sought in making culling decisions, but a much stronger practice of external advisor involvement (usually semen sales representative) occurs in mating decisions.

A range of data sources at both individual cow and herd level were reported by participants as being used in making culling and mating decisions. Here, we focus on individual cow information. Participants most often reported using herd test (or other milk recording) data – especially somatic cell count and yield to make culling decisions. Other information used included: fertility information (i.e. days open, pregnancy test results), age/parity, udder health temperament, and genetics/genomics. For mating decisions, data used included: days in milk, age/parity, health records, genomics,

Table 1. Summary of insights across key themes identified through qualitative analysis.

Theme	Insight
Current use of data	• Farmers are heterogeneous in recording and use of data. • Three potential data user groups identified (Table 2). • Advisors often used for mating but not culling decisions. • Wide variety of data sources used; milk recording data most often mentioned
Demand and interest in new tools	• Generally positive, enthusiasm varied across data user groups. • Appeared to be influenced by herd dynamics, economic, social, and business factors. • Mating and culling seen as interlinked (Figure 1), no overarching preference for one tool seen. • Need to illustrate value of tool highlighted.
Tool features and preferences	• Range of data sources and features for tool identified (Table 6). • Less than half of requested data currently captured in central data repository. • No requirement to duplicate data entry.
Barriers to engagement	• Lack of data access, interoperability and integration across software programs and platforms, on-farm & at industry level. • Many data sources, especially novel data sources, not linked to central data repository. • Farmers do not want to have to duplicate data entry. • Variable levels of data recording across herds.
Other insights	• Opportunity to contribute to discussion and engage with scientists valued. • Gaps in education and training including inbreeding knowledge, best practice with sexed semen, additional uses of cow genomic test results. • Utilising existing events (i.e. scheduled discussion groups) effective for hearing diverse viewpoints. • Service providers had rich and valuable insights.

Table 2. Overview of potential data user groups and their use of data in decision making.

▯Data-driven▯	▯Data-dippers▯	▯Data- disconnected
• Responded to targeted emails. • Described systems, processes and strategies for decision making. • Multiple farm software programs. • Described data management as labour intensive.	• Recording some data • Shared some features of ‘data-driven’ and ‘data-disconnected’ • Can move between groups.	• Limited data recording or use of data in decision making. • Could have smaller herd size. • Group recognised by both farmers & advisors.
Use of data in decision making		
• Used multiple data sources, often compiled in Microsoft Excel.	• Data not often used in decision making.	• Make decisions on limited/incomplete data.

phenotypic attributes (i.e. confirmation) and data from heat detection devices (i.e. collars). A key question farmers reported asking themselves when making mating decisions was; "*Do I want to breed a replacement from her?*"

Individual farm and regional factors appeared to influence demand and interest in 'next-gen' tools

Participants responded positively to proposed new tools and opportunities to bring genetic, non-genetic and environment information together in one place, with beliefs like: '*there's no one tool that integrates all of that data*," and that, "*It would be easier to have one app or one tool which gives you all the information*." However, variation existed amongst participants. 'Data-driven' users saw value in the more systematic approach 'next-gen' tools would offer, the opportunity to compare their current approaches to proposed tools, and were enthusiastic about opportunities to engage further with the project. The next most interested user group 'data-dippers' appeared to see most value in having something that would help them bring their existing data together, with one participant saying that while they collected all the information, they weren't really using it in decision making so; "*I (they) would find that tool very handy, we collect all that data, would be good to have the snapshot*". The 'data-dippers' are perhaps the most significant target audience for these tools because such a tool could help them move into 'data-driven' user group. Adoption by this user group could deliver greater potential benefits to the individual and industry than adoption by the 'data-driven' user group who already have manual processes to bring their data together. Finally, as the 'data-disconnected' group are comfortable making decisions with little or no data, it will be much harder to develop a successful value proposition to collect data and use it in tools. There are emerging opportunities for this group to 'passively' record more data via new technologies such as automated dairy equipment, sensors and virtual fencing, i.e. Bell and Tzimiropoulos (2018); Cabrera and Fadul-Pacheco (2021).

A recurring theme in conversations was how interlinked mating and culling decisions are. Key factors participants articulated that impacted both culling and mating decisions included: herd replacement rate, optimum herd age structure, herd reproductive performance, markets for non-replacement animals and calving pattern (extended lactations). The linkages between these factors and culling and mating decisions is shown in Figure 1. This connectedness could also contribute to why it was hard to see an overall clear preference across all participants for either a culling or a mating tool. However, participants were divided on value of a combined culling and mating tool with some fearing it could become too complicated.

Farmers' interest in the proposed tools – and their decisions about culling and mating – appeared to be context-specific in that it related to their current herd dynamics, business circumstances, social factors and market conditions. Herd fertility came up in conversations often. For example, '*I would say that we don't really have much choice in culling and breeding decisions if you don't have good fertility,'* illustrating how herd dynamics may influence farmer interest in engaging with tools. Farmers who were already using sexed dairy semen and/or beef semen in their herds appeared more interested in the proposed mating tool. However, this interest was in turn influenced by availability of markets for dairy-beef calves or surplus heifers which varies from season to season. The development stage the dairy business was also a consideration. For example, a business focused on growing herd size had limited use for a culling tool and was less likely to be investing in data capture. The relevance of tools in the context of the time of year, was also mentioned. For example, it was suggested that in Tasmania there were more potential applications of tools to support culling decisions in Autumn as involuntary culls (i.e. empty cows, chronic mastitis and lameness) have already been removed from the herd. Changing demand and interest in tools over time aligns with our previous research (Newton *et al.* 2021). Also, while participants could see value in a tool, feedback was received that for farmers to engage with a

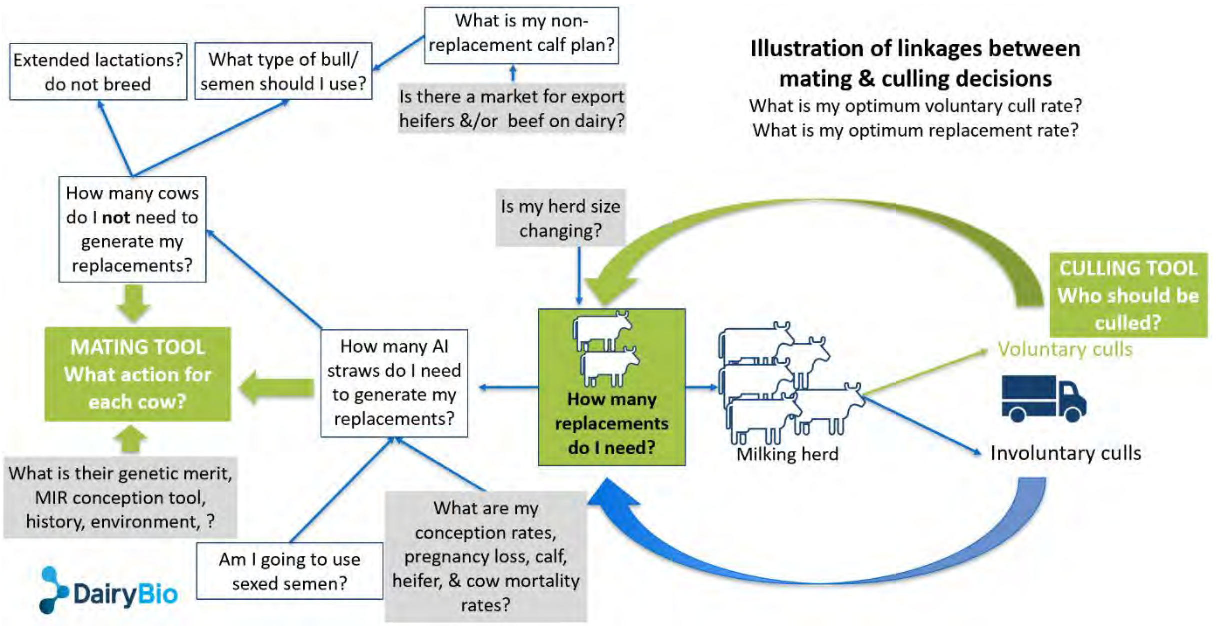

Figure 1. Diagrammatic illustration of some of the linkages between mating and culling decisions on farm and some of the other factors influencing that decision. Three core questions are shown in green text boxes, grey and white text boxes show some of the factors impacting the answer for those questions.

tool they needed to perceive that the tool will provide them benefits and value above current process. The importance of education and extension resources that illustrated this was also discussed.

Participants articulated many features of 'next-gen' tools they saw as important

Where interest in proposed tools existed, participants clearly articulated features of tools or required data inputs they saw as most important. This included:

1. Milk yield, fat and protein from herd test results.
2. Alternate milk recording sources (in line meters and automatic milking system).
3. Mid-infrared (MIR) spectral data (including MIR Conception tool (Ho and Pryce 2020)).
4. Clinical mastitis cases and somatic cell counts.
5. Other health information (i.e. lameness, metritis, antibiotic use).
6. Fertility (i.e. insemination events, calving events, calving interval).
7. Pregnancy scanning results.
8. Novel sensor data (i.e. collars and smart tech).
9. Genetic and genomic information (i.e. breeding values).
10. On-farm software recording systems

11. Temperature-humidity records.
12. Economic parameters.

Of primary importance was the ability to draw upon milk recording data captured from herd testing as well as inline meters and automatic milking systems. Features of the tool that participants identified as important included:

- Ability to pull data from existing sources – no duplicate data entry.
- Ability to consider lifetime data, not just lactation information.
- Account for the flow on effects of decision making (i.e. how value of extra pregnancy changes over a lactation, poorer conception rates with sexed semen).
- Ability for user to manually adjust starting parameters, economic assumptions etc.
- A dashboard for easily visualisation.
- A traffic light or grouping system to facilitate management of cow groups not individual cows in large herds.

When the data sources identified through this study were cross-referenced against the information currently available in Australian dairy's central data repository only 4 were fully accessible (1, 4, 6 and 9 in the list above), and a further 4 had partial or limited availability (3, 5, 7 and 10 in the list above). By 2026, improved accessibility was only expected from: addition of milk recording data from in-line meters and automatic milking machines, and improvements to availability of MIR spectral data. Not all milk samples are processed on machines with MIR capabilities. Historically, only data collected from Bentley machines has been utilised with incorporation of data from FOSS brand MIR machines in DataGene's 2023/24 Operating Plan (DataGene 2023). Data access, availability and integration was identified as a key barrier to 'next-gen' tool development and usage and will be discussed further in the next section.

Data access, availability and integration is a key barrier to 'next-gen' tool development

"I was thinking the last 20 years have been very, very bad for data in Australia because now we've got data sitting in many places and almost no one's talking to each other." These sentiments shared by a service provider highlight the biggest barrier to 'next-gen' tool development. At individual farm level as well as wider industry level, data access, interoperability and integration across software programs and platforms was identified as a key barrier to development and uptake of 'next gen' tools. Participants were very clear in the message that they did not want to have to duplicate data entry - a point raised during nearly all workshops; *'the biggest issue I've got is how certain systems/apps don't talk to one another. How does it get into the system without having to double handle it?'* A key implication of this is that any tool developed needs to integrate into existing data pipelines. This represents a significant challenge. There is also no easy way to combine data from different farm software systems on-farm. This is challenge shared by dairy herds and other farming enterprises globally (Wolfert *et al.* 2017). This means that not all data is being used in decision making – especially for data user groups lacking skills set or motivation to manually combine data themselves.

Compounding this challenge, not all data collected on-farm is currently entering the central data repository in Australia. For example, veterinarians provided feedback that much health information is missing from the repository, believing a major barrier was health record data formats not aligning to data formats/reporting structures used for transferring data in and out of the repository. The proprietary nature of some data being collected by new technologies (i.e. heat detection collars and automatic milking systems) hinders development of data sharing agreements (and pipelines) and makes data centralisation difficult. Big variation in digital literacy also exists and service providers advised that it is likely that some data is still being captured on paper only. The ongoing use of paper-based records in dairy recording has previously been identified by Zottl *et al.* (2015). Such data challenges are not unique to either Australia or the dairy industry, as previously reviewed by Wolfert *et al.* (2017). Locally, the DataConnect project aims to explore opportunities for the Australian herd improvement industry to work pre-competitively on data exchange and integration (DataGene Pty Ltd. 2023). More broadly, initiatives like iDDEN (International Dairy Data Exchange Network) which was launched in 2020 with the aim of streamlining data exchange between dairy herds, milk recording organisations, dairy equipment manufacturers, farm software providers and other service providers have potential to help address these challenges (Reents and Pekeler 2021). While these initiatives are encouraging, overall the dairy industry has made slow progress in adopting data integration technologies (Cabrera *et al.* 2021). So, at present this remains a key barrier to successful development and adoption of ‘next-gen tools’ in Australia.

A further challenge to ‘next-gen’ tool development highlighted by participants was the need to build a tool that can accommodate the big variability in the amount on-farm data captured by individual farms. A key point made was that the farms that would benefit most from ‘next-gen’ tools may not be collecting the data needed to drive the tool effectively (i.e. ‘data-disconnected’) users. For this user group it was suggested that support to determine basic parameters such as herd replacement rate would be helpful. In comparison, while time savings could be expected for ‘data-driven’ user group, the value gained from improved decision making would be smaller. Possible solutions included: developing a tool that accommodates variable levels of data recording, for example a tiered tool with access determined by data recorded. A further discussion point was whether Australia needed more mandatory recording on-farm. Whilst likely to receive negative pushback from some farming groups, mandatory recording is widely used throughout Europe and additional recording may also soon be required as part of anticipating greenhouse gas emissions reporting requirements.

Semi-structured facilitation style provided diverse insights across many areas

The semi-structured facilitation style created opportunities to uncover valuable feedback on areas related to culling and mating. In addition to the discussion around optimum replacement rate and herd age structure discussed previously, feedback on the need for ongoing education and training was received. Areas highlighted by participants included:

1. understanding what best practise use of sexed semen is, including access to more resources and case studies;
2. how to use genomic data to make decisions in the milking herd (i.e. uses beyond choosing heifer replacements); and
3. limited knowledge about the impact of inbreeding, where to seek advice or report concerns about potential new lethal/detrimental conditions.

A further finding from this approach was the value that participants placed on having access to a forum to discuss the use of data in decision-making and the opportunity to engage directly with researchers. One discussion group provided feedback that it was the first time a scientist had attended one of their monthly meetings in 14 years of discussion group operations. Another participant said, “*it’s not often I get to sit down and have a discussion with a geneticist…. Probably the first time we’ve done it, I’m* *enjoying it."* These findings are supported by a recent review of literature on agricultural adoption which found scientists can influence adoption by engaging with a range of service providers, supporting group learning (i.e. discussion groups) and through direct involvement with on-farm trials and demonstrations (Nettle *et al.* 2022).

One limitation of this project is the potential for bias in respondents, however several approaches were used to help with getting feedback from a representative sample. Firstly, including service providers in the participant group. With a large client base, often over multiple dairy regions, they provided perspectives representing a diverse cross-section of dairy farmers and were a rich and valuable source of information. Farmers who respond to an open invitation to discuss 'next-gen' management tools are already likely to be motivated to engage with these tools. The second approach, tapping into existing events, such as dairy discussion group meetings, was found to be an effective strategy to hear more diverse viewpoints in workshops. Participation at these events was driven by other activities of the discussion group such as a free lunch, discussing regional challenges and peer-to-peer learning.

Conclusion

The stakeholder engagement work undertaken here has found participant interest and demand for 'next-gen decision support tools – to enable more informed mating and culling decisions – varied, though has generally positive. Individual farms' current data recording practices, business stage, herd and economic factors appear to influence interest in the tools being proposed with no clear consensus on whether greater demand for culling or mating tools existing overall. A key barrier identified in this study was lack of data integration and access across software platforms on-farm and at lack of pipelines to aggregate data in the central data repository - particularly for novel sensor devices. Coupled with a clear message that farmers do not want to double enter their data, this represents a major barrier to being able to compile the dataset needed to develop 'next-gen' decision support tools. Low levels of data recording in some herds and limited data aggregation will also hinder the number of farms able to use the tool. When these insights were reviewed by funders and key stakeholders, a decision has been made to not continue further tool development in the short term. This is primarily due to the fact delivering a 'next-gen' tool accessible to most farmers is not yet possible and other industry priorities have since emerged. Attention now turns to ensuring the findings of this study are documented and disseminated – particularly to support industry initiatives to improve data connectedness. We will continue to explore other work that may be possible to answer some of the questions raised in this study.

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New algorithm to plan easier and faster weighing for French breeders and technicians

A. Delpeuch[1], M. Legris[1,] G. Augier[2], L. Jeannot[3], D. Delgoulet[3], M. Le Hung[3] and L. Griffon[3]

[1]*Institut de l'Elevage, 149 rue de Bercy, 75 595 Paris, France*
[2]*Eliance, 149 rue de Bercy, 75012 Paris, France*
[3]*Races De France, 149 rue de Bercy, 75012 Paris, France*
Corresponding Author: Arnaud.delpeuch@idele.fr

Abstract

Beef cattle breeding accounted for 3.7 million cows in France in 2022 (1), including 1 million with certified pedigree (CPB), and 435,000 in Beef Cattle Recording (BCR). Each year (2022), more than 1 million calves were weighed up to weaning, giving 331,000 Adjusted Weights at 210 days (AW210). In 2021, the CALPAT project provided new flexible rules for calculating AW and a reliability indicator was developed (2). These recent developments have increased the number of AWs but have heightened the already existing difficulties for technicians and breeders to plan weighing sessions. To solve these problems and facilitate weighing planning, the PATApi project was adopted by «France Genetique Elevage», to provide a high-performance tool for planning weighings up to weaning.

At the beginning, we initially focused on the needs of network advisors with regard to weighing planning. The main features expected from the algorithm are:

- A forecasting tool, able to provide theoretical weight dates based on the previous year's births.
- Regular updates based on actual births and weighings.
- A list of animals with dates of birth and parameters.

At the output, provide optimal passage dates, decision-support elements such as the ratio of animals with AWs, their average reliability, and graphical representations enabling dynamic adjustment by the user.

At the same time, an exploratory analysis of available methods identified the exhaustive search for dates as a solution, particularly for providing dynamic graphical representations. However, this method would involve considering more than 4 billion possible combinations for a 4-month distribution of births and require 4 different passage dates to estimate AWs. An initial optimisation phase enabled us to detect the optimum periods (represented by their median date) rather than the exact dates, and to considerably reduce the number of combinations tested. Finally, the stochastic 'simulated annealing' method completely adapted to this problem, provided the optimum number of weighings and the corresponding weighing dates, with a very significant improvement in calculation times. On the downside, this method does not allow dynamic adjustment by the user. In practice, the performances obtained are satisfying between 3 and 5s for a farm of 60 animals with 3 weighing dates.

Once improved, this algorithm, developed in R language, was packaged and encapsulated in an API. This will interact with any software able to transmit a list of animals and the expected input parameters. Commissioning is expected as from 2024

and it will facilitate the work of technicians and breeders alike from the start of the next birth campaign.

Keywords: algorithm, weighing planning, adjusted weight, beef cattle recording.
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Introduction

In 2022, Beef Cattle Recording (1) in France accounted for 6,119 breeders and almost 435,000 cows in VA4. The VA4 formula, which combines weighing and scoring around weaning, concerned around 14% of calves born in France. Around 400,000 pre-weaning Adjusted Weights (AW) are calculated each year for just over 1 million weighings, 20% of which are carried out by the farmers themselves. This represents an average of 2.5 weighings for pre-weaning AWs (AW120 days or AW210 days).

Over the last ten years or so, the Beef Cattle Recording business has undergone a threefold change:

- Breeders' expectations of services are increasingly heterogeneous,
- Farms are becoming more specialised, herd sizes are increasing, and labour is becoming scarcer, all of which tends to increase the constraints involved in carrying out the weighing required to obtain technical and genetic results,
- Breeding Societies (BSs), the prime contractors for Beef Cattle Recording, want data collection to be able to meet the selection objectives of their breeds, bringing a new differentiation in process management.

These three points illustrate the major changes underway in Beef Cattle Recording at every level.

Material and methods

The aim of weighing planning on farms is to obtain as many AWs as possible (AW120 days, AW210 days or both), and as reliably as possible (2) with as few weighings as possible, depending on the distribution of births.

Collecting user needs and expectations

To identify user expectations, a stakeholder consultation was organised. After that, 5 main principles were retained:

- The algorithm must allow for a predictive simulation of the dates of passages at the start of the campaign, based on the births of animals from the previous year (N-1).
- It must then be possible to adjust the schedules during the season according to the actual births on the farm and any weighings carried out, depending on the date of the scheduling request.
- It must be able to consider the exclusion of periods from the planning (e.g. summer grazing or grassing phase) and early exits of animals.
- It must be able to operate autonomously based on an API that can be used by any application, based on the minimum data transmitted as input.
- It must offer the possibility of weighting the number of weighings required and their positioning according to the situation of the farm and its structure.

Developing the algorithm in R

The algorithm was developed iteratively. Regular exchanges between the teams (development, management, users) meant that progress could be made step by step, considering the expectations and constraints expressed.

Algorithm input

The algorithm needs 3 files to run (Figure 1).

- The first input file concerns the characteristics of the calculation request. It contains the identifier of the farm and the organisation to which it belongs, the season and breed concerned, and the date and group of the calculation request.
- The calculation parameters are contained in the second file. They specify the AWs to be optimised (search for dates to obtain the maximum of AWs) and the minimum level of reliability of the AWs required. It also contains the dates that are potentially forbidden for weighing (unfavourable periods) or those that are desired, the usual exit ages for calves and the number of weighings envisaged (0 lets the algorithm decide). Finally, it also allows to configure the output according to the desired level of detail.
- The last input file concerns the characteristics of the animals on the farm. It contains their birthdate and previous weighing dates, whether they should be excluded from the planning and whether their birth weight can be used in the AW calculation as first weight.

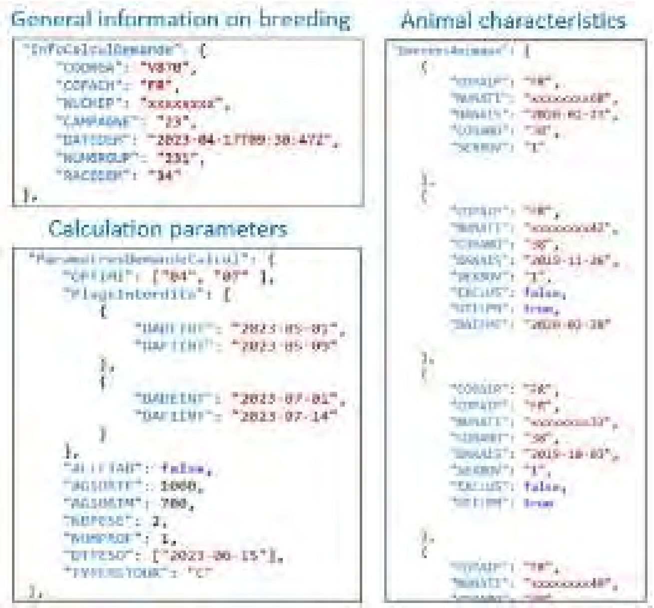


Figure 1. Algorithm input files.

A sorting and selection index

A Sorting and Selection Index (SSI) has been proposed to help objectively choose the best solution. It combines the ratio of AWs obtained, the level of their reliability and the number of weighings required. The SSI is expressed as a value between 0 and 1. Two equations are proposed, depending on whether optimisation of a single or both AWs (AW120 or AW210) is desired:

- For single AW to be optimised:

$$SSI = A * (cC + dD + eE) * \frac{1}{\sqrt{J}}$$

- For two AWs to be optimised

$$SSI = (A * B) * (cC + dD + eE) * (fF + gG + hH) * \frac{1}{\sqrt{J}}$$

With:

A, B: % of AWs (AW120 or AW210) respectively obtained,

C, F: % of these AWs with a high level of reliability,

D, G: % of these AWs with a medium level of reliability,

E, H: % of these AWs with a low level of reliability,

c, d and e: weights applied to each reliability level for AW120,

f, g and h: weights applied to each reliability level for AW210,

J: the number of weighings of the forecasting

Core algorithm

The algorithm has two calculation modes:

1. **Exhaustive and detailed mode**

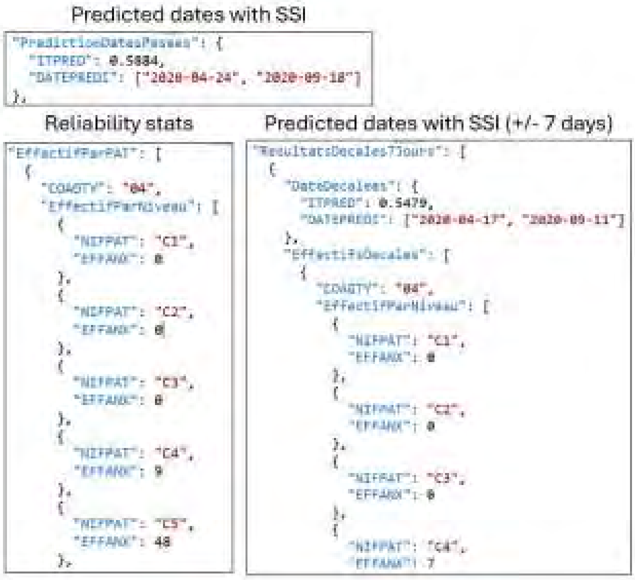
   In this mode, the calculation is based on a grid containing all solutions according to animal characteristics and demand parameters. The best solution is selected on the basis of the sorting index detailed above. This mode provides more details on the chosen solution, as well as elements for interactive date adjustment. On the other hand, it requires a longer calculation time.

2. **Optimized mode**

   This mode speeds up the process of finding only the best solution (and results around +- 7 days) adapted to the input parameters. It is based on the simulated annealing method (1).

Algorithm outputs

The algorithm output comes in 3 different forms, adapted to the expectations and parameters transmitted at the time of the request. In the “optimized” calculation mode (Figure 2), the optimal date(s) of passages associated with their SSI are returned. In addition, a table of animal frequencies by AW and by level is provided. Finally, results +/- 7 days from the optimal dates are provided to help the technician to adjust the final choice.



Predicted dates with SSI

```
"PredictionDatesPesees": {
  "ITPRED": 0.5884,
  "DATEPREDI": ["2020-04-24", "2020-09-18"]
},
```

Reliability stats

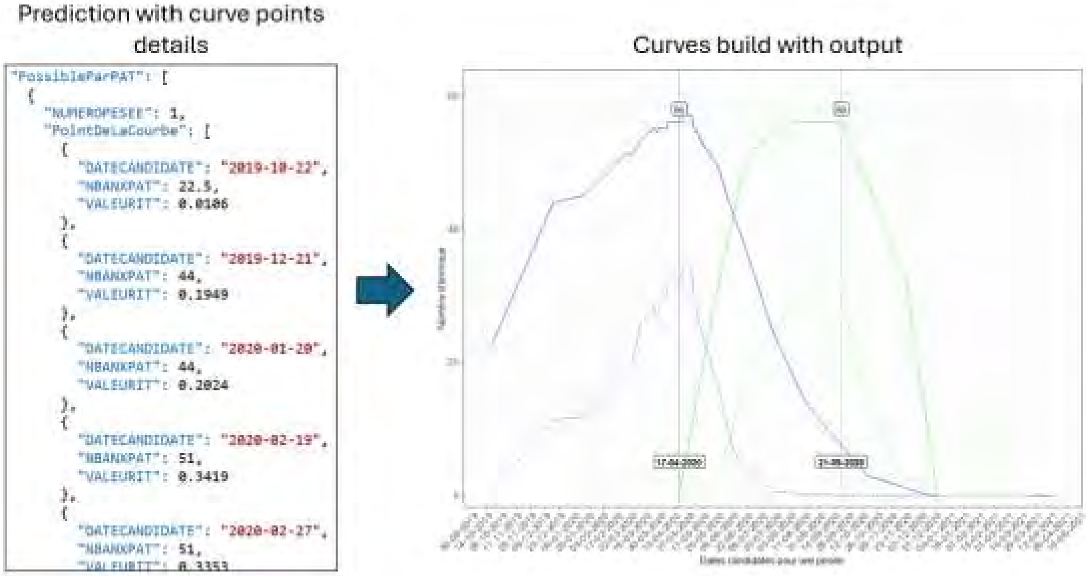
```
"EffectifParPAT": [
  {
    "COAGTY": "04",
    "EffectifParNiveau": [
      {
        "NIFPAT": "C1",
        "EFFANX": 0
      },
      {
        "NIFPAT": "C2",
        "EFFANX": 0
      },
      {
        "NIFPAT": "C3",
        "EFFANX": 0
      },
      {
        "NIFPAT": "C4",
        "EFFANX": 9
      },
      {
        "NIFPAT": "C5",
        "EFFANX": 48
      },
```

Predicted dates with SSI (+/- 7 days)

```
"ResultatsDecales7Jours": [
  {
    "DateDecalees": {
      "ITPRED": 0.5479,
      "DATEPREDI": ["2020-04-17", "2020-09-11"]
    },
    "EffectifsDecales": [
      {
        "COAGTY": "04",
        "EffectifParNiveau": [
          {
            "NIFPAT": "C1",
            "EFFANX": 0
          },
          {
            "NIFPAT": "C2",
            "EFFANX": 0
          },
          {
            "NIFPAT": "C3",
            "EFFANX": 0
          },
          {
            "NIFPAT": "C4",
            "EFFANX": 7
```

Figure 2. Optimized outputs.

The exhaustive mode (Figure 3) provides an additional output which contains an extraction of the calculation grid (left of figure) enabling the reconstruction of the PAT frequency curves obtained according to the date on which the weighings were carried out.



Prediction with curve points details

```
"PossibleParPAT": [
  {
    "NUMEROPESEE": 1,
    "PointDeLaCourbe": [
      {
        "DATECANDIDATE": "2019-10-22",
        "NBANXPAT": 22.5,
        "VALEURIT": 0.0106
      },
      {
        "DATECANDIDATE": "2019-12-21",
        "NBANXPAT": 44,
        "VALEURIT": 0.1949
      },
      {
        "DATECANDIDATE": "2020-01-20",
        "NBANXPAT": 44,
        "VALEURIT": 0.2024
      },
      {
        "DATECANDIDATE": "2020-02-19",
        "NBANXPAT": 51,
        "VALEURIT": 0.3419
      },
      {
        "DATECANDIDATE": "2020-02-27",
        "NBANXPAT": 51,
        "VALEURIT": 0.3353
```

Curves build with output

Figure 3. Specific output exhaustive mode.

An RShiny application for testing and visualization

Right from the start of the project, we realised that we needed to develop an

RShiny application. First, it enabled us to understand the need and its context. It was then very useful for considering the various parameters and constraints expressed by the stakeholders. Finally, it was used to test and demonstrate how the algorithm worked as its development progressed.

Results

To evaluate the algorithm's performance, we randomly selected 45 farms from the 1465 available in the test database. This sample included farms with 40 to 150 calvings and a birth spread of up to 10 months (Figure 5).

Next, the algorithm was called upon 10 times for each mode and each of the selected farms. Then we calculate the median time for each farm, considering only the iterations with the same number of weighings.

Average performance is satisfying, particularly in "optimized" mode. Some farms take a long median calculation time to obtain results (up to 9s) with a large variability range. These are very often large farms with very spread-out birth distributions (up to 180 days). On the other hand, the median calculation time for medium-sized farms, even with an extended birth period, is less than 5s (2.4s vs 4.9s). These tests were carried out on a standard computer and not on a server dedicated to computing (Figure 6).

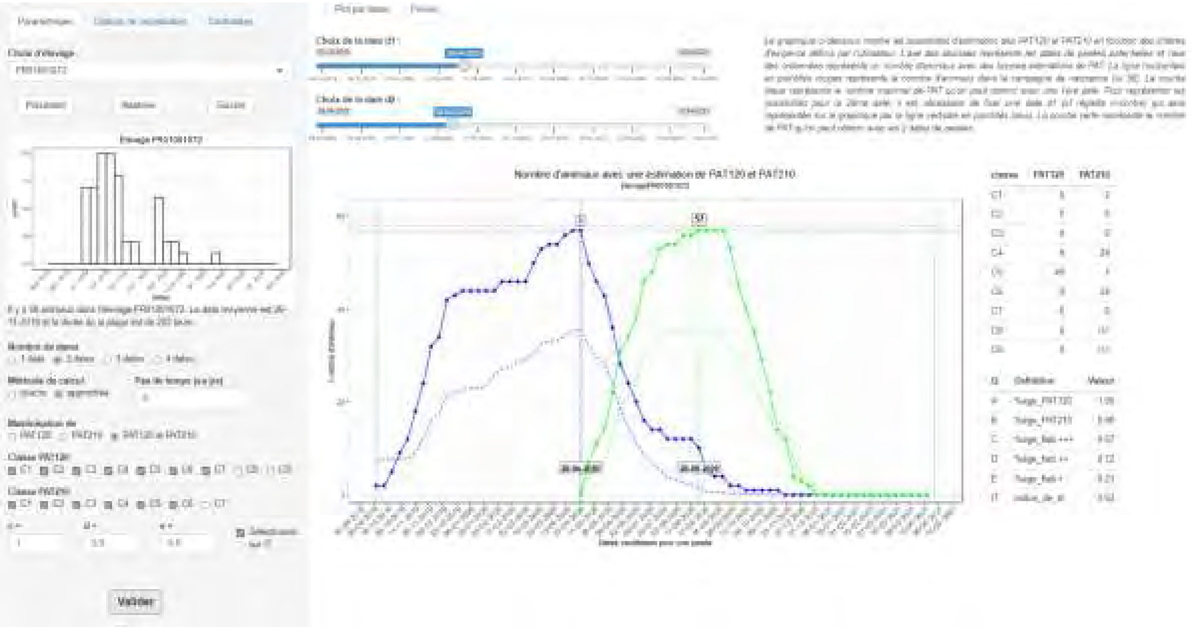


Figure 4. RShiny interface.

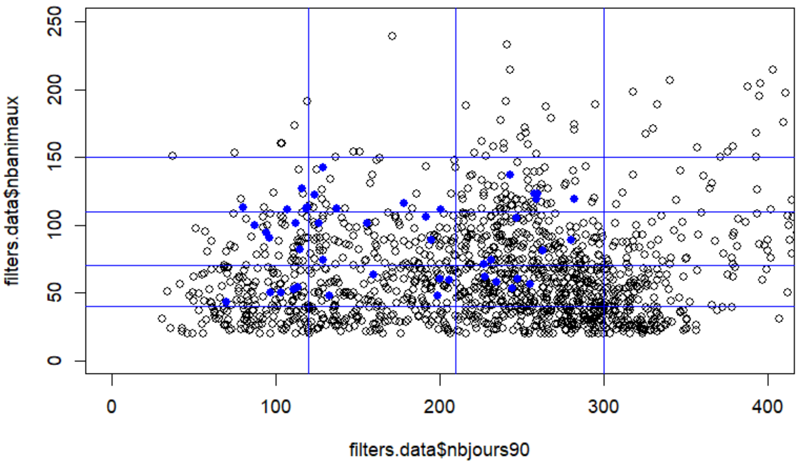


Figure 5. Sampling of test farms.

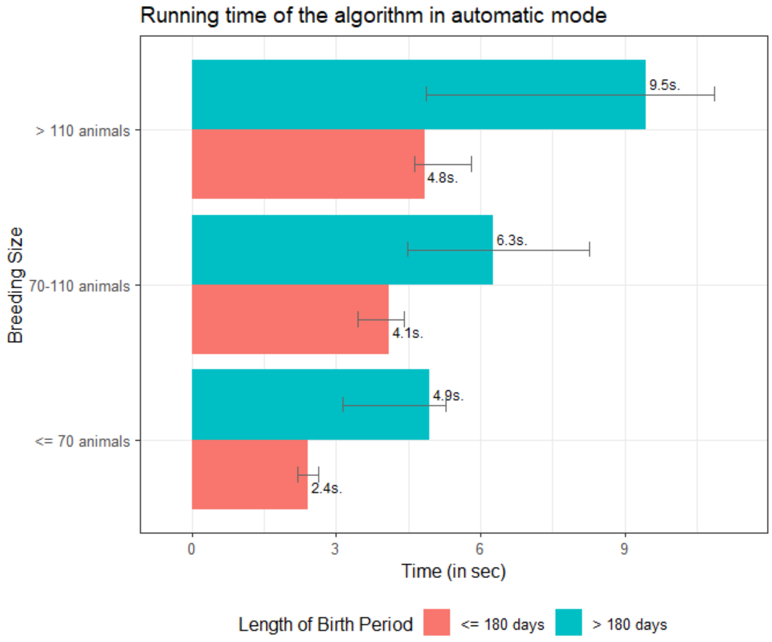


Figure 6. Algorithm runtime in optimised mode comparison.

Discussion and conclusion

We have produced an algorithm (encapsulated in an API) that uses a livestock inventory to determine the ideal weighing dates. This algorithm meets the expectations of professionals. Its flexibility means it can be adapted to a wide range of uses. Some options have not been implemented in this first version, but its modular construction will enable its evolution in the future. The use of parallelized calculations could boost performance and reduce response times. This will certainly be implemented in a 2nd version of the algorithm.

The service is currently being implemented by FGE. The API encapsulating the algorithm should soon be made available to users so that their software can exploit it.

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International benchmarking of technical data from Dairy farmers

H. Jan van der Beek

UNIFORM-Agri, The Netherlands

Introduction

Benchmarking is a widely used method that help's in evaluating mostly technical results from Dairy farmers and can put your own figures into perspective. Farmers and consultants like it, but drawing the right conclusions is not always easy. And it is even dangerous if a farmer makes drastic decisions based upon it.

When benchmark is used in a correct way it can be a serious eye-opener and can stimulate farmers and consultants to make improvements.

Benchmarking

Benchmarking can be done in several ways. It means you can compare your current data or KPI with other KPI's. It is very important that the way the data or KPI's are calculated are identical. This will avoid drawing wrong conclusions. So then we also have to look at the origin of the date we compare.

Values of the data presented in KPI's will differ when you do comparisons. This is because the circumstances are not the same. It is important to distinguish external and internal circumstances. But it is also important with what other data we are comparing. This can be your own data in another timeframe, or other farms in the same timeframe. A combination of them is difficult, because then it is hardly possible to analyze the reason of the differences. It can and mostly will be the combination of internal circumstances and external circumstances. And then it is difficult to make conclusions.

Internal and external circumstances

A typical external circumstance is the weather, this is something that has influence on many aspects on the farm (extreme hot summer has a negative effect on fertility) but the farmer has no influence on it. Ther weather is what it is. How the farmer acts on these circumstances, is however in principle a choice. He can do nothing, or he can change things so the cows will suffer less. Other external circumstances are governmental regulations or diseases like bluetongue.

A typical Internal circumstance is a new barn that is built or a new herdsman that has started or the farm is moving to milk with robots. This has no effect on data from other farmers if you compare it with them. It however has an effect on your own data if you compare the data before and after the change of circumstances. And if all other circumstances stay the same, you can draw the conclusion that the

change in the values of the KPI's is the effect of the specific circumstances that changed. This is in many cases not the situation because not all other circumstances stay the same.

Where do you compare with?

You can compare with your own data in another timeframe. So for example you compare your own data with your own data from a year ago. This can be done in several ways. It is important that the data is stable and in general not over too short a period. For example last year in December you only had 2 cows calving and you had one dead calf (mortality rate is 50%) and now you had 10 cows calving and you had 3 dead (mortality rate is 30%). You better use the rolling average over a longer period of time if you want to compare your current results with the ones from a year ago.

Comparisons with your own situation a year ago will always be affected by external and internal circumstances. (for example a new Barn and bad weather).

You can also compare your own date with other farmers in the same timeframe. This can have the advantage that most external factors are the same for all farms, at least in the same region or country. The reasoning of bad weather for having lower results cannot be used when all other farmers had the same bad weather.

How a farmer is acting on the external circumstances can be the reason of differences in the results. Some farmers will just play the role of a victim and accept the lower results whilst others take action to minimize the negative effect.

There are also farmers who take care that they are prepared for bad external circumstances which only come up occasionally or are unexpected. On the other hand, we all know that the weather is sometimes bad and milk prices are low. Good managers/businessman are prepared for what to do when this occurs. We call that risk management.

With this background information and the opportunity to have access to data from farms from different countries who are all using the same herd management software we can do interesting things.

This means there are no differences in the calculations. We can compare farms with farms in other countries. Besides that we are also able to have groups of farms who send in their data this year and also last year so we can see the development.

This is a unique set of data and we can learn a lot from that.

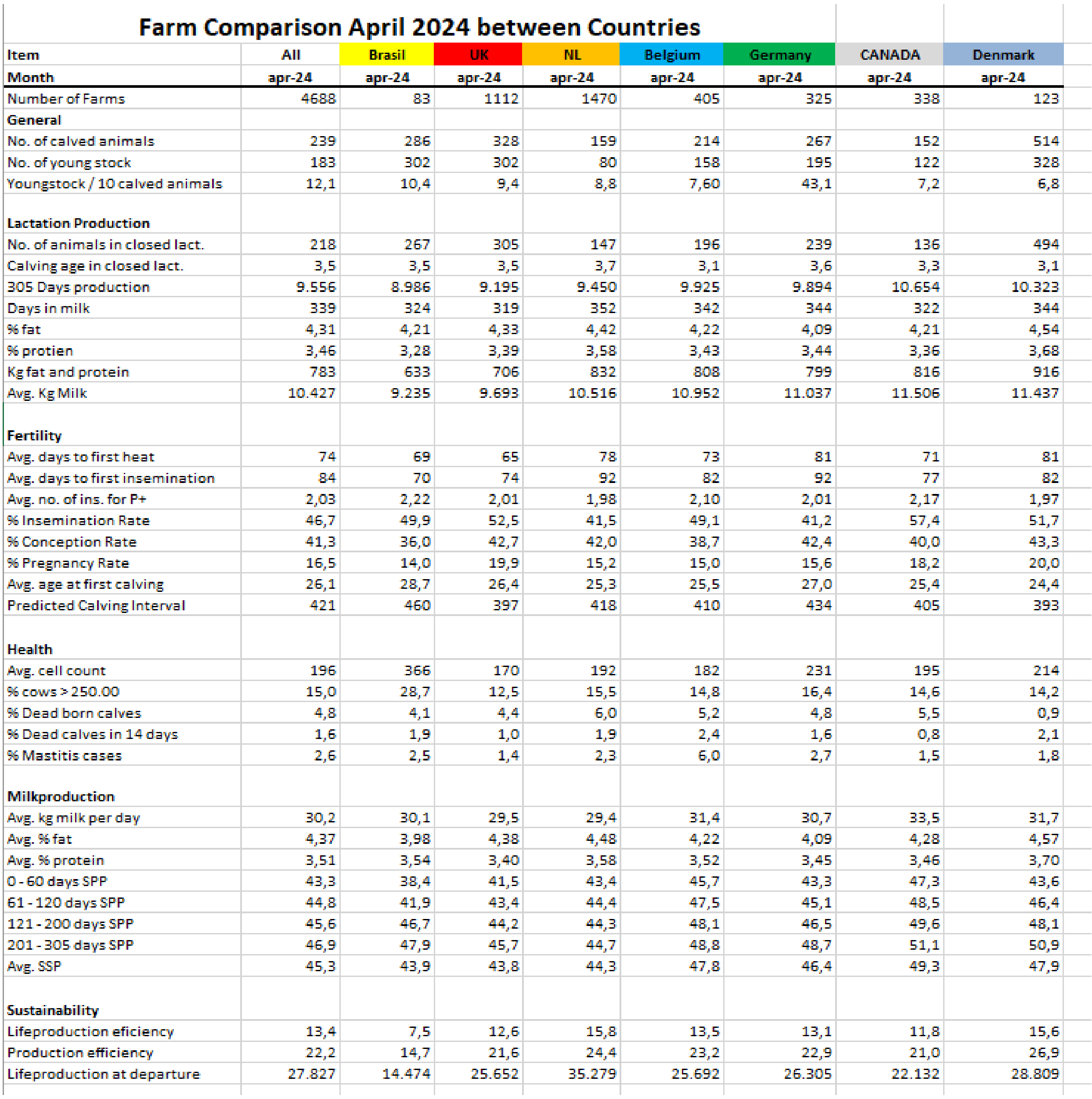
We look at exactly the same group of farms over a period of time in each country, we can see how the farms in the countries evolve, and we can see different focus in countries and the effect of it.

This data collection is there for more than 10 years and that gives us a unique report of the same group of farms who had the same software over a very long period.

This is possible in those countries where there is enough data so the average is stable. In the appendixes there are 3 examples of tables with comparable data.

The first tabie is from dairy farms (more than 100 per country lwho have sent in their data during Aprii.

Looking at the fertility data there is a huge difference between the Danish (20) and Brazilian (14) results in Pregnancy Rate.This is supported by the age at 1st calving with a huge difference 24.4 versus 28,7.

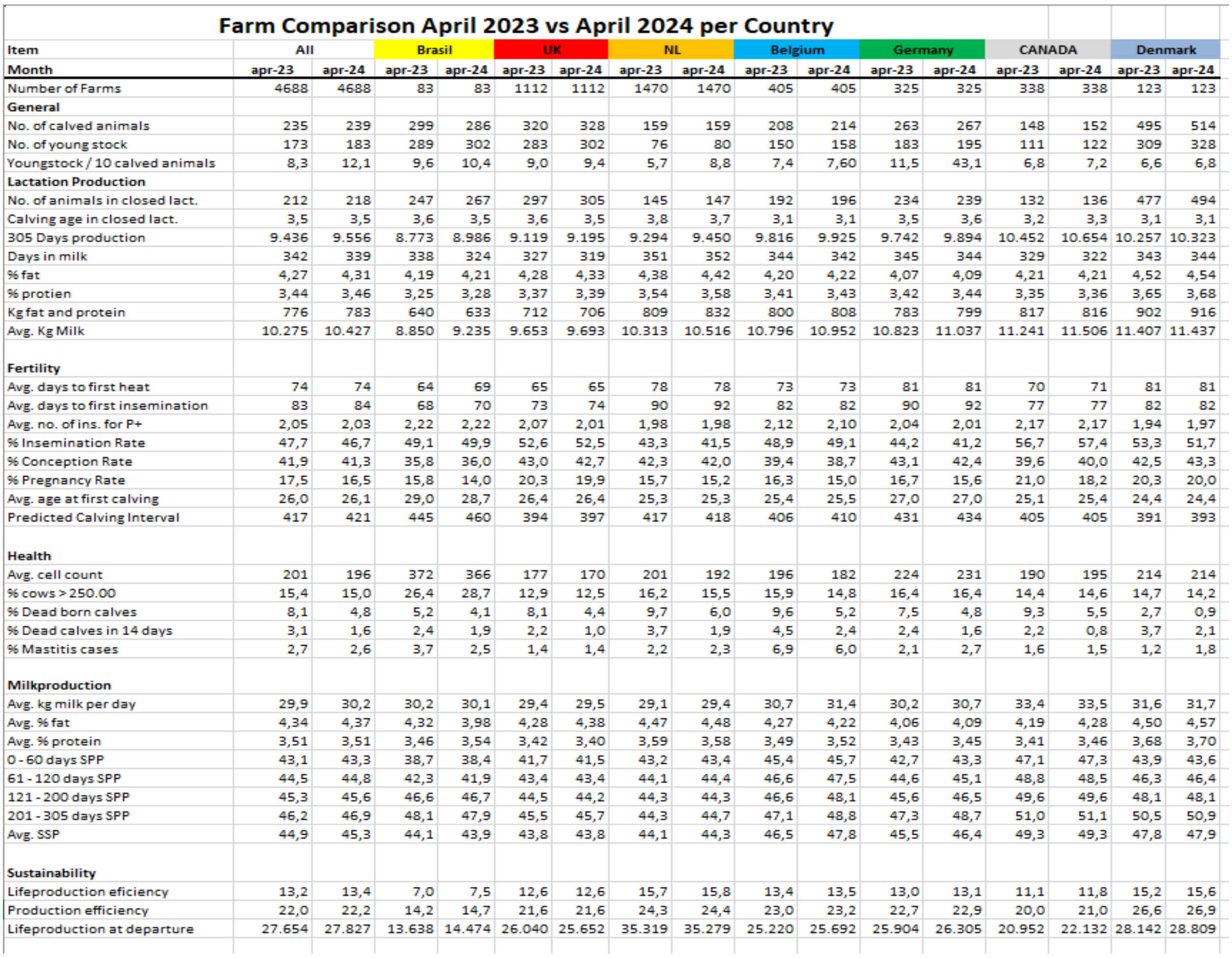


Farm Comparison April 2024 between Countries

Item	All	Brasil	UK	NL	Belgium	Germany	CANADA	Denmark
Month	apr-24	apr-24	apr-24	apr-24	apr-24	apr-24	apr-24	apr-24
Number of Farms	4688	83	1112	1470	405	325	338	123
General								
No. of calved animals	239	286	328	159	214	267	152	514
No. of young stock	183	302	302	80	158	195	122	328
Youngstock / 10 calved animals	12,1	10,4	9,4	8,8	7,60	43,1	7,2	6,8
Lactation Production								
No. of animals in closed lact.	218	267	305	147	196	239	136	494
Calving age in closed lact.	3,5	3,5	3,5	3,7	3,1	3,6	3,3	3,1
305 Days production	9.556	8.986	9.195	9.450	9.925	9.894	10.654	10.323
Days in milk	339	324	319	352	342	344	322	344
% fat	4,31	4,21	4,33	4,42	4,22	4,09	4,21	4,54
% protien	3,46	3,28	3,39	3,58	3,43	3,44	3,36	3,68
Kg fat and protein	783	633	706	832	808	799	816	916
Avg. Kg Milk	10.427	9.235	9.693	10.516	10.952	11.037	11.506	11.437
Fertility								
Avg. days to first heat	74	69	65	78	73	81	71	81
Avg. days to first insemination	84	70	74	92	82	92	77	82
Avg. no. of ins. for P+	2,03	2,22	2,01	1,98	2,10	2,01	2,17	1,97
% Insemination Rate	46,7	49,9	52,5	41,5	49,1	41,2	57,4	51,7
% Conception Rate	41,3	36,0	42,7	42,0	38,7	42,4	40,0	43,3
% Pregnancy Rate	16,5	14,0	19,9	15,2	15,0	15,6	18,2	20,0
Avg. age at first calving	26,1	28,7	26,4	25,3	25,5	27,0	25,4	24,4
Predicted Calving Interval	421	460	397	418	410	434	405	393
Health								
Avg. cell count	196	366	170	192	182	231	195	214
% cows > 250.00	15,0	28,7	12,5	15,5	14,8	16,4	14,6	14,2
% Dead born calves	4,8	4,1	4,4	6,0	5,2	4,8	5,5	0,9
% Dead calves in 14 days	1,6	1,9	1,0	1,9	2,4	1,6	0,8	2,1
% Mastitis cases	2,6	2,5	1,4	2,3	6,0	2,7	1,5	1,8
Milkproduction								
Avg. kg milk per day	30,2	30,1	29,5	29,4	31,4	30,7	33,5	31,7
Avg. % fat	4,37	3,98	4,38	4,48	4,22	4,09	4,28	4,57
Avg. % protein	3,51	3,54	3,40	3,58	3,52	3,45	3,46	3,70
0 - 60 days SPP	43,3	38,4	41,5	43,4	45,7	43,3	47,3	43,6
61 - 120 days SPP	44,8	41,9	43,4	44,4	47,5	45,1	48,5	46,4
121 - 200 days SPP	45,6	46,7	44,2	44,3	48,1	46,5	49,6	48,1
201 - 305 days SPP	46,9	47,9	45,7	44,7	48,8	48,7	51,1	50,9
Avg. SSP	45,3	43,9	43,8	44,3	47,8	46,4	49,3	47,9
Sustainability								
Lifeproduction eficiency	13,4	7,5	12,6	15,8	13,5	13,1	11,8	15,6
Production efficiency	22,2	14,7	21,6	24,4	23,2	22,9	21,0	26,9
Lifeproduction at departure	27.827	14.474	25.652	35.279	25.692	26.305	22.132	28.809

The 2nd data set is fram farmers who use UNIFORM and who have sent in their data in April 2023 and in April 2024.

It is interesting to see the data about sustainability. Due to governmental regulations the Dutch figures show much better figures than the average.



Farm Comparison April 2023 vs April 2024 per Country

Item	All		Brasil		UK		NL		Belgium		Germany		CANADA		Denmark	
Month	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**	**apr-23**	**apr-24**
Number of Farms	4688	4688	83	83	1112	1112	1470	1470	405	405	325	325	338	338	123	123
General																
No. of calved animals	235	239	299	286	320	328	159	159	208	214	263	267	148	152	495	514
No. of young stock	173	183	289	302	283	302	76	80	150	158	183	195	111	122	309	328
Youngstock / 10 calved animals	8,3	12,1	9,6	10,4	9,0	9,4	5,7	8,8	7,4	7,60	11,5	43,1	6,8	7,2	6,6	6,8
Lactation Production																
No. of animals in closed lact.	212	218	247	267	297	305	145	147	192	196	234	239	132	136	477	494
Calving age in closed lact.	3,5	3,5	3,6	3,5	3,6	3,5	3,8	3,7	3,1	3,1	3,5	3,6	3,2	3,3	3,1	3,1
305 Days production	9.436	9.556	8.773	8.986	9.119	9.195	9.294	9.450	9.816	9.925	9.742	9.894	10.452	10.654	10.257	10.323
Days in milk	342	339	338	324	327	319	351	352	344	342	345	344	329	322	343	344
% fat	4,27	4,31	4,19	4,21	4,28	4,33	4,38	4,42	4,20	4,22	4,07	4,09	4,21	4,21	4,52	4,54
% protien	3,44	3,46	3,25	3,28	3,37	3,39	3,54	3,58	3,41	3,43	3,42	3,44	3,35	3,36	3,65	3,68
Kg fat and protein	776	783	640	633	712	706	809	832	800	808	783	799	817	816	902	916
Avg. Kg Milk	10.275	10.427	8.850	9.235	9.653	9.693	10.313	10.516	10.796	10.952	10.823	11.037	11.241	11.506	11.407	11.437
Fertility																
Avg. days to first heat	74	74	64	69	65	65	78	78	73	73	81	81	70	71	81	81
Avg. days to first insemination	83	84	68	70	73	74	90	92	82	82	90	92	77	77	82	82
Avg. no. of ins. for P+	2,05	2,03	2,22	2,22	2,07	2,01	1,98	1,98	2,12	2,10	2,04	2,01	2,17	2,17	1,94	1,97
% Insemination Rate	47,7	46,7	49,1	49,9	52,6	52,5	43,3	41,5	48,9	49,1	44,2	41,2	56,7	57,4	53,3	51,7
% Conception Rate	41,9	41,3	35,8	36,0	43,0	42,7	42,3	42,0	39,4	38,7	43,1	42,4	39,6	40,0	42,5	43,3
% Pregnancy Rate	17,5	16,5	15,8	14,0	20,3	19,9	15,7	15,2	16,3	15,0	16,7	15,6	21,0	18,2	20,3	20,0
Avg. age at first calving	26,0	26,1	29,0	28,7	26,4	26,4	25,3	25,3	25,4	25,5	27,0	27,0	25,1	25,4	24,4	24,4
Predicted Calving Interval	417	421	445	460	394	397	417	418	406	410	431	434	405	405	391	393
Health																
Avg. cell count	201	196	372	366	177	170	201	192	196	182	224	231	190	195	214	214
% cows > 250.00	15,4	15,0	26,4	28,7	12,9	12,5	16,2	15,5	15,9	14,8	16,4	16,4	14,4	14,6	14,7	14,2
% Dead born calves	8,1	4,8	5,2	4,1	8,1	4,4	9,7	6,0	9,6	5,2	7,5	4,8	9,3	5,5	2,7	0,9
% Dead calves in 14 days	3,1	1,6	2,4	1,9	2,2	1,0	3,7	1,9	4,5	2,4	2,4	1,6	2,2	0,8	3,7	2,1
% Mastitis cases	2,7	2,6	3,7	2,5	1,4	1,4	2,2	2,3	6,9	6,0	2,1	2,7	1,6	1,5	1,2	1,8
Milkproduction																
Avg. kg milk per day	29,9	30,2	30,2	30,1	29,4	29,5	29,1	29,4	30,7	31,4	30,2	30,7	33,4	33,5	31,6	31,7
Avg. % fat	4,34	4,37	4,32	3,98	4,28	4,38	4,47	4,48	4,27	4,22	4,06	4,09	4,19	4,28	4,50	4,57
Avg. % protein	3,51	3,51	3,46	3,54	3,42	3,40	3,59	3,58	3,49	3,52	3,43	3,45	3,41	3,46	3,68	3,70
0 - 60 days SPP	43,1	43,3	38,7	38,4	41,7	41,5	43,2	43,4	45,4	45,7	42,7	43,3	47,1	47,3	43,9	43,6
61 - 120 days SPP	44,5	44,8	42,3	41,9	43,4	43,4	44,1	44,4	46,6	47,5	44,6	45,1	48,8	48,5	46,3	46,4
121 - 200 days SPP	45,3	45,6	46,6	46,7	44,5	44,2	44,3	44,3	46,6	48,1	45,6	46,5	49,6	49,6	48,1	48,1
201 - 305 days SPP	46,2	46,9	48,1	47,9	45,5	45,7	44,3	44,7	47,1	48,8	47,3	48,7	51,0	51,1	50,5	50,9
Avg. SSP	44,9	45,3	44,1	43,9	43,8	43,8	44,1	44,3	46,5	47,8	45,5	46,4	49,3	49,3	47,8	47,9
Sustainability																
Lifeproduction eficiency	13,2	13,4	7,0	7,5	12,6	12,6	15,7	15,8	13,4	13,5	13,0	13,1	11,1	11,8	15,2	15,6
Production efficiency	22,0	22,2	14,2	14,7	21,6	21,6	24,3	24,4	23,0	23,2	22,7	22,9	20,0	21,0	26,6	26,9
Lifeproduction at departure	27.654	27.827	13.638	14.474	26.040	25.652	35.319	35.279	25.220	25.692	25.904	26.305	20.952	22.132	28.142	28.809

The 3rd data set is from groups of farms in the UK, NL , Belgium and Germany.

It is interesting to see the change in the number of cows over 10 years in the countries. But even more interesting is the change in the number of youngstock. In the Netherlands this is completely different form other countries. This is the effect of political regulations.

Farm Comparison April 2014 vs April 2024 per Country

Item	**All**		**UK**		**NL**		**Belgium**		**Germany**	
Month	**apr-14**	**apr-24**	**apr-14**	**apr-24**	**apr-14**	**apr-24**	**apr-14**	**apr-24**	**apr-14**	**apr-24**
Number of Farms	1204	1204	60	60	850	850	148	148	24	24
General										
No. of calved animals	138	179	237	312	127	156	124	213	199	239
No. of young stock	100	114	241	351	87	81	102	150	152	172
Lactation Production										
No. of animals in closed lact.	107	161	152	300	105	143	97	191	123	208
Calving age in closed lact.	3,4	3,6	3,9	3,4	3,5	3,7	3,1	3,1	3,3	3,5
305 Days production	8.672	9.579	8.572	9.029	8.629	9.501	8.948	10.044	9.014	10.354
Days in milk	351	349	350	324	351	352	349	340	344	334
% fat	4,31	4,40	4,17	4,41	4,35	4,42	4,15	4,26	4,06	4,15
% protien	3,49	3,55	3,29	3,39	3,51	3,58	3,42	3,47	3,35	3,44
Kg fat and protein	744	831	703	722	746	837	745	824	726	841
Avg. Kg Milk	9.520	10.615	9.453	9.684	9.463	10.563	9.832	11.003	9.781	11.422
Fertility										
Avg. days to first heat	76	75	64	59	76	76	72	72	87	84
Avg. days to first insemination	88	90	76	70	89	92	82	82	91	92
Avg. no. of ins. for P+	2,01	1,99	2,12	2,06	1,99	1,96	2,08	2,11	1,88	2,07
Health										
Avg. cell count	189	192	183	176	180	195	187	170	241	229
% cows > 250.00	15,1	15,2	14,2	12,6	14,9	15,4	15,7	14,2	17,6	16,2
% Dead born calves	7,4	6,3	0,4	0,7	7,7	6,7	6,3	5,9	2,5	4,0
% Dead calves in 14 days	2,0	1,8			2,3	1,9	1,8	2,6	1,8	1,0
Milkproduction										
Avg. kg milk per day	27,1	29,8	28,8	28,1	26,9	29,6	27,8	31,7	27,2	31,7
Avg. % fat	4,32	4,47	4,18	4,74	4,34	4,47	4,10	4,17	4,05	4,88
Avg. % protein	3,48	3,58	3,35	3,55	3,49	3,58	3,43	3,52	3,35	3,65
0 - 60 days SPP	40,1	43,7	39,5	40,1	40,2	43,6	40,7	46,6	39,7	43,8
61 - 120 days SPP	41,4	44,9	41,6	41,7	41,1	44,7	43,1	48,2	42,2	44,9
121 - 200 days SPP	41,1	45,0	42,9	42,3	40,7	44,6	43,1	49,0	41,5	47,4
201 - 305 days SPP	40,3	45,6	42,1	42,9	39,8	45,0	42,3	49,7	41,6	50,4
Avg. SSP	40,7	45,0	42,1	42,1	40,4	44,6	42,3	48,5	41,5	47,8

AgNav - a tool for putting climate action planning in farmers' hands

M. Lynch[1], P. Fenton[1]. J. Herron[2], D. O'Brien[2], E. Murphy[3] and M. Houlihan[3]

[1]ICBF, Link Road, Ballincollig, P31 D452, Cork, Ireland
[2]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark,
Fermoy P61 P302, Co. Cork, Ireland
[3]Bord Bia, 140 Pembroke Road, Dublin 4, D04 NV34, Ireland

Abstract

Agriculture accounts for 37% of Irelands carbon emissions. The Irish government, in its Climate Action Plan, has set a target of a 25% reduction in emissions from agriculture by 2030.

One of the key challenges facing farmers, advisors and processors is having an accurate picture of on-farm emissions as well as understanding the potential carbon mitigation effects of individual farm management practices.

ICBF, Bord Bia and Teagasc are three agencies with key involvement in the research, implementation and promotion of best practice in the Irish agriculture and food industry. They have collaborated to develop an online toolkit, AgNav, that provides individual farmers with an individual assessment of the total carbon emissions of their farm and the carbon footprint of their produce. AgNav adopts the approach of "assess, analyse, act" to provide a holistic decision support tool for farmers.

1. Assess: AgNav is powered by access to the most accurate farm level data available drawing from a range of data sources. It uses certified methodology of the Lifetime Cycle Analysis model to calculate carbon emissions.
2. Analyse: The tool provides on-demand forecasting capability that allows the farmer and their advisor to estimate the impact on farm emissions of different carbon mitigation actions, such as reducing fertilizer, optimizing grazing days or reducing finishing age.
3. Act: Once the farmer, in conjunction with their advisor, has evaluated the most appropriate mitigation practices for their farm, AgNav provides the functionality to develop a farm specific action plan.

Overall the aims of AgNav are to:

1. Encourage and support farmers to implement climate action and sustainability improvement on Irish farms
2. Leverage the most robust inter-agency data, research and resources to drive the most appropriate actions tailored to individual farms
3. Enable the most precise capture and analysis of data allowing accurate calculation of action impact
4. Provide a mechanism to support the quantification of progress towards Climate Action Plan targets for the agri-sector

Support clear communications on positive progress achieved at farm level – giving control to farmers

Introduction

A particular challenge of climate change mitigation in the context of agriculture is the variety of figures and data that need to be understood and acted upon e.g. total carbon emissions, carbon footprints, emissions targets.

There are challenges in getting a consistent, accurate assessment of on-farm emissions, translating sectoral targets down to individual farm level and building a farm specific roadmap on how to arrive at optimal best practice in carbon mitigation practices.

These challenges need to be addressed at different levels : country, sectoral, corporate and farm.

For agriculture the questions to be answered include:

- What are the current greenhouse gas emissions for the sector ?
- Where are the sources of these emissions?
- What are the targets for the sector ?
- What is the pathway to achieving these targets ?
- What does it mean for an individual farmer ?

In Ireland, the national and sectoral targets are set by the Irish Government and set out in the Climate Action Plan.

The Climate Action Plan sets out a target of a 25% reduction in GHG emissions in Agriculture by 2023. A number of state agencies are involved in assessing how this target can be achieved in this timeframe.

One of these agencies is Teagasc, the national body providing integrated research, advisory and training services to the agriculture.

Teagasc has developed a Marginal Abatement Cost Curve Curve (MACC) to display the abatement potential and relative cost of different mitigation measures (Figure 1).

In the MACC, the wider the bar on the x-axis, the more carbon abatement potential for that action. On the y-axis if an action is below 0 it also cost-negative, if it is above 0 then there is a cost to this measure.

The MACC outlines the activities that will have an impact at the macro level. It is then necessary to translate this into actions that can be undertaken at individual farm level.

This involves three steps:

1. Understanding where each farm is at currently in its ghg emissions.
2. Examining the menu of mitigation actions and see what does it mean for that farm.
3. Agreeing on mitigation actions and making a farm specific plan.

The components needed to underpin these three steps include:

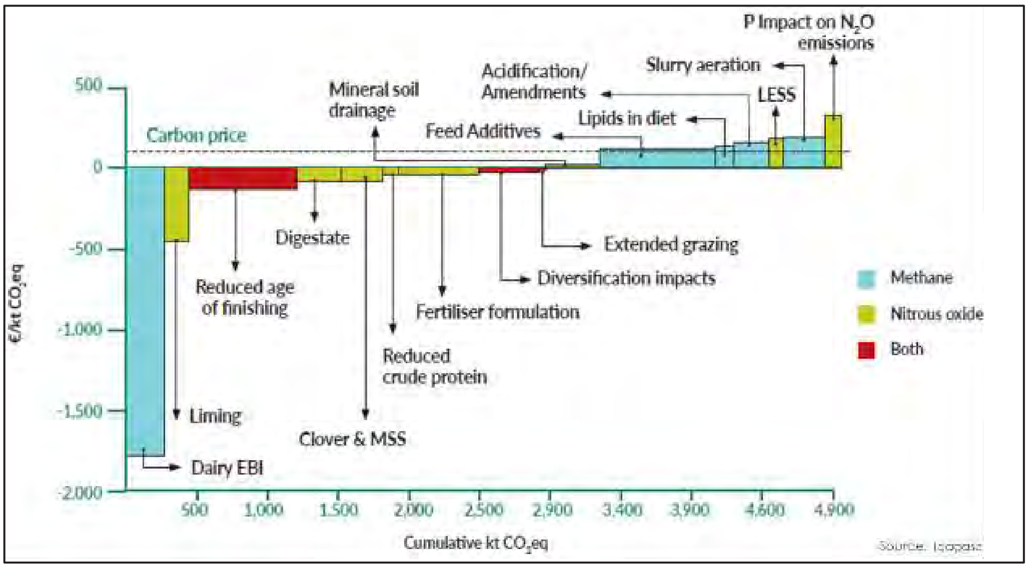


Figure 1. Marginal Abatement Cost Curve – Teagasc 2023..

- Robust, peer reviewed climate emission models.
- Validated sources of input data.
- Maximised use of existing data sources to avoid duplication of effort by farmers.
- Technical capability to run the models at scale.
- Advisory channels to aid in the dissemination of best practice and to provide farmer advice and education.
- Consistent approach to developing farm specific action plans that can be recorded, monitored and followed up.
- Easy to use interfaces for farmers and advisors navigating from the assessment of figures to the development of action plans.

The effort in drawing these components together can be aided by multi-agency collaboration. AgNav is such a collaboration involving three agencies in Ireland. Teagasc as described above, Bord Bia which is the agency that promotes Irish agricultural produce and manages the Quality Assurance Schemes to which the majority of beef and dairy farmers belong, and ICBF, which is the central database for genetic evaluations and for wider data services in the Agrifood sector in Ireland.

The three agencies have developed the AgNav data platform. The contribution from each agency can be summarised as follows:

Teagasc: latest of the scientific research and models for calculating emissions across agriculture. In addition to this there is the link with the Signpost Climate Advisory programme, that is being rolled out across the entire industry.

ICBF: Software engineering and database capabilities in managing data, combining the data from different sources, programming and running the scientific models, testing at scale and updating in line with the latest research. In addition to this it has to technical capability required for development of tools and dashboards for farmers and advisors.

Bord Bia: interface with the existing Quality Assurance Schemes in which farms are audited periodically across a variety of sustainability measures, providing a key source of validating data. In addition to this, as part of the of the Quality Assurance Schemes, farmers develop actions plans for their own farms across a variety of areas covering sustainability and animal welfare. Bord Bia is also the agency for the promotion of Irish agricultural produce both nationally and internationally.

The AgNav tool endeavours to assist the farmer in the three stages of : Assess – Analyse – Act.

Assess: AgNav is powered by access to the most accurate farm level data available **drawing** from a range of data sources. It uses certified methodology of the Lifetime Cycle Analysis model to calculate carbon emissions.

Analyse: The tool provides on-demand forecasting capability that allows the farmer and their advisor to estimate the impact on farm emissions of different carbon mitigation actions, such as reducing fertilizer, optimizing grazing days or reducing finishing age. Act: Once the farmer, in conjunction with their advisor, has evaluated the most appropriate mitigation practices for their farm, AgNav provides the functionality to develop a farm specific action plan.

AgNav is available to farmers at the website https://www.agnav.ie

The user is presented with the latest carbon emissions for their farm: total emissions, emissions per hectare and carbon footprint. These have been calculated using existing data sources to which the farmer has granted permission (Figure 2).

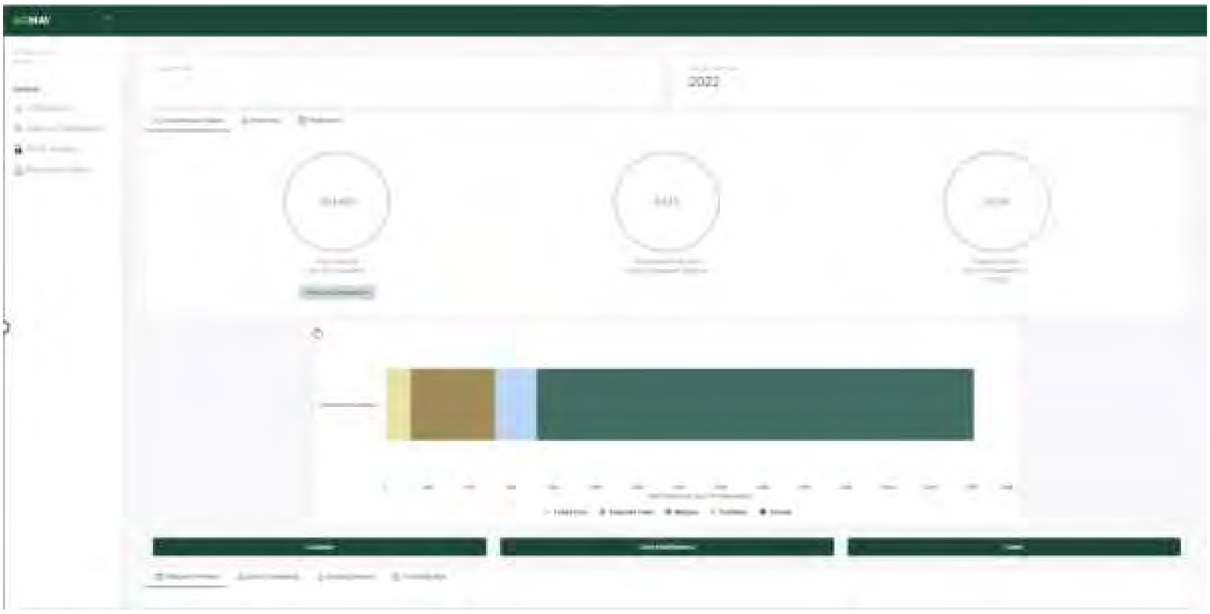


Figure 2: AgNav – Assess: Showing the current on-farm emissions breakdown.

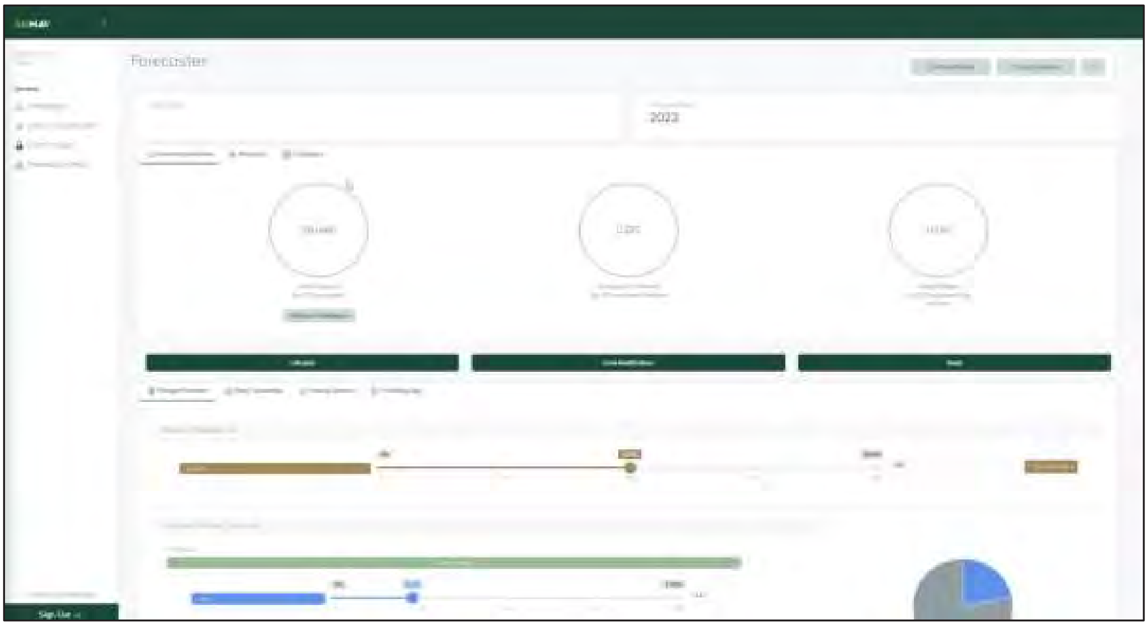


Figure 3. AgNav – Act: Building a specific Action Plan of suitable mitigation measures.

The user can then investigate various mitigation scenarios. Current functionality includes scenario planning across the following areas:

- Reduction in total fertiliser use.
- Modification in the type of fertiliser used.
- Modification to slurry spreading method and time of year of application.
- Modification to the begin/end of the grazing/housing seasons.
- Modifications to the finishing age (age at slaughter) of beef animals.

Following analysis of the effectiveness of different mitigations practices, the farmer can navigate to the Action Planner, and in consultation with their advisor, choose from a menu of options.

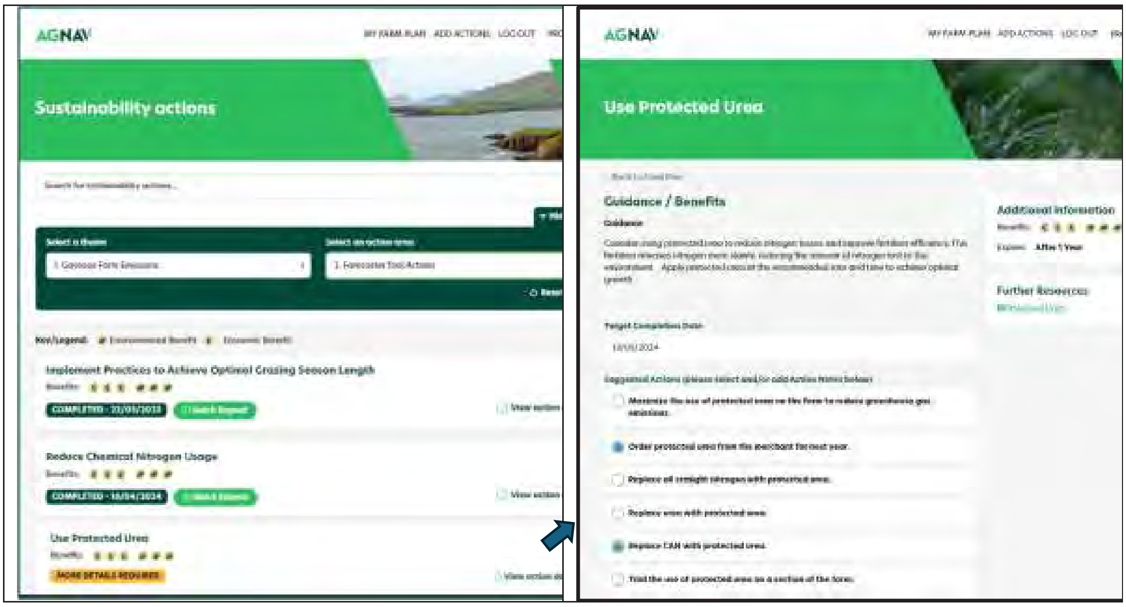
In the example in Figure 4 the user has opted to use protected urea. They are then instructed to select specific actions to help them achieve this and to set a target date for completion.

The Action Plans are saved on the AgNav system to allow for monitoring and further follow up with their advisor.

Future development and rollout

In addition to the functionality outlined thus far, the AgNav platform development plan includes scenario planning in the areas of:

- The effects of increased genetic gain on methane emissions
- The effects of certain feed additives



Sustainability actions

Key/Legend:

Implement Practices to Achieve Optimal Grazing Season Length

Benefits:

Reduce Chemical Nitrogen Usage

Benefits:

Use Protected Urea

Benefits:

MORE DETAILS REQUIRED

Use Protected Urea

Guidance / Benefits

Guidance

Target Completion Date

Additional Information

Benefits:

Expires: After 1 Year

Further Resources

Figure 4. AgNav – Act: Building a specific Action Plan of suitable mitigation measures.

- The effects of different feed concentrate levels

Further development will look at integrating soil type to assess carbon sequestration at farm level.

The development of the AgNav platform began in 2022 building on existing integrations and co-operation between the three agencies. It involved co-design workshops with pilot groups that included farmers and advisors.

It is now included in the training of climate advisors as part of the wider Teagasc Signpost Advisory programme. The Signpost Advisory Programme is a national advisory programme that aims to engage with 10,000 farmers each year from 2024 in the area of climate action and sustainability with a target of 50,000 farm plans by 2030.

AgNav remained in closed pilot phase through 2023 and early 2024 to allow for training of advisors and the gradual onboarding of farmers involved in the Signpost Advisory Programme. It will open to all farmers in the latter half of 2024.

Future developments will see AgNav functionality extend into the sheep, pig, poultry and tillage sectors.

The reality of on-farm hardware when providing tools of the future

J. van der Noord

[1]UNIFORM-Agri, Stationsplein 14 9401 LB Assen, The Netherlands
Corresponding Author: jn@uniform-agri.com

While the dairy industry is making large steps in development of new sensors, data platforms and analysis tools to help farmers manage their farm in a more sustainable way, reality is that the farmer is also using hardware on the farm that has been installed 10-15 years ago. In the current climate of higher costs and interest rates, these old systems are likely to remain on farm for the years to come. *"As long as it works well, we don't have to replace it".* Even though the industry is working step by step on integrating the ICAR ADE standards, making it much easier to exchange data, many older systems don't have these standards in place.

Challenge for all providing new opportunities to the farmers is to connect to these on farm systems. Connecting would make it possible to provide even more powerful tools that combine both the data from on farm hardware, like milk meters, and new sensors that are completely working in the cloud. A second, important, challenge is to make sure data entry doesn't have to happen in two places, as this will reduce acceptance of new tools. Third are the advisors of the farmer who help the farmers in analysing data, to have a complete understanding of the farm they need to be able to see the complete set of data.

During this session the aim is to provide context to the current technological environment of the modern dairy farmer and take a look at developments of the future. Illustrated with examples of how farmers using herd management software are connecting multiple brands of sensors, both old and new and share this data with the advisors around them, including:

- Linking on farm automation.
- Interface techniques.
- Legacy systems examples.
- On farm interfaces divided by age, type and number of interfaces per farm.
- Modern and old technologies combined.

A tool to identify cows eligible for Selective Dry Cow Therapy (SDCT)

M. Fioretti[1], L. Pascarella[1,2], C. Melilli[1], F. Luisi[1,2] and R. Negrini[1,2]

[1]Associazione Italiana Allevatori, via XXIV Maggio 44, Rome, Italy
[2]Dipartimento di Scienze animali, della nutrizione e degli alimenti, Università Cattolica del Sacro Cuore, Via E. Parmense, 29122 Piacenza, Italy

Introduction

Bovine mastitis is an inflammatory condition affecting the udder tissue of the mammary gland, typically triggered by physical trauma or infections from microorganisms. It is the most common disease in the dairy industry, leading to significant economic losses due to reduced milk yield and poor milk quality (Gomes and Henriques, 2016). On average, the total economic loss attributed to bovine mastitis is estimated at $147 per cow annually, primarily due to decreased milk production and culling. This represents 11% to 18% of the gross margin per cow each year (Hogeveen *et al*., 2019). Damage to mammary tissue, resulting in decreased milk production, accounts for approximately 70% of these total losses (Zhao and Lacasse, 2008).

Bovine mastitis can be classified into three categories based on the degree of inflammation: clinical, sub-clinical, and chronic. Clinical bovine mastitis is easily detectable through visible abnormalities such as a red and swollen udder, and fever in the affected cow. The milk appears watery with flakes and clots. Clinical mastitis can be further subdivided into per-acute, acute, and sub-acute, depending on the severity of inflammation. Severe cases of clinical mastitis can be fatal. In contrast, sub-clinical mastitis shows no visible abnormalities in the udder or milk, but milk production decreases with an increase in the somatic cell count (SCC).

Mastitis significantly impacts the profitability of dairy farms. The major economic effects of mastitis in dairy cattle breeding include:

1. **Decreased milk production**: Mastitis reduces milk yield in infected cows, affecting the overall profit potential of the dairy operation.
2. **Loss from discarded milk**: Milk discarded during treatment or deemed unsuitable for human consumption (with SCC >200,000 cells/ml) represents a significant loss. A cow is not profitable unless she produces saleable milk.
3. **Veterinary fees and drug costs**: Expenses for mastitis diagnosis and treatment.
4. **Additional labour demand and related costs**: Managing infected cows, including veterinary treatment and health monitoring, increases labour costs, which can be challenging, especially when labour is scarce or expensive.
5. **Increased risk of culling and cow mortality**: Decisions to cull cows due to mastitis can substantially impact the dairy's economic performance, with premature culling leading to significant losses.

The negative effects of mastitis underscore the importance and necessity of effective management and prevention strategies. The risk of mastitis onset is not constant throughout the productive life of a dairy cow. The dry-off period is a critical time due to a decrease in immune defences (Schukken *et al*., 2011). At the beginning of this period, the absence of physical barriers, such as keratin plugs at the nipple sphincter, allows mastitogenic agents to enter (Schukken *et al*., 2011). According to Green *et al*. (2002), 50% of environmental mastitis cases in the first 100 days of lactation originate from infections contracted during the dry period. Consequently, significant efforts are made during this period to prevent mastitis infections.

Treatments against mastitis during dry-off

The traditional approach to preventing mastitis during the dry-off period has been blanket dry cow therapy (BDCT). This veterinary protocol involves two main criteria for dried-off cows:

1. treating all udder quarters with antibiotics to eradicate existing infections at the time of dry-off and prevent new infections during the dry-off period, and
2. using an external or internal sealant for nipples to prevent pathogen entry into the udder.

BDCT is a prophylactic approach recommended to reduce intramammary infections, decrease the prevalence of contagious pathogens, and contribute to the overall reduction in bulk tank somatic cell count (SCC). While this approach ensures a robust risk protection, it requires high antibiotic usage and associated costs, contributing to microbial antibiotic resistance to both animals and humans.

The growing demand for responsible antimicrobial use and cost reduction for farmers promoted an alternative approach known as selective dry cow therapy (SDCT). This method consists in treating individual cows based on a risk factor analysis and administering antibiotics to cows showing infection symptoms at dry-off only. The correct application of the SDCT protocol reduces antibiotic by 21-60% without compromising health status in the subsequent lactation (Zecconi *et al*., 2020; Cameron *et al*., 2014; Kabera *et al*., 2020; Rowe *et al*., 2020a, 2020b). In 2019, the European Union approved the Prohibition of Antibiotics for Prophylaxis (EU Reg. 2019/6), officially replacing BDCT with SDCT.

SDCT relies heavily on a thorough assessment of mastitis risk factors at the individual level, primarily based on somatic cell counts and, more recently, on differential somatic cell counts, both produced routinely by DHI milk analysis

To promote the adoption of the SDCT approach in Italy, the Italian Breeders Association (A.I.A.), the official Dairy Herd Improvement Association (DHIA), developed a tool called “Report Asciutta Selettiva” (Selective Dry-off Report). This tool is designed to assist farmers and veterinarians in accurately identifying cows eligible for SDCT therapy.

The tool complies with National Veterinary Official Protocols and allows users to input a set of parameters and thresholds, enabling the algorithms to be customize to specific needs and circumstances.

How the tool works

The tool implies two steps:

1. listing candidate lactating cows for drying-off (e.g., pregnant or low production cows), and
2. applying protocols based on SCC and other information to select cows for treatment based on their specific risk.

Step 1. Use DHI data to list candidate lactating cows to be dried off

Farmers can access A.I.A.'s performance recording data (both current and historical) through the dedicated software Si@lleva, which includes the selective dry cow therapy (SDCT) report. This tool enables farmers to select a specific date, either the current day or a future date, to list the candidate cows candidate for SDCT treatment based on two independent eligibility criteria

Pregnancy status

Among the lactating cows those with a positive pregnancy diagnosis are selected and their conception date is calculated to predict:

1. The expected calving date as "Conception date plus 283 days" where 283 days is the average gestation length in Italy.
2. The expected dry-off date as "Expected calving date minus Average farm dry-off period" in days, where the average farm dry-off period is selected by the farmer.
3. The number of days between the current date and the expected dry-off date.

If the number of days between the current date and the expected dry-off date is less than the average farm dry-off period, the cow is eligible for drying off. Otherwise, the cow is not eligible (Figure 1).

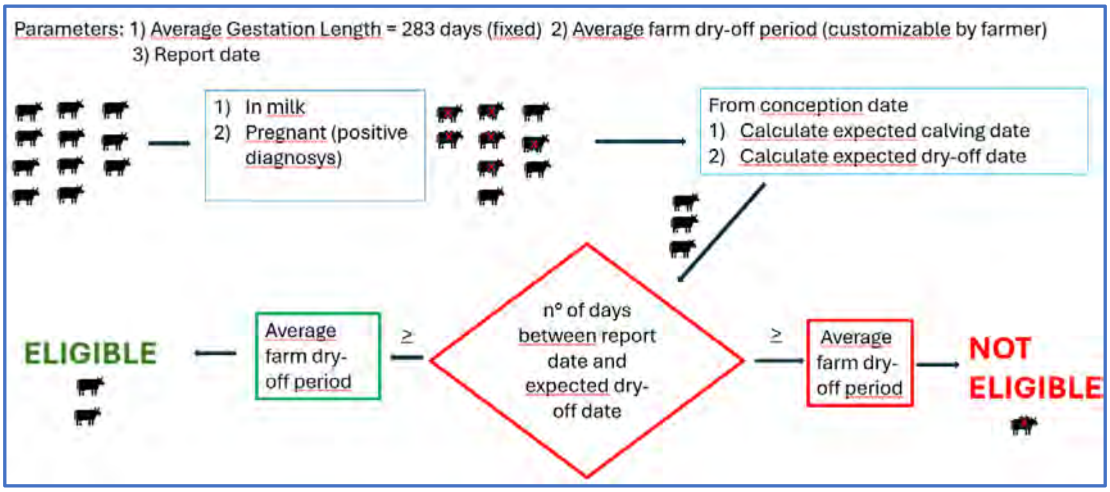


Figure 1. Identification process of lactating cows eligible for dry-off basing on pregnancy status

Daily milk yield individual production

The second criterion (Figure 2) for identifying cows eligible for dry-off is low milk yield. Cows no longer profitable for their milk production should be dried off. In this step, two main parameters must be set by the farmer:

1. the threshold milk yield (m) below which the cow is no longer profitable (for Italian Friesian, the default is 14 kg), and
2. the stage of the lactation curve to monitor is specified as the number of test days (n) after which monitoring begins (default setting:4 test days).

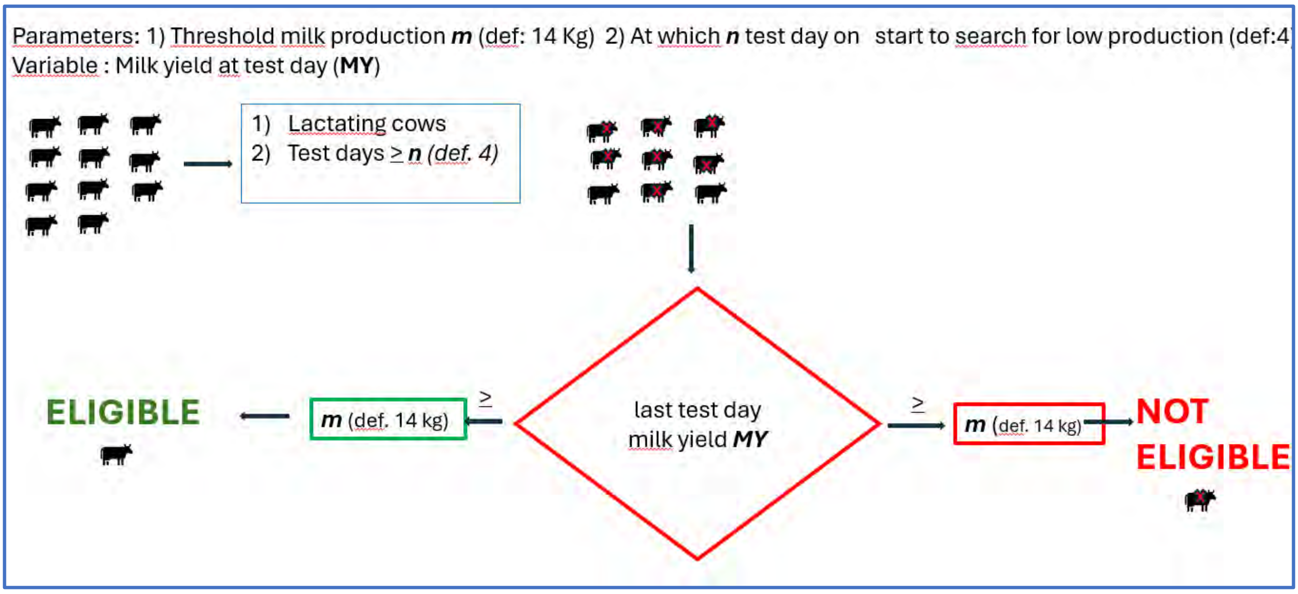


Figure 2. Identification process of lactating cows eligible for dry-off basing based on milk yield.

If , during the monitored lactation period the MY is less than the threshold value, the cow is eligible for dry-off. Farmers can modify the parameters n and m to set different productivity thresholds at various stages of lactation.

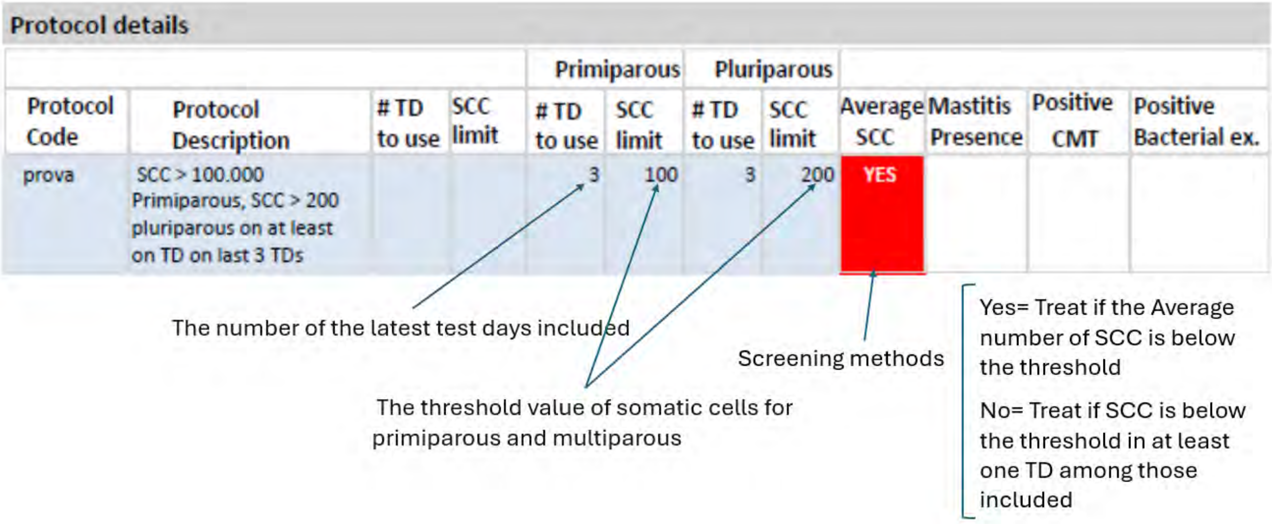
Step 2. Elicit the cows to be treated among the selected

To select cows to be treated with SDCT among those eligible for dry-off (step 1) we combine data from DHI with all other available information on mastitis such as the California Mastitis Test or antimicrobial scans. More in detail, if DHI data only are available, cows will be treated if:

1. the average somatic cell count calculated on a fixed number of sequential Test Day data is above the threshold
2. the value of somatic cell count is above the threshold in at least one among a fixed number of sequential test days

Both the number of sequential test days and somatic cell count threshold can be user-modifiable

One the Eligible criteria and the protocols parameters are set, the system generate the comprehensive report in Figure 4.



Protocol details

				Primiparous		Pluriparous					
Protocol Code	Protocol Description	# TD to use	SCC limit	# TD to use	SCC limit	# TD to use	SCC limit	Average SCC	Mastitis Presence	Positive CMT	Positive Bacterial ex.
prova	SCC > 100.000 Primiparous, SCC > 200 pluriparous on at least on TD on last 3 TDs			3	100	3	200	YES			

The number of the latest test days included

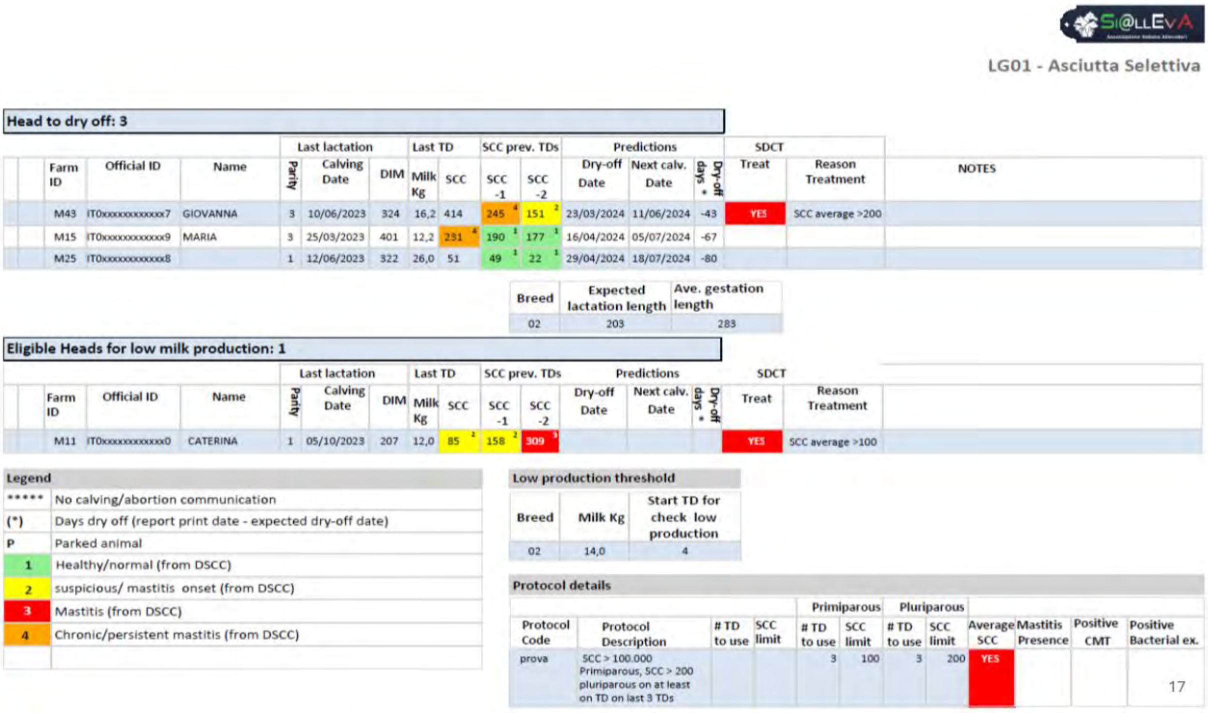
The threshold value of somatic cells for primiparous and multiparous

Screening methods

Yes= Treat if the Average number of SCC is below the threshold

No= Treat if SCC is below the threshold in at least one TD among those included

Figure 3. Example of a customized setting.



LG01 - Asciutta Selettiva

Head to dry off: 3

			Last lactation			Last TD		SCC prev. TDs		Predictions			SDCT		
Farm ID	Official ID	Name	Parity	Calving Date	DIM	Milk Kg	SCC	SCC -1	SCC -2	Dry-off Date	Next calv. Date	Dry-off days *	Treat	Reason Treatment	NOTES
M43	IT0xxxxxxxxxxx7	GIOVANNA	3	10/06/2023	324	16,2	414	245 [4]	151 [2]	23/03/2024	11/06/2024	-43	YES	SCC average >200	
M15	IT0xxxxxxxxxxx9	MARIA	3	25/03/2023	401	12,2	231 [4]	190 [1]	177 [1]	16/04/2024	05/07/2024	-67			
M25	IT0xxxxxxxxxxx8		1	12/06/2023	322	26,0	51	49 [1]	22 [1]	29/04/2024	18/07/2024	-80			

Breed	Expected lactation length	Ave. gestation length
02	203	283

Eligible Heads for low milk production: 1

			Last lactation			Last TD		SCC prev. TDs		Predictions			SDCT	
Farm ID	Official ID	Name	Parity	Calving Date	DIM	Milk Kg	SCC	SCC -1	SCC -2	Dry-off Date	Next calv. Date	Dry-off days *	Treat	Reason Treatment
M11	IT0xxxxxxxxxxx0	CATERINA	1	05/10/2023	207	12,0	85 [2]	158 [2]	309 [3]				YES	SCC average >100

Legend	
*****	No calving/abortion communication
(*)	Days dry off (report print date - expected dry-off date)
P	Parked animal
1	Healthy/normal (from DSCC)
2	suspicious/ mastitis onset (from DSCC)
3	Mastitis (from DSCC)
4	Chronic/persistent mastitis (from DSCC)

Low production threshold

Breed	Milk Kg	Start TD for check low production
02	14,0	4

Protocol details

				Primiparous		Pluriparous					
Protocol Code	Protocol Description	# TD to use	SCC limit	# TD to use	SCC limit	# TD to use	SCC limit	Average SCC	Mastitis Presence	Positive CMT	Positive Bacterial ex.
prova	SCC > 100.000 Primiparous, SCC > 200 pluriparous on at least on TD on last 3 TDs			3	100	3	200	YES			17

Figure 4. Based on one the eligible criteria and the protocols parameters are set, the system generate the comprehensive report.

Adoption of the tool in Italy

The tool is freely available to all dairy farmers enrolled in A.I.A.'s official milk recording activity through the proprietary software Si@lleva. Each recorded farm can access all the information collected during milk recording up to the last test day.

Currently, about 14,000 farms are enrolled in the dairy milk performance recording activity. Of these, more than 500 farms (approximately 4% of the total recorded herds) are using this tool, involving about 7% of the total recorded dairy cows in Italy. The regional distribution of herds using this tool is presented in Figure 5.

Figure 5. Regional distribution of herds using the tool.

Conclusions

The tool is freely available to all herds participating in A.I.A.'s milk performance recording program. It complies with National Veterinary Official Protocols and allows the setting of parameters and thresholds, according to specific needs and conditions. Its availability promotes the adoption of the Selective Dry Cow Therapy (SDCT) in Italy, helping farmers and veterinarians accurately identify cows at lower risk of mastitis and reduce unnecessary antibiotic treatments. Of course, Veterinarians are the only professionals authorized to prescribe medical treatments,

By incorporating additional diagnostic tools such as the California Mastitis Test (CMT) or PCR analysis, the accuracy of mastitis risk assessments and treatment decisions is further enhanced. This improvement will help promote best practices based on more precise diagnostic data still not very common.

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Association of individual cow milk fatty acid proportion and variance with milk production

M.J. Caputo[1], A.M. Miles[2], J. Mattison[3], S.J. Sievert[3], Xiao-Lin Wu[1,4], R.L. Baldwin VI[2], J. Burchard[1] and J. Dürr[1]

[1]Council on Dairy Cattle Breeding, Bowie, MD, USA
[2]USDA Animal Genomics and Improvement Laboratory, Beltsville, MD, USA
[3]National Dairy Herd Information Association, Fitchburg, WI, USA
[4]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, USA
Corresponding Author: malia.caputo@uscdcb.com

Abstract

Milk fatty acid (FA) fractions, de novo (DN), mixed, and preformed (PF), are grouped based on chain length, as < C16:0, C16:0, and > C16:0, respectively. These groups reflect the origin of milk FA synthesis, with DN representing FA synthesis within the mammary gland, PF representing FA coming from the diet or body tissue reserves and mixed representing all three sources. Understanding the associations of milk FA groups with milk and component yields at the individual cow level may provide insight into making management and dietary decisions. To investigate these associations, milk samples (n = 14,091) were collected during the morning milkings from 1,737 Holstein cows from a herd milking 3x daily and averaging 41 kgs milk/cow. Milk samples were analyzed for FA groups (g/100g fat), fat, true protein, and lactose. Time periods of the first test (FT; 30 ± 2 DIM), peak milk (PT; 68 ± 31 DIM), and mid-lactation (MT; 100 ± 2 DIM) were selected. The variance of the FA groups was calculated for each animal as the variance of FA proportion between tests days within the first 305 DIM. Linear models were fit with FA group (proportion or variance), parity (1 vs $\geq$ 2), their interaction, and DIM (FA proportion models only) as the fixed effects and the month of sampling (FA proportion models) or month of calving (variance models) as the random effect. Across all periods, PF was positively associated with test day milk yield and cumulative milk yield through 305 DIM. In contrast, DN was negatively associated with test day milk yield and cumulative milk yield through 305 DIM across all periods ($P < 0.1$). Interestingly, increased variation in DN within cows across the first 305 DIM was positively associated with cumulative milk yields through 305 DIM ($P< 0.01$). The relationship between FA and component yields differed among the periods. Energy-corrected milk yield had a significantly negative association with DN at FT ($P = 0.04$) and a highly significantly positive association with DN at MT ($P < 0.01$). Still, it was not significantly associated with DN at PT ($P = 0.24$). The fat yield was negatively associated with DN at FT but was positively associated with DN at PT and MT ($P \leq 0.01$). In contrast, fat yield was positively associated with PF at FT and negatively associated with PF at PT and MT ($P \leq 0.03$). Protein yield was positively associated with DN for multiparous cows, negatively associated with DN for primiparous cows at FT2 ($P < 0.01$), and positively associated with DN for all parities at MT ($P < 0.01$). In contrast, protein yield was negatively associated with PF for multiparous cows, positively associated with PF for primiparous cows at FT ($P = 0.02$), and not significantly associated with PF at PT and MT ($P > 0.1$). The association of milk FA groups with milk and component yields suggests that milk FA groups may be a useful management tool for making pen grouping decisions, cow selection and breeding decisions, and informing dietary adjustments. However, the variable associations between FA groups and milk performance outcomes by parity and at different lactation stages highlight the importance of considering these factors when making decisions based on a single milk test. Routine milk testing across lactation may allow for tailored management decisions at the individual cow level using these FA groups.

Keywords: de novo, preformed, milk analysis.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

Of the 9.4 million dairy cows in the United States, 41.9% are on routine Dairy Herd Improvement (**DHI**) testing (CDCB, 2024). When deciding to participate in DHI testing, the producer must consider not only the cost of testing, but also the value of the data they receive. Routine DHI testing typically provides milk yield and major component (fat, protein, lactose, SCC) information back to the producer, but there is considerably more information that can be extracted from the same milk sample using the existing technology. Of these additional traits, milk fatty acid origin groups are already available on most milk analysis instruments and have been of recent interest (Dorea and Armentano, 2017; Woolpert *et al*., 2017; 2018). Milk fatty acid origin groups, *de novo*, mixed, and preformed, are grouped based on chain length, as < C16:0, C16:0, and > C16:0, respectively. These groups reflect the origin of milk fatty acid synthesis. De novo represents fatty acid synthesis within the mammary gland, preformed represents fatty acids coming from the diet or body tissue reserves, and mixed represents all three sources. At the herd level using bulk tank milk, these fatty acid groups can be informative when making dietary and management decisions (Dorea and Armentano, 2017; Woolpert *et al*., 2017; 2018). However, less is understood about these fatty acids at the individual cow level. Understanding the associations of milk fatty acid groups with milk and component yields at the individual cow level may provide insight into making management and dietary decisions. Therefore, our objective was to identify the association of milk de novo and preformed fatty acids with milk and milk component production at different stages of lactation and investigate within-cow variability of fatty acids across lactation.

Materials and methods

Data collection

Data were collected from a privately-owned Holstein dairy in New York, USA. Milk samples were collected for 18 weeks between May and September of 2023 from all three milkings within the day. Milk samples were analysed on the same Milkoscan 7 RM instrument for milk fat, true protein, lactose, de novo fatty acids, mixed fatty acids, and preformed fatty acids (FOSS Analytical, Hilleroed, Denmark). Milk yields were recorded electronically using electronic meters (SmartControl meter, BouMatic).

Data editing

The raw dataset comprised of 63,312 milk samples from 2,687 cows. Records were removed when > 305 DIM or < 2 DIM, component records were > 3 standard deviations from the mean within milking session, and when the daily record of 3 milk yields and component values were incomplete. The cleaned dataset consisted of 42,273 records representing 14,091 daily records from 1,747 cows.

Milk fatty acids (g/100g milk) were calculated on a fat basis as:

$$\frac{fatty\ acid\ (g/\ 100g\ milk)}{milk\ fat\% \times 0.95} \times 100 = fatty\ acid\ (g/\ 100g\ fat) \qquad (1)$$

effectively calculating the milk fatty acid groups as a proportion of milk fat.

In order to investigate the associations at specific stages of lactation, the data were categorized as first test (**FT**; 30 ± 2 DIM), peak milk (**PT**; 68 ± 31 DIM), and mid-lactation (**MT**; 100 ± 2 DIM). Peak test represents the milk test nearest in time to the individual cow's actual peak milk production. The first morning milk sample was used for fatty acid proportions to better reflect normal DHI test day data.

Statistical analysis

Data analysis was conducted using the lmer package of R 4.3.1 (R Core Team, 2024). For each production outcome, the model contained the fixed effects of fatty acid proportion (de novo, mixed, or preformed), parity (primiparous or multiparous), their interaction, day in milk, and the random effect of month of sampling.

Individual cow variation in fatty acid proportion across lactation was calculated in two steps. First, a Wilmink curve was fit for each cow using all fatty acid data and the deviance was determined as the difference between the observed and predicted values. Second, the variance was calculated as the log variance of the deviance values. For each production outcome, the mixed model contained the fixed effects of fatty acid variance, parity, their interaction, mean fatty acid proportion, and the random effect of month-year of calving.

Results

Overall, mean (SD) lactation number was 2.4 (1.4), representing cows from 1 to 9 lactations.

Mean milk production was 47.0 kg (9.2 kg), 50.4 kg (9.3 kg), and 46.4 kg (8.7 kg) at FT, PT, and MT, respectively (Table 1). Mean 305-day cumulative milk yield was 13,177 kg (2,041 kg). Proportion of de novo fatty acids was lowest at FT and greatest at MT, whereas proportion of preformed fatty acids was greatest at FT and lowest at MT (Table 1).

De novo fatty acids were significantly associated with milk and component yields across all periods. At FT, there was a significant interaction of de novo fatty acids and parity on test day and 305-day cumulative milk yields, in which the association

Table 1. Descriptive statistics of production performance and fatty acids (FA) at the selected lactation stages: first test (30 ± 2 DIM), peak test (68 ± 31 DIM), and mid test (100 ± 2 DIM).

	First Test		Peak Test		Mid Test	
Variable[1]	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
De novo FA, % of fat	25.4	2.5	26.5	2.0	27.2	1.8
Mixed FA, % of fat	37.7	2.9	39.5	2.4	40.9	2.3
Preformed FA, % of fat	37.0	4.8	34.0	4.0	32.0	3.4
Test day yield, kg	47.0	9.2	50.4	9.3	46.4	8.7
Test day fat yield, kg	1.9	0.4	2.0	0.4	1.8	0.3
Test day ECM, kg	50.4	9.2	53.1	8.5	49.4	8.0

[1] Energy Corrected Milk = Yield × 0.3237 + Fat × 12.95 + Protein × 7.65.

was negative for both parties but more negative for primiparous animals ($P \leq 0.01$; Table 2). Test day and cumulative milk yields were negatively associated with de novo fatty acids at PT and were negatively associated and tended to be negatively associated with de novo fatty acids at MT, respectively ($P \leq 0.06$). Association of de novo fatty acids with component yields changed based on the DIM period. Milk fat yield and de novo fatty acids were negatively associated at FT ($P \leq 0.01$), not associated at PT ($P = 0.68$), and positively associated at MT ($P \leq 0.01$). Energy corrected milk yield followed similar patterns, although there was a significant interaction of de novo fatty acids and parity at FT, wherein ECM was negatively associated for both parities but more negative for primiparous animals. There was a significant interaction of de novo fatty acids and parity on milk protein yield at FT, in which multiparous animals had a positive association and primiparous had a negative association with yields of milk protein ($P \leq 0.01$). Later in lactation at MT, there was a positive association for all parities with de novo fatty acids and milk protein yield.

Preformed fatty acids were also significantly associated with milk and component yields across all periods. Generally, the opposite association was detected for preformed fatty acids compared with de novo fatty acids. Test day and 305-day cumulative milk yields were positively associated with preformed fatty acids across all three periods ($P \leq 0.01$). Yields of ECM were positively associated with preformed fatty acids at FT and PT but tended to be negatively associated at MT ($P \leq 0.07$). Milk fat yields were positively associated with preformed fatty acids at FT ($P \leq 0.01$) and negatively associated with preformed fatty acids at MT ($P \leq 0.01$), but an interaction between preformed fatty acids and parity was detected at PT ($P = 0.03$). The interaction effect indicates a positive association for multiparous cows and a negative association for primiparous cows at MT for fat yield. Different associations by parity were also observed for milk protein yield at FT, wherein yields of milk protein were negatively associated with multiparous cows and positively associated with primiparous cows for preformed fatty acids ($P = 0.02$). At PT and MT, milk protein yield was not associated with preformed fatty acids ($P > 0.1$).

Table 2. Least square means and standard errors of production performance for de novo fatty acids (FA) at the selected lactation stages: first test (30 ± 2 DIM), peak test (68 ± 31 DIM), and mid test (100 ± 2 DIM).

	FA		Parity		Interaction		P-value		
Yield[1], kg	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**FA**	**Parity**	**Int.**
First Test									
Milk	-0.39	0.14	1.19	6.76	-0.68	0.28	0.01	0.86	0.01
Fat	-0.03	0.01	-0.54	0.03			<0.01	<0.01	
Protein	0.009	0.004	0.23	0.18	-0.02	0.01	0.02	0.22	<0.01
ECM	-0.31	0.15	2.71	7.05	-0.71	0.29	0.04	0.70	0.01
305-day	-113	45	2682	2024	-234	82	0.01	0.19	<0.01
Peak Test									
Milk	-0.53	0.14	-14.72	0.62			<0.01	<0.01	
Fat	-0.002	0.01	-0.36	0.03			0.68	<0.01	
Protein	0.004	0.004	-0.33	0.02			0.27	<0.01	
ECM	-0.16	0.14	-11.92	0.67			0.24	<0.01	
305-day	-151	46	-3167	222			<0.01	<0.01	
Mid Test									
Milk	-0.39	0.18	-11.67	0.65			0.03	<0.01	
Fat	0.04	0.01	-0.30	0.02			<0.01	<0.01	
Protein	0.012	0.005	-0.28	0.02			0.01	<0.01	
ECM	0.44	0.16	-9.90	0.58			0.01	<0.01	
305-day	-128	67	-2817	234			0.06	<0.01	

[1] Energy Corrected Milk = Yield × 0.3237 + Fat × 12.95 + Protein × 7.65

Table 3. Least square means and standard errors of production performance for preformed fatty acids (FA) at the selected lactation stages: first test (30 ± 2 DIM), peak test (68 ± 31 DIM), and mid test (100 2 DIM).

	FA		Parity		Interaction		P-value		
Yield[1], kg	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**FA**	**Parity**	**Int.**
First Test									
Milk	0.36	0.06	-15.43	0.60			<0.01	<0.01	
Fat	0.01	0.003	-0.52	0.03			<0.01	<0.01	
Protein	-0.002	0.002	-0.73	0.15	0.01	0.004	0.22	<0.01	0.02
ECM	0.26	0.06	-14.62	0.63			<0.01	<0.01	
305-day	81	21	-7024	1622	104	42	<0.01	<0.01	0.01
Peak Test									
Milk	0.40	0.07	-14.83	0.61			<0.01	<0.01	
Fat	0.01	0.003	0.15	0.23	-0.01	0.01	0.06	0.52	0.03
Protein	0.000	0.002	-0.34	0.02			0.81	<0.01	
ECM	0.18	0.07	-12.14	0.66			0.01	<0.01	
305-day	104	23	-3186	215			<0.01	<0.01	
Mid Test									
Milk	0.37	0.09	-12.07	0.64			<0.01	<0.01	
Fat	-0.02	0.003	-0.30	0.02			<0.01	<0.01	
Protein	-0.003	0.002	-0.29	0.02			0.22	<0.01	
ECM	-0.16	0.08	-10.07	0.59			0.07	<0.01	
305-day	105	35	-2881	227			<0.01	<0.01	

[1] Energy Corrected Milk = Yield × 0.3237 + Fat × 12.95 + Protein × 7.65

Table 4. Association of within cow variance in de novo and preformed fatty acids with 28-day and 305-day cumulative milk yields.

Cumulative yield, kg	**Estimate**	**SE**	**P-value**
De novo			
28-day	81.1	54.0	0.14
305-day	1733.4	604.7	<0.01
Preformed			
28-day	-4.0	51.8	0.94
305-day	59.2	613.3	0.92

Conclusions

The proportion of de novo and preformed fatty acid groups exhibited considerable variation amongst cows both within week of lactation but also across lactation (Figure 1). Proportion of de novo fatty acids reached a maximum mean (SD) of 27.2% (1.8%) at 14 weeks of lactation and had a minimum mean of 23.8% (3.0%) at 2 weeks of lactation. Proportion of preformed fatty acids had the opposite relationship, wherein the minimum mean was reached at 16 weeks of lactation (31.5 ± 3.5%) and the maximum mean was achieved at 4 weeks of lactation (38.4 ± 5.2%). Individual cow variation in fatty acids across lactation was high. Variance in individual cow de novo proportion ranged from -0.66 to 0.85 and from -0.24 to 1.43 for preformed fatty acids. Individual cow lactational variance in de novo fatty acids were positively associated with 305-day cumulative milk yields ($P < 0.01$; Table 4). However, individual cow lactational variance in preformed fatty acids was not associated with either 28-day or 305-day cumulative milk yields ($P > 0.1$).

Milk fatty acids groups, de novo and preformed, are strongly associated with milk production outcomes at different stages of lactation. This suggests that these milk fatty

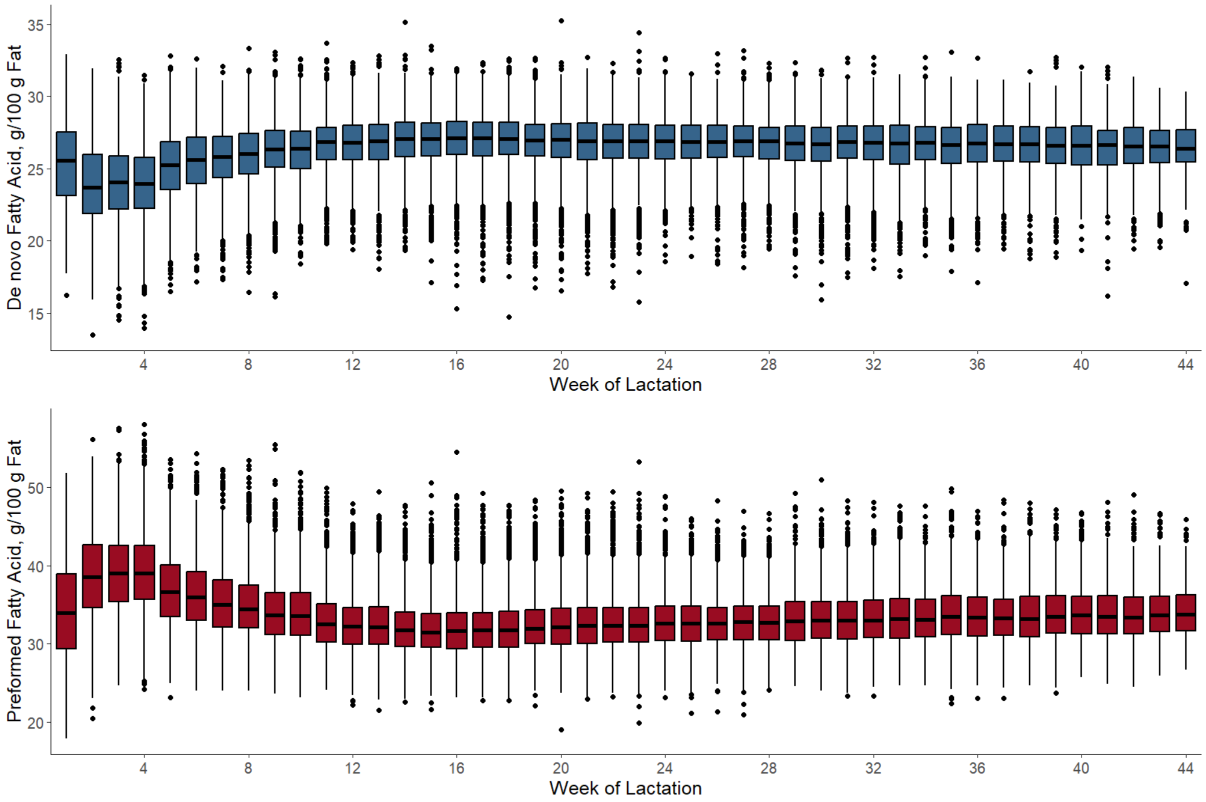

Figure 1. Box plots of de novo (top panel) and preformed (bottom panel) fatty acids of individual cows by week of lactation.

acids may be a useful management tool to aid in making pen grouping decisions, cow selection and breeding decisions, and informing dietary adjustments. Changes in the direction of the association between milk fatty acids and milk components at different lactation stages, as well as interactions with parity, underscores the importance of considering these factors when making management decisions based on a single milk test. A positive association of individual cow lactational variance in de novo fatty acids with 305-day cumulative milk yields is an interesting finding and warrants further investigation to understand the biology behind this association. Combined, the results herein indicate that routine milk testing across lactation may allow for tailored management decisions at the individual cow level using these fatty acid groups.

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Predicting glutamate concentration in milk using mid-infrared spectrometry for routine detection of energy-deficient cows

M. El Jabri[1], A. Leduc[1,2,3], M. Boutinaud[2], F. Le Provost[3], A. Fischer[1], C. Leroux[4], J. Pires[4] and M. Gelé[1]

[1]Institut de l'Elevage, 149 rue de Bercy, 75012, Paris, France
[2]INRAE, Institut Agro Rennes Angers, PEGASE, Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, 35590, Saint-Gilles, France
[3]Université Paris-Saclay, INRAE, AgroParisTech, GABI, Génétique Animale et Biologie Intégrative, 78350, Jouy-en-Josas, France
[4]INRAE, Université Clermont Auvergne, Vetagro Sup, UMRH - Unité Mixte de Recherche sur les Herbivores, 63122, Saint-Genès-Champanelle, France
Corresponding Author: marine.gele@idele.fr

Abstract

Identifying energy-deficient cows is difficult on farm. To calculate their energy balance, it is necessary to know the feed intake of each cow and their energy requirements. An alternative way would use biomarkers of energy deficiency, that are more accessible on farm. Candidate biomarkers have previously been identified as metabolites, proteins, and microRNAs. Among them, glutamate proved to be an interesting biomarker. The present project aimed to predict the concentration of glutamate in milk based on the mid-infrared (MIR) spectra. 577 MIR spectra were available with known glutamate concentration. Of these, 514 data were from a 6-day feed restriction trial conducted on 18 mid-lactating cows. The feed allowance was restricted to 50% of the energy requirements estimated during the previous *ad libitum* week. The 63 remaining data were from 26 cows that calved next autumn. For these cows, the feed restriction was based on a dilution of the diet with straw during mid-lactation the following spring. Two datasets were then created: a calibration and a validation dataset. The calibration dataset included 70% of the data (mean glutamate concentration = 338.7 µM/L; standard deviation (SD) = 177.3 µM/L) and was used to develop the equation using spare partial least squares regression.

The validation dataset included 30% of the data (mean glutamate concentration = 346.0 µM/L; SD = 138.9 µM/L) and was used to apply the equation to calculate its accuracy [coefficient of determination (R^2) and residual standard deviation (Sy,x)]. Milk glutamate concentrations were predicted with a calibration $R^2 = 0.78$ (Sy,x = 82.7 µM/L) and a validation $R^2 = 0.65$ (Sy,x = 82.5 µM/L). This equation is original. Its inclusion in panel of biomarkers paves the way for its use in the detection of energy-deficient dairy cows.

Keywords: dairy cattle, feed restriction, milk metabolite, mid-infrared spectrometry. Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

Dairy cows are very sensitive to negative energy balance. A negative energy balance is the state reached by an animal when the energy provided by its diet is lower than its energy requirements. It can be physiological, such as in early lactation, when requirements are higher than the energy intake, or environmental, when a significant decrease in intake occurs, for example during a feed shortage. Energy deficiency has a negative impact on the profitability of the dairy farm, particularly when it is severe or long-lasting, leading to a drop in milk production, health disorders or reproductive problems.

To prevent or correct energy deficit in dairy cows, we first need to detect it. Breeders detect it through body condition score decrease, but it is usually not sensitive enough for early detection and consequently for early intervention. Blood tests are accurate to diagnose consequences of energy deficit such as ketosis but are invasive. Milk analyses have the advantage of being easy to access but are not specific enough.

In this context, the Biomarq'lait project aimed to identify new biomarkers of energy deficit. A review of the literature (Leduc *et al*., 2021a) has shown that energy deficit has an impact on hormonal regulation, mobilisation of body reserves, mammary gland activity and milk production and composition. Panels of biomarkers, including macro-components, proteins (Leduc *et al*., 2022), microRNAs (Leduc *et al*., 2023), and metabolites (Billa *et al*., 2020; Leduc *et al*., 2021b) have been proposed.

Among milk metabolites, glutamate appears to be a good indicator for several reasons. A rapid decrease in its concentration during dietary restriction, followed by a return to baseline levels during *ad libitum* re-feeding, has been observed (Billa *et al*., 2020, Leduc *et al*., 2021b, Pires *et al*., 2022). In addition, the positive correlation between glutamate concentration and energy balance is very strong (coefficient of correlation = 0.61 according to Billa *et al*., 2020). The response range seems to depend on the intensity of the feed restriction and on the lactation stage. Finally, no difference of glutamate concentration was observed at the beginning of lactation compared to the mid-lactation with ad libitum feeding, suggesting that glutamate could be an indicator of dietary restriction following an ad libitum period (Leduc *et al*., 2021b). Therefore, the objective was to determine the potential of measuring milk glutamate concentrations to detect energy-deficient cows using mid infrared (MIR) spectra already obtained routinely.

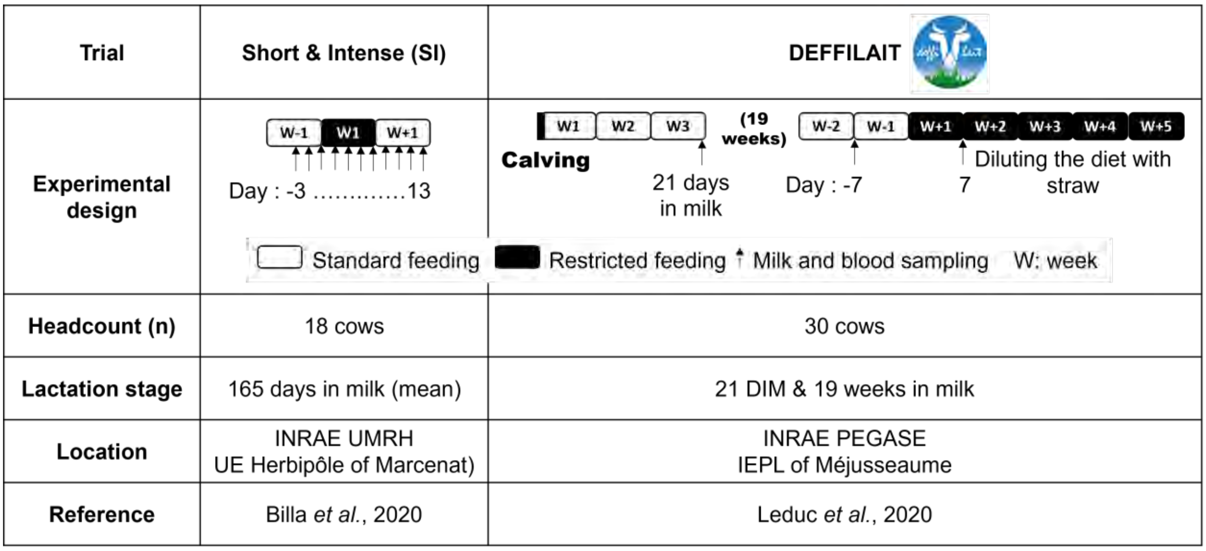
Material and methods

To carry out this study, two feed restriction trials were set up. The glutamate concentration was measured in milk, and MIR spectra of milk were collected. A mathematical model was then developed to predict the glutamate concentration in milk from the MIR spectra.

Data collection

The data used come from two trials set up at INRAE (Figure 1): a short and intense restriction protocol (SI) and a protocol set up in a project called DEFFILAIT.

SI protocol was led in 2016 at the Marcenat experimental farm (INRAE, Herbipôle), on 18 cows in mid-lactation. During pre- and post-restriction, the cows were fed an *ad libitum* ration consisting of maize silage (66% of diet DM), barley straw (8% of diet DM), maize grain (8% of diet DM), soybean meal (17% of diet DM) and minerals and vitamins (1% of diet DM). During the experimental period (W1 in Figure 1), feed intake was limited to 50% of cows estimated energy requirements during the previous 6 days (W-1 in Figure 1). Milk and blood sampling kinetics (one sample per day) were used to



Trial	Short & Intense (SI)	DEFFILAIT
Experimental design	W-1 W1 W+1 Day : -3 13	W1 W2 W3 (19 weeks) W-2 W-1 W+1 W+2 W+3 W+4 W+5 Calving 21 days in milk Day : -7 7 Diluting the diet with straw
Headcount (n)	18 cows	30 cows
Lactation stage	165 days in milk (mean)	21 DIM & 19 weeks in milk
Location	INRAE UMRH UE Herbipôle of Marcenat)	INRAE PEGASE IEPL of Méjusseaume
Reference	Billa *et al.*, 2020	Leduc *et al.*, 2020

▭ Standard feeding ■ Restricted feeding ↑ Milk and blood sampling W: week

Figure 1. Presentation of the experimental protocols.

study responses to restriction and refeeding. The short duration of the feed restriction period meant that each cow could be considered as its own control (Billa *et al*., 2020).

DEFFILAIT protocol was carried out as part of a trial set up during the ANR DEFFILAIT programme at the INRAE experimental farm of the UMR PEGASE (IEPL), on around thirty Holstein cows chosen to be representative of the herd's variability (Fischer *et al*., 2020). These cows calved in autumn 2017 and were fed *ad libitum* during the first few months of lactation with a constant total mixed ration based on maize silage (65% of diet DM), dehydrated alfalfa (18% of diet DM), soybean meal (18% of diet DM) and production concentrate (9% of diet DM). In mid-lactation, all cows underwent a change of diet in March 2018, moving from the control diet to a restricted diet after a week of dietary transition. For the current work, only the 2 weeks prior to feed restriction and the weeks 2 to 5 (week+1 being the transition week) of feed restriction were kept. The restricted ration was diluted in energy and protein with inclusion of straw (11.5% of diet DM) and aimed to reduce milk production by 20% while maintaining *ad libitum* DM intake. Milk and blood samples were taken at 22 ± 1 days of lactation (D21) from 34 cows that calved in autumn 2017, then at D-7 before dietary change and D+7 after the end of the transition week (Leduc *et al*., 2020).

Immediately after milk sampling, the samples were divided into two aliquots. The first was used to analyse milk glutamate concentrations using an enzymatic-fluorometric method (Larsen and *Fernández, 2017). The second was used to perform the MIR spectrum in milk analysis labs (MyLab, Chateaugiron, France for DEFFILAIT trial; Agrolab's, Aurillac, France for SI trial), which was then standardised (Grelet et al., 2015).* Finally, 577 spectra with an associated measurement of milk glutamate were used for this study (Table 1). Of these, 514 came from the SI trial and 63 from the DEFFILAIT trial.

Table 1. Presentation of the data used.

Trial	Number of data	Number of cows	Lactation number	Days in milk
Short and Intense	514	10 Montbéliarde + 8 Holstein cows	2 to 7	114 to 215 days
DEFFILAIT	63	26 Holstein cows	1 to 6	22 to 205 days

Data processing

Two sets of data were randomly generated from all the 577 spectra. We used 70% of the data from each trial (45 data from DEFFILAIT and 361 data from SI) to constitute the calibration set (n = 406). The remaining 30% (n = 171) constituted the validation set. Figure 2 shows that glutamate levels in milk and the trial of origin of the data are homogeneous between the calibration and validation sets.

An equation was developed by sparse partial least square regression on the data from the calibration set to predict the glutamate concentration in milk from MIR spectra. This equation was then applied to the validation set to calculate its performance, described by the coefficient of determination (R^2), the residual standard deviation ($S_{y,x}$), and the ratio between the standard deviation of the calibration set and the residual standard deviation (RPD).

Results and discussion

The predicted glutamate using the developed equation was compared with the measured values of glutamate obtained using the enzymatic-fluorometric method (Figure 3).

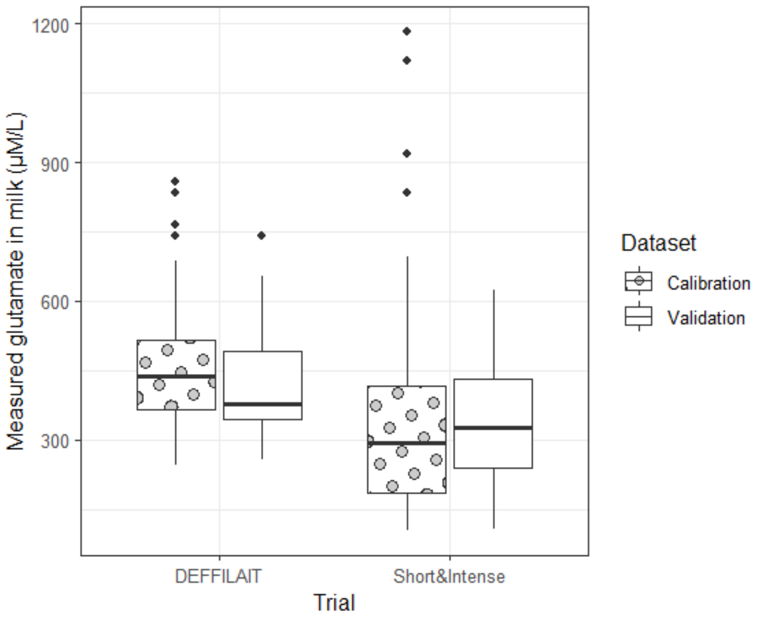


Figure 2. Glutamate levels in milk according to trials in the calibration and validation sets.

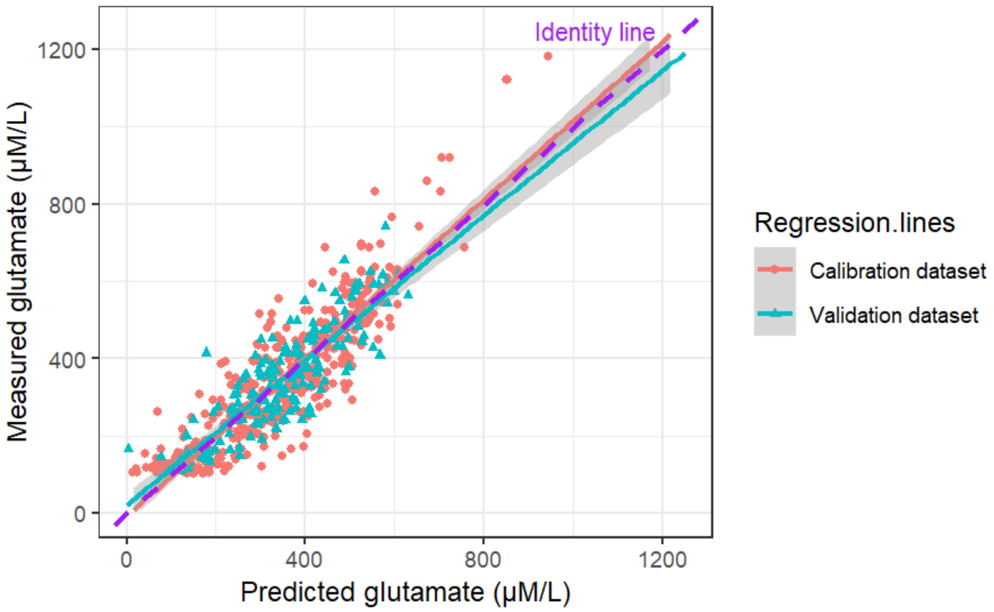


Figure 3. Scatter plot of measured (y-axis) versus predicted (x-axis) values for the glutamate concentration in milk using a partial least squares model built on all 577 milk samples. 1 point = 1 individual milk sample. Circle = data from calibration set; triangle = data from validation set; solid lines = regression lines; dashed line = first bisector.

Table 2. Performances of the milk glutamate prediction equation in the calibration set and validation set.

Dataset	Mean of measured glutamate	SD of measured glutamate	Mean of predicted glutamate	SD of predicted glutamate	SD of Residuals	R²	RPD
Calibration set	338.7	177.6	340.6	152.2	82.7	0.78	2.15
Validation set	346.0	138.9	348.2	123.4	82.5	0.65	1.68

The performances of the milk glutamate prediction equation developed are showed in Table 2. The equation achieved a calibration coefficient of determination (R²) of 0.78 and a residual standard deviation ($S_{y,x}$) of 82.7 µM/L. On the validation set, R² reached 0.65 with a $S_{y,x}$ of 82.5 µM/L. There is no equivalent equation in the literature to compare with our results. However, this equation appears promising.

To detect cows with energy deficits, the energy status of the animals was projected in Figure 4, with a distinction being made between cows in early lactation, cows in mid-lactation with energy deficit and cows in mid-lactation with energy surplus. The low glutamate values (less than 200 µM/L) only concern cows in mid-lactation with an energy deficit, i.e., on feed restriction. Milk glutamate therefore appears to be specific to feed restriction. This finding opens interesting prospects for improving the prevention of energy deficit when advising livestock farmers.

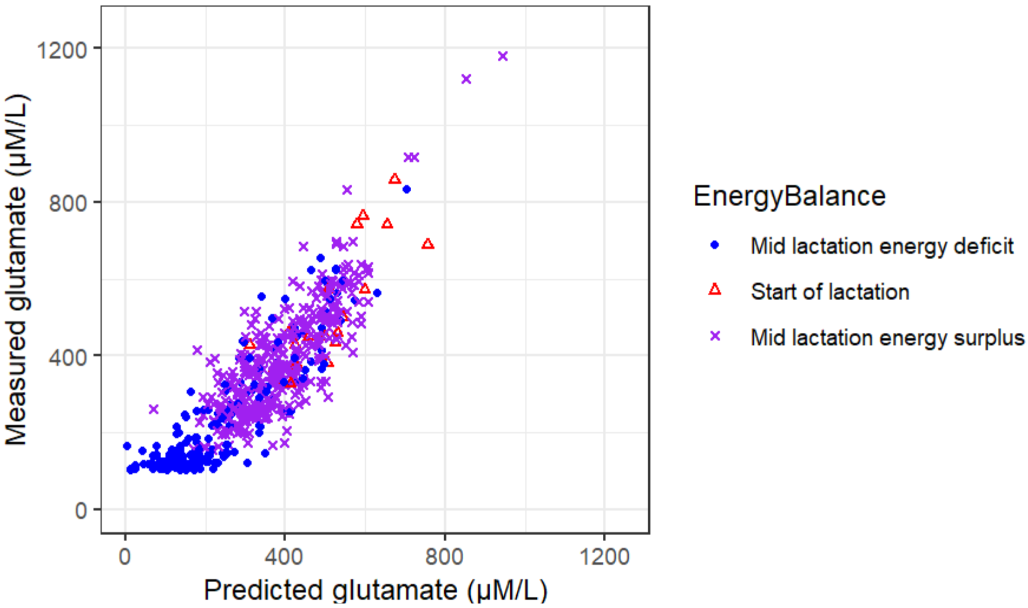


Figure 4. Scatter plot of measured (y-axis) versus predicted (x-axis) values for the glutamate concentration in milk using a partial least squares model built on all 577 milk samples. 1 point 1 individual milk sample. Circle = Mid lactating cows in energy deficit; triangle = early lactating cows; cross = Mid lactation cows without any energy deficit.

To go further, it would be interesting to explore the possibility of predicting the variation in glutamate content to detect situations penalising cows at an early stage, or even the possibility of qualitatively identifying milks with low glutamate concentrations.

Conclusion

Glutamate concentration in milk seems to be an interesting indicator of energy deficit in dairy cows, when it is caused by feed restriction, and can be predicted using MIR spectrometry is possible. However, as the accuracy of the equation is not high enough for routine use, this indicator could be used in combination with other MIR-based indicators to provide more accurate information about the physiological state of the cows.

Acknowledgement

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FeMIR: MIR Spectral predictions for feed and energy efficiency. Practical Application

L. M. Dale[1], A. Werner[1], C. Natterer[1], eMissionCow[2], ReMissionDairy[3], E.J.P. Strang[1], A. Al Baqain[1], K. Drössler[1] and J. Bieger[1]

[1]Regional association for performance testing in livestock breeding of Baden-Wuerttemberg Heinrich-Baumann-Str. 1-3, 70190 Stuttgart, Germany
[2]Association for Bioeconomy Research (FBF), Adenauerallee 174, 53113 Bonn, Germany
[3]Federal Office for Agriculture and Food (BLE), Deichmanns Aue 29, 53179 Bonn, Germany
Corresponding Author: ldale@lkvbw.de

Abstract

Mid-infrared spectrometry (MIR) is a commonly used and cost-effective method for analyzing milk composition. By utilizing infrared light, MIR spectroscopy reveals specific absorption patterns in milk samples, providing detailed information on its chemical constituents. European Milk Recording (EMR) is a key provider of MIR services, offering standardization and predictions through collaboration with former OptiMIR Project - milk recording organizations (MROs). Researchers have shown the potential of MIR in predicting milk components like fatty acids and minerals, as well as biomarkers and complex traits such as ketosis and mastitis. By analyzing fatty acid patterns in milk spectra, MROs can estimate various traits related to animal health and productivity. Recent projects like eMissionCow and ReMissionDairy optimized calibration equations, improved farm management practices and validated the MIR methane (CH_4) equation developed in CRA-Wallonie. The data derived from these equations can help in making informed decisions on feeding practices and climate impact at different levels of animal production. Continuous updates and inclusion of reference data are essential for the advancement of this field in the global context. FeMIR is a new tool developed by LKV Baden-Württemberg for farmers and consultants to improve monitoring of animals' metabolism. The FeMIR report, developed as part of the ReMissionDairy and eMissionCow projects, offers a comprehensive overview of the herd's energy status throughout lactation phases. Over the past two years, the FeMIR report has been successfully tested in practice by four field workers and three consultants. A field test was conducted comparing new parameters such as energy, feed and nitrogen efficiency, and fatty acids (FA). DeNovo and Preform FA were compared with livestock on trial farms. Farm visits were made to different parts of Baden-Württemberg to establish guidelines for these new parameters. The report included MIR spectral data from monthly milk recordings, feed samples, and examinations of animals according to FeMIR. Thresholds and limits for individual parameters were determined to define an optimal framework for farms. The physical condition of animals on site confirmed experts' expectations derived from the report's efficiency and energy parameters. Participants rated the FeMIR report as a valuable tool for feeding and metabolic control of animals.

Keywords: MIR, spectral data, dairy cows, FeMIR, feed efficiency, methabolic control, herd management.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

Climate change presents numerous challenges across all sectors of human activity today. The increasing frequency and duration of extreme weather events, such as droughts, along with the rise in global temperatures, pose a greater risk to farms in terms of water scarcity and animal well-being (Huber and Gulledge, 2011). This is particularly evident in livestock systems, which are impacted by the consequences of global warming (Kuczynski *et al*., 2011) and contribute significantly to greenhouse gas emissions, including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Lesschen *et al*., 2011). In this context, it is crucial to mitigate the impact of climate change on agriculture and animal production, as well as address the contribution of these sectors to the issue. This is essential in ensuring a sustainable food supply for a rapidly expanding global population (Bauer *et al*., 2016).

An essential aspect of achieving these objectives involves utilizing Mid Infrared (MIR) spectral data. Studies have demonstrated the capability of MIR in forecasting milk components such as fatty acids and minerals, along with biomarkers and intricate traits like ketosis and mastitis. Recent initiatives like eMissionCow and ReMissionDairy have been enhancing calibration equations, refining farm management techniques, and validating the MIR methane (CH_4) equation established at The Walloon Agricultural Research Center (CRA-Wallonie) (Dale *et al*., 2023). The information obtained from these equations can assist in making well-informed decisions regarding feeding practices and the environmental impact at various stages of animal production. Regular updates and the incorporation of reference data are crucial for the progress of this field on a global scale. Feed and energy efficiency Mid Infrared (FeMIR) is a novel tool created by the Regional Association for Performance and Quality Inspection in Animal Breeding of Baden Württemberg (LKV BW) to enhance the monitoring of animals' metabolism for farmers and consultants.

The FeMIR report provides a thorough assessment of the herd's energy levels throughout various lactation stages. Field workers and consultants have successfully trialed the FeMIR report over the last two years. The report incorporates Mid Infrared (MIR) spectral data from monthly milk recordings, feed samples, and animal assessments based on FeMIR criteria. Threshold values for specific parameters were established to establish an ideal framework for farms. The actual state of the animals on the premises validated the experts' predictions based on the report's effectiveness and energy metrics. Participants deemed the FeMIR report as a valuable resource for managing the feeding and metabolism of dairy cows. Consequently, ongoing efforts will assist farmers in the Upper Rhine region and beyond in enhancing feed efficiency, lowering greenhouse gas emissions, and mitigating their contribution to climate change.

Material and methods

In recent years, LKV BW has been involved in various international and national projects focused on calibrating different MIR models. One such project was eMissionCow, which aimed to enhance feed intake, feed efficiency, and reduce CH_4 emissions in German cattle populations. Accurate individual animal data from experimental farms played a crucial role in developing the models integrated into the LKV BW FeMIR tool. Approximately 900 cows with standardized MIR spectral data provided information on energy balance (EB), the EB were calculated in accordance with GfE (2001), feed efficiency (FE) calculated as kg ECM/kg dry matter, and energy efficiency (EE) calculated as kg ECM/MJ NEL (Lidineck, *et al.*, 2021) for key German breeds like Holstein, Simmental (Fleckvieh), and Brown Swiss (Braunvieh) across 13 German teaching and experimental farms (Dale *et al*., 2019). Additionally, with the help of the eMissionCow project 20 Simmental cows were studied in climate chambers to enhance the robustness of the CRA-Wallonie model for CH_4 emissions.

Figure 1. Milk analysis using mid-infrared (MIR) spectra.

Another MIR project involving LKV BW was ReMissionDairy, which aimed to leverage modern technologies to develop practical tools for feed management that assist dairy farmers in optimizing production efficiency and reducing their farm's emission impact. Both ReMissionDairy and eMissionCow were complementary initiatives focused on decreasing CH_4 emissions and enhancing feed efficiency. ReMissionDairy was focussed on the individual herd management, while the eMissionCow was focussed on the individual animal.

How does MIR spectral analysis function? It is both straightforward and somewhat intricate. As illustrated in Figure 1, when infrared light is directed at a sample, only specific frequencies are absorbed, resulting in a characteristic spectrum for that substance. The intensity of absorption is influenced by the substance's concentration in the sample. A significant development from the OptiMIR project is the capability of MIR spectrometers to not only analyse primary components but also fatty acids, minerals, lactoferrin, beta-hydroxybutyric acid (BHB), acetone, and citrates. Additionally, complex attributes can be identified. For instance, models for ketosis, energy balances, and CH_4 emissions were established as part of various projects.

The data utilized for modeling was prepared by TiDa (Tier und Daten GmbH) as part of the OptiKuh, OptiKuh2, and eMissionCow projects. This data now serves as the foundation for calculating the FeMIR parameters. Through collaboration with OptiMIR/EMR, the Methagene group, and the European project GplusE at CRA-Wallonie, a methane equation incorporating SF6 and climate chamber measurements was developed. This equation was made accessible not only to EMR members but also to the German Association for Performance and Quality Testing (DLQ) through the eMissionCow project. Within this project, the methane model was expanded to include climate chamber measurements on Simmental cows. The inclusion of the southern breed Simmental and various feed rations enhanced the data variability and strengthened the equation's reliability.

The data used for statistical analyses and machine learning consisted of spectral data from Bentley FTIR analysers, which had been standardized using the European Milk Recording (EMR) and CRA-Wallonie procedures (*Grelet et al., 2014*). The first derivative was computed from the absorbance values of the spectra, and 212 relevant wave numbers were selected. New parameters have been predicted with the help of the spectral models such as energy balance (EB-NEL), energy, feed, and nitrogen efficiency (EE, FE and NE - calculated as ratio of the amount of milk protein produced to the amount of crude protein consumed), and fatty acids (DeNovo and Preform FA). Different packages in the "R" statistical analysis software were employed to validate the models. Subsequently, the data was utilized to provide guidance to farmers at both herd and individual levels.

Results and discussion

The LKV BW identified a growing necessity to enhance herd management concerning energy provision by incorporating additional MIR parameters. The FeMIR report emphasizes the energy balance within the herd, integrating various MIR parameters such as EB-NEL, energy efficiency, and feed efficiency from collaborations with DLQ, the OptiKUH Consortium, and LKV BW. Additionally predictions for fatty acids from the OptiMIR/RobustMilk equation, methane emission per day from the MethaMIR equation developed by CRA-Wallonie, and nitrogen efficiency utilizing forage analysis and milk recording data were included (see Figure 2).

The report presents fatty acids as a percentage of total fat (100%) rather than in relation to their content in milk, as per Barbano *et al*. (2019). DeNovo fatty acids are directly linked to rumen functionality and were therefore chosen for visualization in the FeMIR tool. In addition to utilizing and interpreting the report, specific limits for each parameter were identified and established to define an optimal framework for farm management (see Table 1).

Understanding the factors at play, it is important to note that the breakdown of plant cell walls in the rumen primarily produces acetic acid and butyric acid. These acids travel to the udder through the bloodstream, where the mammary gland converts acetic acid into short and medium-chain fatty acids, indicating the conversion of feed protein into milk protein. Low values may suggest low feed intake, excessive crude fat content, poor digestibility of cell walls, or inadequate rumen fermentation. Preform

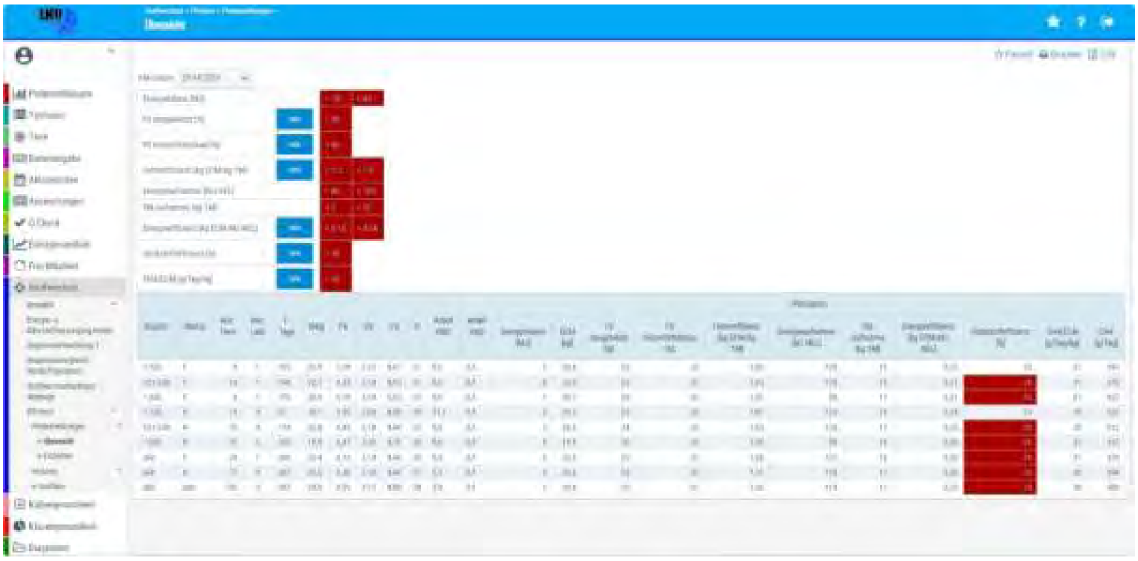

Figure 2. FeMIR - MIR parameters that indicate the energy condition of the entire herd..

fatty acids provide insights into the conversion of fatty acids broken down from body tissues. Elevated values indicate a high breakdown of pre-formed (long-chain) fatty acids from body fat.

Table 1. Optimal ranges of values for herd management.

Parameter	Low Value	High Value
EB [MJ NEL]	< -30	> 40
FA Denovo [%]	< 20	
FA Preform [%]		> 50
FE [kg ECM/kg DM]	< 1.2	> 1.8
EI [MJ NEL]	< 80	> 180
DMI [kg DM]	< 5	> 25
EE [kg ECM/MJ NEL]	< 0.12	> 0.36
NE [%]	< 30	
CH4/ECM [g/day/kg]	< 10	

x Achse: Laktag

Parameter: Futtereffizienz [kg ECM/kg TM]

PM_Datum: 29.04.2024

Lebensnummer	SNR	Name	Rasse	Lakt	L-Tage	ECM	Status	Laktationstage	Futtereffizienz [kg ECM/kg TM]
DE 08 755	201		SBT	4	48	32,9	K	48	1,90
DE 08 599	226		FL	3	49	32,3	K	49	1,81
DE 08 594	228		FL	3	62	29,6	K	62	1,74
DE 08 803	208		SBT	3	87	35,7	K	87	1,73
DE 08 064	135		BV	7	60	30,5	K	60	1,73

Figure 3. FeMIR - MIR parameters that reflect the energy status of the cow or herd.

As part of the ReMissionDairy project, the FeMIR tool was developed for use by farmers and consultants. The FeMIR report shows cases with milk MIR parameters that indicate the energy status of either the individual cow or the entire herd. The variation of the values are larger on individual cow level, than on herd level.

The DeNovo and Preform fatty acids are presented alongside results from milk recording data, including energy corrected milk (ECM), fat (F%), protein (E%), lactose (L%), urea (H), NEL energy balance, feed efficiency, energy efficiency, as well as CH_4 and CH_4 per kg ECM. Energy-related MIR parameters are color-coded to highlight values outside the normal range. It is evident from the visualization that issues arise at the start of lactation, with negative EB-NEL values indicating a problem.

During fieldwork within the ReMissionDairy project, the FeMIR report underwent testing to establish the limits for each parameter and determine an optimal framework for herd management. Consultants and techniciens of the LKV were involved in evaluating the output on test animal and -herd level.

The physical condition of the animals observed on-site aligned with the experts' expectations derived from the efficiency and energy parameters in the report.

Feed efficiency, as shown in Figure 3, is the ratio of milk produced in kg ECM to feed consumtion in kg dry matter (DM). In individual animals, high efficiency can be falsely indicated by fat decomposition. To improve feed efficiency at the farm level, it is important to reduce maintenance requirements (such as cow weight relative to performance) and avoid overconsumption. This information applies to all components mentioned in the introduction. The next most important component after feed efficiency is energy balance (EB), which can be visually observed when animals have lower EB values during mid-lactation. DeNovo fatty acids provide insight into newly formed fatty acids in the udder and rumen functionality, with lower values indicating low feed intake and cell wall digestibility. Preform fatty acids indicate high digestibility degradation with high values. The final component in the FeMIR report is methane (CH_4) emissions and CH_4 per kg ECM, which can help monitor emissions and implement measures to reduce methane production. All participants rated the FeMIR report as a valuable and effective tool for managing feeding practices and monitoring animal metabolism. But differences between the informative value of the parameters have been found: according to the consultants of the LKV the feed efficiency value should not be used as "stand alone" parameter due to higher variation, but can be used combined with the energy balance. The energy balance in contrary is a meaningful value regarding to feed management, as is methan emission (CH_4). The parameters are particularly expressive on herd level, while critically seen on animal level. The farmers themselves however benefit most of the output CH_4. The LKV herd manager was created as part of the Cattle Network cooperation (RDV) and is planned to be utilized by all RDV partners. FeMIR has the potential to be used for all RDV cows in the future, totalling approximately 2.3 million cows. Additionally, the pilot farms' initial data and research data for each MIR parameter are also accessible.

Conclusions

The FEMIR report tested on-site showed, that the physical condition of the animals observed confirmed the experts' expectations derived from the efficiency and energy parameters in the report. A distinction is made between the usefulness and informative value of the individual parameters. While the feed efficiency can only be used together with the values of the energy balance, the latter is informative enough on its own. On animal level, the report has still to be improved. But all participants agree, that the FeMIR report is a valuable and effective tool for managing feed and monitoring animal metabolism on herd level. Further research is needed on animal level and to investigate the impact of heat stress on efficiency indicators to assess how climate change and heat stress affect cows feeding systems.

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Predicting the likelihood of conception to first insemination using milk mid-infrared spectroscopy: a model for FOSS instrument

P.N. Ho[1], A.J. Chamberlain[1,2], M. Haile-Mariam[1,2] and J.E. Pryce[1,2]

[1]Agriculture Victoria, AgriBio, Centre for AgriBioscience,
Bundoora, Victoria 3083, Australia
[2]School of Applied Systems Biology, La Trobe University,
Bundoora, Victoria 3083, Australia
Corresponding Author: phuong.ho@agriculture.vic.gov.au

Abstract

An ability to predict the likelihood of conception of dairy cows in early lactation would help farmers make informed breeding decisions. Cows predicted to be most fertile, for instance, could be inseminated with sexed or high premium semen while those predicted to be least fertile could be mated with beef semen. Previously, we developed such a model using data from commercial milk testing, which included milk yield, milk composition (fat, protein, and lactose percentages), somatic cell count, calving age, days in milk, days from calving to first insemination, and milk mid-infrared (MIR) spectra generated from a Bentley instrument. The model shows a good prediction accuracy and has been implemented by Australian herd-test centres who provide reports to farmers. This study extended the analysis to FOSS instrument, which is the other major instrument used for MIR in Australia. Firstly, we tested if the previously developed Bentley model would be applied directly to spectral data obtained from a FOSS machine. Secondly, a new model trained specifically using FOSS MIR data was developed and evaluated. Finally, various genomic and phenotypic measures were compared for cows predicted to have most and least likelihood of conception compared to herd average. A total of 9,120 records of milk MIR spectra, milk yield, milk composition, somatic cell count, calving age, days in milk and days from calving to first insemination of 3,518 cows from 31 dairy herds were used. The new model was developed in the same way as the Bentley model which included initial training on “extreme data” and then validating against field data. Specifically, the “extreme data” only include cows that conceived to first insemination (“good”) and cows with no conception event recorded and had only one insemination (“poor”), whereas field data include all cows in the herd. The model performance was evaluated by first ranking the cows within a herd for their predicted likelihood of conception and then selecting the top and bottom 10% of records and compared to actual values. The accuracy was measured as the proportion of selected records being correct. When applying the Bentley model to FOSS data, the prediction accuracies of identifying the top and bottom 10% of cows were around 0.37 and 0.62, respectively. Such a poor prediction accuracy using the Bentley model implies the need to develop a separate model for FOSS. The new model was able to achieve an accuracy of around 0.53 and 0.77 when used to identify the top and bottom 10%, respectively, which is comparable to the published Bentley model. It could also correctly identify the top 10% of cows conceiving following two inseminations with an accuracy of 0.70. Compared to herd average, the top 10% of cows ranked by the model were significantly younger and had lower somatic cell cows while the opposite pattern was observed for cows in the bottom 10%. Interestingly, there was no significant differences in 305-day milk yield, milk composition, days from calving to first insemination, days in milk, and other breeding values and national selection indices. In conclusion, a model

for predicting the likelihood of conception to first insemination of Australian dairy cows using milk MIR spectra and other on-farm data has been developed and validated for further implementation for farmers who use FOSS instrument.

Keywords: Likelihood of conception, mid-infrared spectroscopy, cows. Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

Along with genomic selection , numerous management tools have been developed to help farmers optimized their breeding decisions (Giordano *et al*., 2022). One important category of such models is those that aim to predict the potential outcome of insemination (Shahinfar *et al*., 2014, Hempstalk *et al*., 2015, Blavy *et al*., 2018, Ho and Pryce, 2020). The information used to develop these models vary from easy to obtain on-farm (e.g. milk production, milk composition, and milk mid-infrared (MIR) spectroscopy), to moderately easy to measure (e.g., milk progesterone) or comparatively difficult to measure (e.g., body weight and body condition score). In Australia, milk recording is routinely practiced by nearly half of Australian farmers with resulting data being utilized to support a range of management decisions such as culling or ending a cow's lactation (Newton *et al*., 2020). As such, the prediction models that make use of data from the current milk-testing program offer most advantage as this information is readily available on-farm. In 2019, Ho *et al*. (2019) developed a model for predicting the likelihood of a cow to get pregnant at first insemination with a good prediction accuracy (~0.76).

A potential application of this model is to rank cows within-herd for their probability of conception to subsequently prioritize them for insemination with different types of semen (sexed, beef or conventional semen). Newton *et al*. (2024) showed that MIR-fertility outperformed calving date when being applied to optimize semen allocation.

The model has been implemented by Australian herd-test centres who provide reports to farmers (DataGene, 2022). However, the current model is only available to farmers that have their milk tested with herd-test centres operating Bentley instrument, which means the other half of farmers who use FOSS instrument is missing (Peter Thurn, personal communication).

The major objective of this study was therefore to extend the analysis to FOSS instrument. First, the transferability of the current model trained using spectra generated by Bentley instruments was validated against data collected from FOSS brand. Then, a new model using FOSS MIR data was developed and evaluated. We also examined the differences in various genomic and phenotypic attributes between cows predicted to be most and least fertile compared to the herd average.

Materials and methods

Animal Data

All the data used for this study were obtained from DataGene (https://www.datagene.com.au/). Because the aim of this model is to predict how likely a cow is going to conceive to first insemination (i.e. a future event), only milk-testing records collected before the first insemination were retained. Accordingly, there were 9,120 records of insemination, calving date, days in milk (DIM) at herd-test, days from calving to first insemination (DAI), age at calving, herd-test day milk yield (MY), fat, protein, and lactose percentages, somatic cell count (SCC), and MIR spectra, from 3,518 cows of 31 commercial herds. The data were collected in the years of 2021 (n = 1,032) and 2022 (n = 7,989). Other information was also available on these cows, including 305-d milk yield, Banlance Performance Index (BPI), Health Weighted Index (HWI), and breeding values of milk, fat, protein, SCC and daughter fertility.

The cows were milked twice daily with milk samples (either a.m. or p.m.) being sent to the National Herd Development Co-Op Ltd (Cohuna, Victoria, Australia) and Dairy Express (Armidale, New South Wales, Australia) to be analyzed for fat, protein, and lactose concentrations and SCC using the MilkoScan FT+ spectrometer (Foss, Hillerød, Denmark). The corresponding spectra from the milk composition analysis were obtained for this study.

Data processing

The pregnancy was confirmed by a calving event in the subsequent lactation and was coded binarily as 1 (pregnant) or 0 (open). In addition, the inseminations that resulted in abortions were removed. Prior to modelling, several mathematical treatments were applied to the raw spectra. First, the spectral regions previously specified to be noisy or non-informative (regions between 1,710 and 1,600 cm−1, between 3,690 and 2,990 cm−1, and >3,822 cm−1) caused by a high-water absorption were eliminated, which led to 531 wavenumbers remained. Thereafter, a global Mahalanobis distance (GH) between each spectrum and the population average was calculated as an indicator of potential outliers. The spectra with GH > 3 were assumed to be outliers and excluded (n = 50). Lastly, first-order Saviztky–Golay derivative was applied to the reduced spectra. The final dataset included 9,070 records from 3,498 cows of 31 herds to be used for future analyses.

Model development and evaluation of performance

In the first scenario, we tested the transferability of the current Bentley model on the spectra generated from a FOSS instrument This was done by first matching each FOSS MIR wavenumber to the closest one generated from a Bentley machine. Then, the current Bentley model was applied directly to these spectra and other predictors including milk yield, milk composition, DIM, calving age, DAI, and SCC to derive predictions.

The second scenario included training and evaluating a new model specific to FOSS instrument, using the methodology described in Ho and Pryce (2020). Briefly, the model was first trained using only data from cows that conceived to first insemination (coded as 1) and cows with no conception event recorded and with only 1 insemination (coded as 0). A fresh dataset with all cow's data regardless of the conception statuses was used to test the ability of this newly developed model for identifying cows that conceived or did not conceive to first insemination. To do this, we first extracted the probability of conception from the model and used this to rank cows within each herd from highest to lowest (i.e. most and least fertile). Next, varying proportions of records from 5 to 40% were selected and validated against the actual observations.

In both scenarios, the model performance was evaluated as the proportion of selected records to be truly predicted. For example, if the purpose is to predict 10% of least fertile cows (i.e. potentially fail to get pregnant to first insemination) from a herd of 1000 cows, 100 of these will be selected from the bottom of the predicted list and compared with the actual values. The performance in this case will be calculated as the proportion of cows being non-pregnant over 100. For the scope of this study, we only reports the prediction accuracy obtained from the external herd-by-herd validation, which has been concluded to provide realistic performance compared to random cross-validation (Wang and Bovenhuis, 2019). In this validation approach, for each round, data of a given herd was excluded to be subsequently used as a validation set against the model trained using the data of the remaining herds (i.e. 30 herds), and this process was continued until all herds have been validated once.

Finally, to further understand the model's behaviour, we extracted and compared various genomic and phenotypic characteristics of cows ranked as top and bottom 10% in comparison to the herd average. The comparisons were done using 1-way ANOVA tests with pairwise comparisons. All analyses in this study were performed using R statistical software version 3.6.1 (R Development Core Team, 2020).

Results and discussion

When applying the Bentley model directly to the FOSS data, regardless of the proportions selected, the prediction accuracies for all three categories (conceived to first insemination, conceived following two inseminations, and open to first insemination) were around 0.37, 0.49, and 0.62, respectively (Tabe 1). The corresponding figures of model performance reported by Ho and Pryce (2020) were 0.48, 0.76 and 0.69. The poor performance obtained in the current study implies the need to develop a separate model for farmers that milk-test their herds with herd-test centres operating FOSS instrument.

On the new model, good performance was obtained and the results were comparable with that of Bentley model (Ho and Pryce, 2020). The prediction accuracies of the model to rank and identify cows that conceived to first and second insemination, and cows not conceived to first insemination ranged between 0.49–0.54, 0.66–0.72, and 0.75–0.81, respectively. Also, the higher the selected proportions, the lower the model performance. However, the prediction accuracies of this FOSS model were considerably variable (the standard deviation of around 0.20) indicating that more data is needed to improve the robustness of the model, as also concluded by Pralle and White (2020).

To further understand the model's behaviour, we examined various phenotypic and genomic features of cows that were predicted to be high and low fertility compared to the herd average. This is an important step as it gives farmers confidence in using

Table 1. Accuracy of models (mean ± standard deviation) for identifying cows with good likelihood of conception to first and second insemination and cows with poor likelihood of conception to first insemination.

Proportions	Cows with good likelihood of conception at first insemination	Cows with good likelihood of conception at second insemination	Cows with poor likelihood of conception at first insemination
Scenario 1: Bentley model applied to FOSS data			
5	0.31 ± 0.34	0.39 ± 0.36	0.59 ± 0.29
10	0.31 ± 0.32	0.40 ± 0.35	0.58 ± 0.26
15	0.32 ± 0.32	0.41 ± 0.35	0.56 ± 0.24
20	0.32 ± 0.32	0.41 ± 0.35	0.55 ± 0.24
25	0.32 ± 0.32	0.41 ± 0.35	0.54 ± 0.24
30	0.32 ± 0.32	0.41 ± 0.35	0.54 ± 0.23
Scenario 2: new FOSS model			
5	0.54 ± 0.25	0.72 ± 0.21	0.81 ± 0.27
10	0.52 ± 0.20	0.68 ± 0.18	0.77 ± 0.26
15	0.49 ± 0.21	0.68 ± 0.16	0.76 ± 0.23
20	0.49 ± 0.20	0.67 ± 0.17	0.76 ± 0.20
25	0.48 ± 0.19	0.66 ± 0.16	0.76 ± 0.22
30	0.49 ± 0.13	0.66 ± 0.14	0.75 ± 0.23

Table 2. Mean and standard deviation of genomic and phenotypic traits for predicted high fertility subgroup, herd-average, and predicted low fertility subgroups.*

	Top 10%	Herd average	Bottom 10%
MY305 (kg)	6,548 ± 1,270	6,880 ± 1,355	7,107 ± 1,427
Fat percent	3.95 ± 0.51	4.00 ± 0.43	4.05 ± 0.53
Protein percent	3.27 ± 0.28	3.22 ± 0.26	3.20 ± 0.36
SCC	63.7 ± 49.3^{a}	164.5 ± 103.3^{b}	676.4 ± 716.5^{c}
MY24 (kg)	24.0 ± 4.2^{a}	27.2 ± 4.5^{b}	27.5 ± 6.1^{b}
DIM	47.2 ± 26.6^{a}	55.6 ± 21.6^{b}	66.7 ± 28.4^{b}
Calving age (month)	32.3 ± 8.9^{a}	49.9 ± 10.1^{b}	77.1 ± 20.3^{c}
Calving to first AI	95.5 ± 22.8^{a}	108.9 ± 29.3^{ab}	124.7 ± 39.5^{b}
Conception to first AI	0.47 ± 0.19^{a}	0.40 ± 0.16^{a}	0.23 ± 0.22^{b}
BPI	157 ± 100	152 ± 66	136 ± 69
HWI	161 ± 99	154 ± 64	133 ± 55
ABV$_{milk}$	58.2 ± 161	73.1 ± 64	74 ± 242
ABV$_{fat}$	11.1 ± 12.1	12.1 ± 7.5	12.3 ± 9.3
ABV$_{protein}$	7.2 ± 5.6	7.2 ± 5.1	6.9 ± 7.3
ABV$_{scc}$	129.8 ± 19.4	126.7 ± 12.1	122.2 ± 10.1
ABV$_{daughter\_fertility}$	104.1 ± 3.3	103.6 ± 2.5	102.8 ± 2.5

*The results are similar for other selected proportions (i.e. 5%, 15%, 20%, 25%, and 30%), only those that obtained when 10% of records were selected being presented here.

MY305 = 305d milk yield (kg), SCC = somatic cell count, MY24 = 24 hours milk yield on the herd-test day (kg), DIM = days in milk at herd-test, BPI = Balance Performance Index, HWI = Health Weighted Index.

the model to make breeding and management decisions. For example, in conjunction with BPI or daughter fertility, the cows predicted to have high likelihood of conception could be inseminated with sexed or premium semen. In this context, it is essential to make sure that the fertile cows are not the low milk producers. Because the results are similar when other proportions of cows being selected, we only present here the statistics for the top and bottom 10% of cows selected to compare with that of herd average. In general, the results were similar to our previous analysis on the Bentley model (Bird *et al.*, 2023). Compared to the herd average, cows predicted to be most fertile by the model were significantly younger and had a lower somatic cell count while the opposite patterns were observed for cows that were predicted to be least fertile. These results might partially explain for the higher conception rate to first insemination of the top-ranking cows compared to herd average and bottom-ranking individuals (0.47, 0.40, and 0.23, respectively). Numerous previous publications have reported the negative association between the cow's age as well as SCC and their health and reproductive performance (Lomander *et al.*, 2013, Golder *et al.*, 2021). Interestingly, while 24-hrs milk yield was significantly between the three groups, MY305 were not, which would be explained by the difference in DIM, i.e. predicted fertile cows were milked earlier while the infertile cows were milked later than all cows within the herd (47.2, 66.7 versus 55.6, respectively). In addition, predicted high fertility cows had a decreased calving to first insemination interval compared to the herd average and low fertility cows (Table 2). This is highly desirable especially in the pasture-based and seasonal calving system, for example, in Australia, because this allows farmers to match the cow's high energy requirements in early lactation to the peak pasture growth rate (Shalloo *et al.*, 2014).

While there were no statistically significant differences between the three groups regarding the genomic features, it is interesting to note that the cows predicted to be most fertile had higher BPI and daughter fertility breeding values. These results are consistent with the previous analysis of Bird *et al*. (2023).

Conclusions

This study shows that the MIR-fertility prediction model developed using data from a Bentley instrument could not be transferred directly to the spectra generated from a FOSS instrument. Further, we have successfully developed a new model specific to FOSS instrument with good prediction accuracy. However, more data is needed to improve the robustness of the model.

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Daily standardization of milk mid-infrared spectra in a comprehensive regression model framework considering animal related data

A. Mensching[1], J. Braunleder[1], E. Bohlsen[2], S. Schierenbeck[1] and R. Reents[1]

[1]Vereinigte Informationssysteme Tierhaltung w.V.(vit), IT Solutions for Animal Production, Heinrich-Schröder-Weg 1, 27283 Verden, Germany
[2]Landeskontrollverband Niedersachsen, Großstr. 30, 26789 Leer, Germany
Corresponding Author: andre.mensching@vit.de

Abstract

Milk mid-infrared (MIR) spectrometry has been utilized worldwide and for decades to analyze the components of milk. In a routine use, the method demonstrates a very high precision and repeatability, particularly for the main milk components. This is substantially supported by repeated analyses of standard milk samples with known reference values, whereof a slope-intercept (S/I) correction is derived for regular samples. However, this does not apply to routinely collected spectral data, where deviations and drift can be observed both between different instruments and within an instrument over time. The aim of this study was to demonstrate a new approach for standardization of MIR spectra using a framework of regression models considering results of laboratory analyses and information on the animal, such as days in milk (DIM) or parity, to estimate daily and instrument-wise standardization coefficients for the individual wavelengths.

The data were provided by the Landeskontrollverband Niedersachsen (LKV Niedersachsen, Leer, Germany) and included spectral data from 5 spectrometers (FOSS, Hillerød, Denmark) as well as the corresponding data from the dairy herd improvement (DHI) testing, which were routinely collected in the first half of 2022 (dataset I, DS-I). In addition to the total of 2.3 M spectra from routine DHI testing, triple analyses of the same 5.3 k DHI milk samples on 3 of the 5 spectrometers were carried out on samples of 7 different farms during the same period (DS-II). Furthermore, 61.0 k spectra of standard milk samples were available (DS-III). In the daily laboratory routine, these samples of the weekly changing North German Standard Milk (NGSM) were analyzed 3 times in a row every 200 regular samples (reference analysis by LUFA Nord-West, Oldenburg, Germany).

In a first step, the DHI spectra of DS-I were used to quantify and eliminate day-specific instrument effects in a complex framework of regression models, considering information on the animal as well as data obtained from the lab analyses to finally estimate instrument-, day- and wavelength-wise standardization coefficients. With the aim of demonstrating the effect of standardization, the dataset with triple analyses (DS-II) was utilized in a second step to develop calibration models for both raw and the standardized spectra using the S/I-corrected fat values obtained from the laboratory. Based on this, two separate analyses were performed: first, the dataset with the triply analyzed DHI samples (DS-II) was used for principal component analyses (PCA) and a comparison of the estimability of fat in each case for raw and standardized spectra. Second, the dataset with the analyses of standard milk samples (DS-III) was used to compare the S/I-corrected laboratory fat values with estimates from the developed fat models based on both raw and standardized spectra across all 5 instruments and over time.

It could be shown that the standardization led to a harmonization of the spectra between instruments as well as over time and thus corrected both general and temporary instrument effects. In addition, the estimability of milk fat, which was used as an example trait for the validation of the methodology, was optimized by the standardization of the MIR spectra. Results showed that the root mean squared error (RMSE) in a leave-one-instrument-out cross-validation (LOIO-CV) could be reduced from 0.110 to 0.032% fat during calibration and from 0.045 to 0.020% fat during validation. Regarding the standard milk analyses, the RMSE was also reduced from 0.038 to 0.019% fat and thus closely approximates the RMSE of 0.014% fat of the S/I-corrected laboratory values. This study showed that there is not only a high demand for standardization across instruments, but also within instruments over time. Therefore, vit-standardization as a statistical procedure featuring a daily standardization seems to be a promising novel approach for future estimation of traits that are not covered by standard milk samples.

Keywords: standardization, milk mid-infrared spectrometry, regression model framework. Presented at the ICAR Anual Conference 2024 in Bled at the Session 2: New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

For decades, Fourier transform MIR spectrometry of milk has been used globally to determine milk components. Its accuracy for payment-relevant main milk components is essentially attributable to the check sample tests carried out regularly in the laboratory routine (Gengler *et al*., 2016; Nieuwoudt *et al*., 2021). By comparing target and actual values of standard milk samples with known composition, these tests are used to derive a S/I-correction for regular milk samples. The requirement for S/I corrections is well known, as recorded spectra the same sample are neither comparable between instruments nor over time (Young, 1978; Wang *et al*., 1991). In addition to fundamental differences between various manufacturers, there can be differences even within the same manufacturer between different instruments of the same model generation (Grelet *et al*., 2015; Nieuwoudt *et al*., 2021). Apart from this, environmental effects such as temperature and humidity, mechanical wear, sample handling and constructional differences of the instruments as well as mechanical and electronic effects, can result in spectral deviations or drift over time (Wang *et al*., 1991; Nieuwoudt *et al*., 2021).

General and temporary instrument effects are a particular hindering when estimating traits beyond those known from the standard milk samples, as no simple S/I correction is possible. To handle this, there are various approaches that focus on the standardization of MIR spectra. In theory, this shall allow the transfer of models between different instruments to obtain reliable estimates over time. The manufacturer FOSS, for example, has been using its own patented process for instrumental standardization, which should be carried out regularly, e.g. monthly, by analyzing a chemical liquid, the so-called ‘equalizer’, for which a reference spectrum is known (FOSS, 2014). In general, piecewise direct standardization (PDS) can be considered the gold standard of non-instrumental standardization methods (Wang *et al*., 1991). In this process, spectra from secondary instruments (“slaves”) are translated into spectra from primary instruments (“masters”) with the aid of statistical models. However, PDS requires the analysis of common milk samples. An example of the use of PDS is the standardization service offered by European Milk Recording (EMR, 2024, Ciney, Belgium), which is based on the procedure described by Grelet *et al*. (2015, 2017). In the EMR service, common standardization samples are analyzed in repetition on all instruments approximately every month. Based on the corresponding recorded spectra, standardization coefficients are derived in a centralized procedure, that can be applied on regular milk samples. An alternative standardization method that does not require analysis of common samples is the procedure described by Bonfatti *et al*. (2017). In this so-called retroactive standardization of spectra, temporary homogeneous subsets are identified first. Using a PCA-based method, transformations are then determined which translate the various subsets in the form of "slave" datasets into a "master" by means of an S/I-based correction of the absorbance values.

It is important to emphasize that particularly short-term instrument effects can occur during spectrometric measurements. In this regard, Nieuwoudt *et al*. (2021) showed in their method for weekly monitoring of spectral data that there is a great need for continuous monitoring, as conspicuous drifts or deviations can already be detected between weekdays.

The objective of this study was to demonstrate a novel approach for the daily standardization of milk MIR spectra, in which general and temporal instrument effects are estimated and eliminated using a regression model framework to determine instrument-, day- and wavenumber-specific standardization coefficients.

Material and method

All the data was provided by the LKV Niedersachsen (Leer, Germany) and can be differentiated in 3 sub-datasets. The first dataset (DS-I) included data of 2.3 M samples from routine DHI testing in the period from 01/01/2022 to 31/06/2022. The related milk samples were preserved with Bronopol (Georg Hansen e.K., Wrestedt, Germany) and were taken according to the ICAR guidelines (ICAR, 2022). The laboratory of the LKV Niedersachsen in Leer was equipped with a total of 5 FOSS instruments, of which 2 were MilcoScanTM 7 RM (A and B) and 3 were MilcoScanTM FT+ instruments (C, D, and E). Besides the MIR spectra, fat, protein, and lactose contents of the milk determined by the laboratory were available. These MIR spectra-based values were determined by the manufacturer's equations but were adjusted in a further step as part of the laboratory routine using analyses of NGSM and a thereof derived S/I correction. Furthermore, animal identification and the affiliation to the farm, the date of calving and therefore the DIM, parity, the information about the milk testing scheme including milking time as well as the milk performance were provided. The second data (DS-II) set also contained DHI data, but the associated samples were analyzed sequentially on 3 different instruments (A, B, and C). The 5.3 k samples were taken from 7 different farms whose DHI tests covered the same period from 01/01/2022 to 31/06/2022. The third dataset (DS-III) consisted of 61.0 k records from routine check sample tests of the weekly changed NGSM as well as their laboratory reference measurements analyzed, which were analyzed 3 times in a row every 200 regular samples. The reference analysis for the main milk components of the NGSM samples, which were also preserved with Bronopol (Georg Hansen e.K.), was carried out at LUFA Nord-West (Oldenburg, Germany). Depending on the dataset, plausibility checks and outlier removals were carried out at the spectral level with the global H-value (Soyeurt *et al*., 2019), regarding the agreement of the S/I-corrected fat values in the triplicate analyses of the DHI samples or in terms of completeness of the control samples (3 analyses of NGSM per test).

The dataset DS-I was used to determine instrument-specific, daily, and wavenumber-related standardization coefficients of the MIR spectra. To quantify and eliminate both general and temporal instrument effects from raw spectra, a regression model framework was developed to obtain the standardized spectra. In this context, wavenumber-wise regression models were used to differentiate the observed variance of MIR spectral absorbance values into fixed effects associated with the milk sample and random effects related to the respective instruments. For the milk sample-associated effects, S/I-corrected laboratory values and information on the sample origin, such as

DIM and parity of the cow, were considered. In a further step, the absorbance values corrected for instrument effects were regressed on the raw absorbance values by simple linear regression to obtain slope and intercept correction factors as standardization coefficients. This was done by day, instrument, and wavenumber and thus allowed a translation of raw spectra into the standardized spectra.

Furthermore, fat models were developed using dataset DS-II for both raw and standardized spectra to evaluate the effect of spectral standardization. In each case, partial least squares (PLS) regression models were used, considering n = 6 latent variables. The 1st gap derivative according to Soyeurt *et al*. (2011) was used as spectral pretreatment. In addition, the spectral ranges were reduced to 516 of 1,060 WN according to the selection by Grelet *et al*. (2015). The dataset was split so that 80% of the data was used for calibration and 20% for validation.

In the first part of analysis, the data of repeated analyzed DHI samples (DS-II) were used to investigate the effects of standardization. On spectral level, PCA was used similar as in the work of Grelet *et al*. (2017). Separate PCA were performed both for untreated spectra and for spectra pretreated with a 1st gap derivation. The scores of the standardized spectra were projected into the same vector space as that spanned by the raw spectra. For comparison, the results were graphically displayed with score plots. To evaluate the estimability of fat, common statistics such as the RMSE at calibration, LOIO-CV and validation were determined for both raw and standardized spectra-based fat calibration models. For graphical investigation, the observed S/I-corrected laboratory fat values were compared with the MIR-based estimates in scatter plots. In the second part of the analysis, the dataset DS-III of analyzed check samples (NGSM) was utilized. For visualization, aggregated mean values of the differences between fat reference values of the NGSM and the S/I-corrected laboratory fat values as well as the fat estimates based on raw and standardized spectra were calculated at the daily level and displayed individually for each instrument over time using line plots.

Results and discussion

PCA was utilized to assess the impact of standardization on MIR spectra. Figures 1A and C display variations in the spectra data across different instruments, notably in the 1st and 2nd principal components (PC) for non-derived raw spectra and particularly in the 5th and 6th PC of gap-derived raw spectra. In contrast, standardized spectra (Figures 1B and 1D) exhibit greater alignment between instrument scores, indicating a successful harmonization through standardization. These results are therefore basically comparable with those of Grelet *et al*. (2017). Instrument similarities are notably higher between instruments A and B (both MilcoScan^{TM} 7 RM, diamond cuvette) compared to instrument C (MilcoScanTM FT+, CaF_2 cuvette), possibly due to the different material of the cuvette or other marginal constructional distinctions. An influence of the cuvette material, for example, was also described by Nieuwoudt *et al*. (2021). A closer look at the data revealed that the separate point clouds of the analyses with instrument B (see Figure 1C) were each carried out on the same days and thus reveal temporary instrument effects on the spectra. These were significantly minimized by standardization, as can be seen in Figure 1D. Thus, these results underline the effectiveness of standardization for harmonization of spectra between different instruments and over time.

The calibration and validation metrics determined during model development as well as the comparison of the generated estimates with the S/I-corrected laboratory fat values were used to evaluate the effect of standardization on the estimability of fat. In Figure 2, the S/I-corrected laboratory values are plotted against the estimates from the calibration models based on raw (Figure 2A) and standardized spectra (Figure 2B). In general, the values lie close to the identity in both cases, but the estimates based

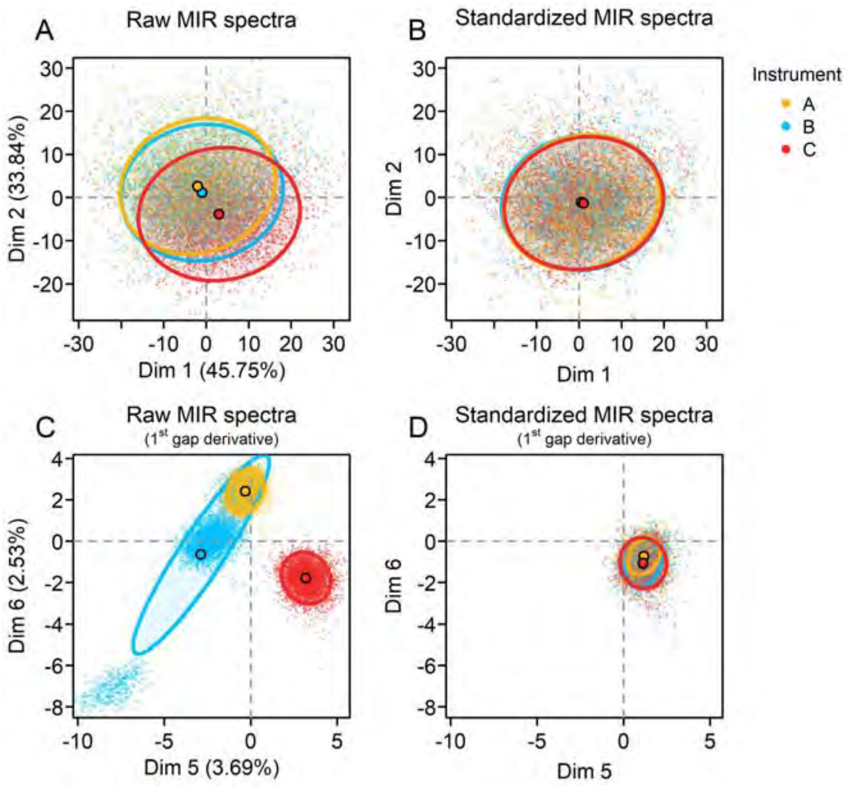

Figure 1. Results of principal component analyses of the spectra of triple analyzed milk samples before (Figures 1A and 1C) and after (Figures 1B and 1D) standardization (n = 4,471 per instrument) on 212 of 1060 wavenumbers. The instrument-wise points correspond to the means in the respective principal components, where the respective ellipses represent the 95% two-dimensional density contour. Dim = Dimension, MIR = mid-infrared.

on the raw spectra reveal larger scatter and deviations. As in the previous part of the analysis, instrument B in particular shows considerable outliers in the estimated values based on raw spectra, but not in the estimated values based on standardized spectra. Thus, the standardization shows a substantial contribution to the reduction of overall and temporary instrument effects for the MIR-based fat estimates, which is also reflected in a significant reduction of the RMSE values. The RMSE of LOIO-CV during calibration decreased from 0.110 to 0.032% fat and from 0.045 to 0.020% fat at validation. These results can thus be compared with those of Grelet *et al*. (2015), who also observed a notable reduction in the RMSE in pre- and post-standardization comparisons of fat.

Differences between reference values from the NGSM samples to S/I-corrected lab fat values as well as fat estimates based on raw and standardized spectra were aggregated per instrument into daily mean values and plotted over time in Figure 3. Minimal scatter can be observed for S/I-corrected lab fat values, while strong short-term instrument effects with temporal daily mean biases up to 0.2% fat occur in estimates based on raw spectra. The differences between the reference values and the fat estimates based on standardized spectra scatter only slightly larger around 0 than of the S/I-corrected fat values but much smaller than for the estimates based on non-standardized spectra. This is also confirmed by the calculated mean RMSE values. The standardization led

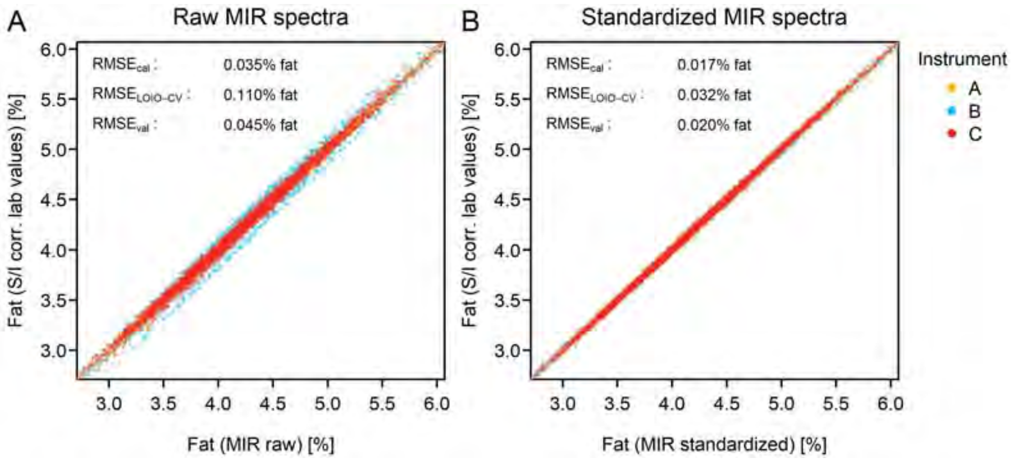

Figure 2. Comparison of the slope-intercept corrected laboratory fat values of triple analyzed milk samples to the fat estimates based on raw (Figure 2A) and standardized (Figure 2B) spectra (n = 4,471 per instrument). MIR = mid-infrared, RMSE$_{val}$, RMSE$_{LOIO-CV}$, and RMSE$_{val}$ = root mean squared error of calibration, leave-one-instrument-out cross-validation and validation, S/I = slope-intercept.

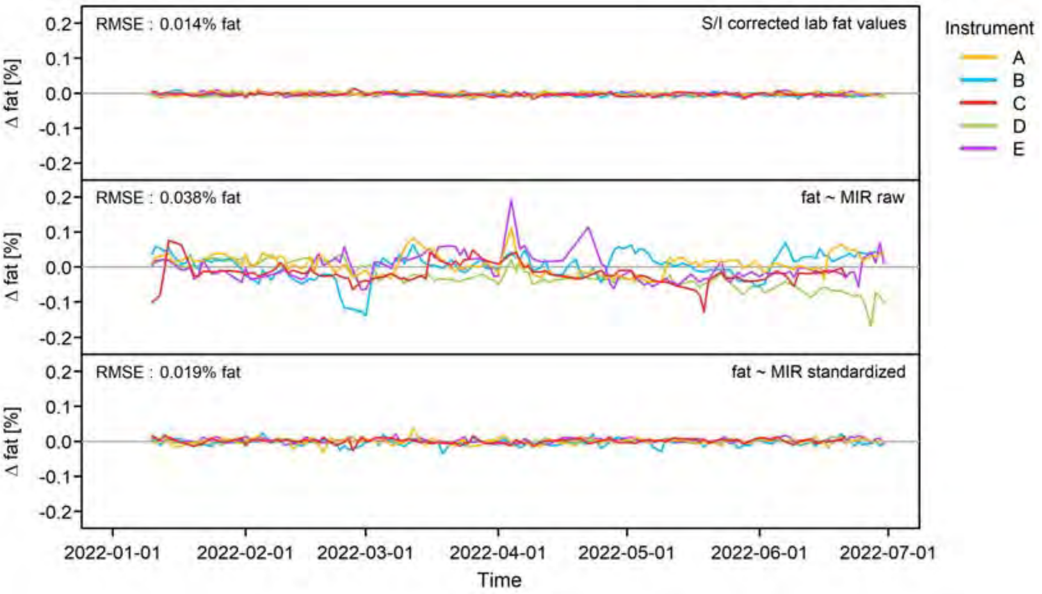

Figure 3. Daily mean differences between reference values of the North German Standard Milk samples to the slope-intercept corrected laboratory fat values as well as the fat estimates based on raw and standardized mid-infrared spectra (n = 53.5 k). lab = Laboratory, MIR = mid-infrared, RMSE = root mean squared error.

to a reduction from 0.038 to 0.019% fat and thus almost corresponds to the RMSE of 0.014% fat of S/I-corrected laboratory fat values. As the NGSM samples neither were used for standardization nor for model development, this part of the analysis served as external validation both for the models and for the standardization approach. The estimated standardization coefficients based on DHI samples thus also show a potential for application to bulk milk samples analyzed with the same instrument and on the same day.

Conclusions and outlook

MIR spectrometric measurements are often influenced by general and temporal effects. This is why there is a high need for regular standardization to reduce drifts in both spectra and estimations of traits, that are not covered by standard milks and therefore cannot be adjusted via S/I correction. The presented procedure for daily standardization based on a regression model framework showed the ability to harmonize MIR spectra both across instruments and over time. This was confirmed by an improved estimability of milk fat, that was used as an example trait to validate the methodology. The underlying standardization method therefore has great potential to generate reliable MIR-based predictions of further phenotypes in the future, both to promote the development of herd monitoring tools for feeding and animal health and to serve as a data source for genomic evaluations.

The work presented here can be seen as the first part of a proof of concept for the “vit- standardization”. In the meantime, the procedure has been upgraded so that standardization can be carried out not only on a closed dataset but also with daily new incoming data. Furthermore, a first MIR-based tool for monitoring of ketosis on routine DHI data has been released for over 3000 farms of the LKV Niedersachsen.

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Predicting dairy cattle heat stress indicators using machine learning and mid infrared spectral data

L.M. Dale[1], E.J.P. Strang[1], P. Lemal[2], M. Jattiot[3], A. Werner[1], HoliCow Consortium[4], ResKuh Consortium[5], H. Amamou[6], N. Gengler[2] and H. Soyeurt[2]

[1]Regional association for performance testing in livestock breeding of Baden-Wuerttemberg Heinrich-Baumann-Str. 1-3, 70190 Stuttgart, Germany
[2]Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés 2, 5030 Gembloux, Belgium
[3]INNOVAL, 35538, Noyal-sur-Vilaine, France
[4]https://holicow.nweurope.eu/, 4 Rue des Champs Elysées, 5590, Ciney, Belgium
[5]https://agroecologie-rhin.eu/de/klimaco-2/, 2 rue de Rome, 67013, Schiltigheim, France
[6]High School of Agriculture of Kef, University of Jendouba, 7119, Le Kef, Tunisia
Corresponding Author: ldale@lkvbw.de

Abstract

Heat stress negatively affects the well-being, productivity, and profitability of dairy cows and farms. Previous studies have primarily focused on indicators such as respiration rate, skin temperature, rectal temperature, and milk yield, with limited research on the impact of heat stress on milk composition. Initial findings from various locations like Belgium, Tunisia, and Germany have explored the mid-infrared (MIR) responses to heat stress in dairy cows. This study aimed to investigate the effects of heat stress on LKV Baden-Württemberg (LKVBW) farms within a 20 km radius of weather stations using all available datasets related to milk production traits and predicted milk biomarkers derived from MIR spectra. Additionally, the study sought to determine if there are differences in heat stress indicators between data collected from barn weather stations versus public weather stations. Meteorological data from public weather stations in Baden-Württemberg (BW) and barn weather stations from MobiMets and Pessl Instruments devices, including temperature and humidity, were combined with data collected by the milk recording organization. THI values were calculated using mathematical calculations for daily averages, and a three-day average was linked to farm data based on the day of milk recording collection. These datasets were then linked to each animal using monthly spectral data for each cow from 500 selected LKVBW farms. The model was developed as part of the HappyMoo project using MIR spectral data from Bentley Instruments devices collected in the LKVBW database from 2012 to 2019, with external validation conducted on a dataset containing MIR spectral data from 2020 to 2022. Barn weather data was collected in Projekt KlimaCO, with MobiMets data from 2020 to 2022 and Pessl Instruments data from 2021 to 2023. A machine learning algorithm was implemented in R using the “glmnet” package. The spectral data were standardized using the EMR method and preprocessed with the first derivative algorithm using the Savitzky-Golay filter. Differences were observed in MIR spectra recorded under THI and thermoneutral conditions, with certain wavenumbers of the MIR spectrum showing varying responses. The THI index was established based on the relationship between the THI value of individual cows and the mean THI value of the farm. Pearson correlations were calculated using the THI index and milk parameters in the R environment with the “corrplot” library. The THI index showed negative correlations with milk yield (0.15), lactose (0.12), acetate (0.33), blood NEFA (0.2) and positive correlations with fat content (0.59), protein content (0.40), blood BHB (0.25), blood glucose (0.30), blood calcium (0.21) and fatty acids (0.35). No differences were found between public weather stations and barn weather stations. Additional analysis is required within the scope of upcoming projects like HoliCow and ResKuh to identify possible MIR heat stress phenotypes derived from milk. These phenotypes could be utilized for herd management and breeding purposes to pinpoint animals that are resilient to heat stress.

Keywords: MIR, spectral data, dairy cows, heat stress.
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Introduction

The decline in small and medium-sized dairy farms has led to the HoliCow1 project's emphasis on assisting these farmers. The primary objective is to transform intricate Big Data into accessible, cost-effective, and remote decision-making tools for farmers. This project aims to combat farm abandonment, improve animal welfare, and tackle climate-related challenges. By offering a novel solution, HoliCow strives to equip farmers with the tools needed to make informed choices, fostering sustainability in agriculture, enhancing livestock welfare, and safeguarding the environment.

To achieve this goal, a cross-border database has been set up to encompass various dairy breeds on farms across North-West Europe. The objective is to gather sufficient data to establish a reliable alert system. It is crucial to acknowledge the significant role that farms play in fostering robust and eco-friendly communities. Farmers are key advocates for animal welfare and climate resilience. HoliCow aims to consolidate all incoming data sources and streamline them effectively to provide a comprehensive overview of the welfare status of cows. In addition, HoliCow will incorporate animal-related data, milk spectral predictions refined through enhanced equations from previous projects like Robust Milk, OptiMIR, GplusE, HappyMoo, or D4Dairy, as well as external data such as climate information sourced from public weather stations. Moreover, the project will leverage interactive machine learning models to promote transparent farming practices. Therefore, the efficient processing of vast amounts of Big Data, including climate and weather data, is essential to assist farmers in making informed decisions and implementing sustainable practices that enhance their operations and benefit the broader community. This integration will empower farmers to remain engaged with their local communities and embrace modern agricultural techniques using cost-effective tools.

HoliCow will enable farmers to remotely monitor individual cows and their surroundings. Recognizing the importance of each animal compared to larger farms, receiving "early warnings" about an individual's status is economically vital. HoliCow will drive the collaborative adoption of integrated and innovative methods to facilitate this transformation for farmers, as well as the integration of these methods into regional agricultural innovation strategies and advisory services. HoliCow will help establish NWE as a pioneering force in innovative solutions for rural areas with a diverse cultural landscape. The approach will also address climate change mitigation and resilience by incorporating climate data, nitrogen efficiency, and methane predictions. Heat stress is a crucial factor affecting dairy cow performance and productivity, leading to decreased milk yields and metabolic disorders. As global warming trends continue, the impact of heat stress is receiving more attention even in temperate regions like central Europe. Traditionally quantified by the temperature-humidity index (THI), heat stress is also known as the discomfort index. Different countries have varying THI thresholds for negative effects on milk production; for example, the US uses a threshold of 72, while Luxembourg and Germany use thresholds of 62-60 that are often exceeded during the summer months. Studies have shown that for each THI unit increase, there can be a loss of 0.08 to 0.26 kg of milk per cow. In the HappyMoo project, a THI of 68 resulted in a 21% decrease in milk production and a 9.6% reduction in dry matter intake on average.

Material and methods

Experimental data

Through the HappyMoo Project, Amammou *et al*. (2021) have created a milk MIR spectra based THI model to forecast the cow's THI as a 3-day average before the testing day, aiming to determine the cow's resilience to heat stress. The primary goal of the HappyMoo research was to analyse the changes in milk MIR spectra linked to THI within a specific subset of the southwest German dairy herd under milk monitoring and explore the potential for identifying clear indicators of heat stress in individual milk samples.

The data utilized for the modelling was sourced from 120 farms across Baden-Württemberg, representing a subset of data from approximately 4500 dairy farms in the region. THI values were determined using data from 67 local German weather stations (DWD) for each farm. In Figure 1, the map on the left displays coloured dots representing all 4,500 farms participating in milk recording, while the map on the right highlights selected farms and weather stations with red dots. The geographical coordinates and measurements from these sensor points are openly available on the DWD server. Milk analysis and milk MIR spectral data were accessible for the farms between 2012 and 2019, encompassing the primary breeds and production systems in the area. To link to temperature and humidity data, the closest active sensor point at the time of milk recording was selected.

Statistical approach

The statistical analyses and machine learning utilized spectral data from Bentley FTIR analysers that were standardized using the EMR/CRA-W procedure. Absorbance values from the spectra were used to calculate the first derivative, and 212 relevant wave-numbers were selected. Additional input parameters included breed, parity, milking time, days in milk (DMI) categories, and age at calving. As a reference point, a three-day average of THI values prior to the day of milk recording was determined. Various linear regression methods such as PCR, PLS, CPPLS (pls package in R), and GLMNET (glmnet package in R) were employed. Three different calibration and validation subsets were created for validation purposes, based on spectra, animals, and cross-validation selection.

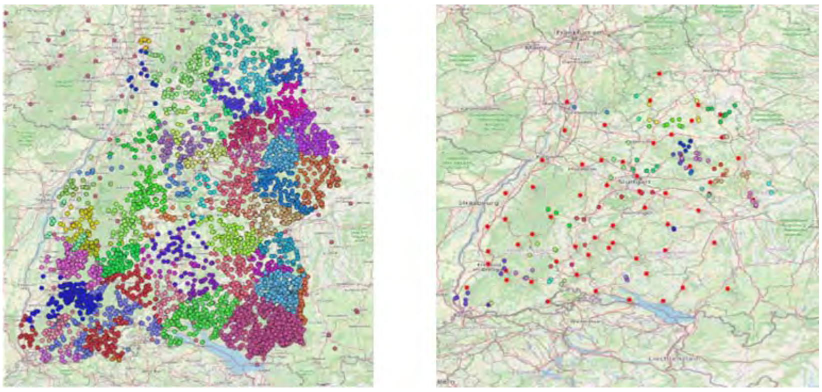

Figure 1. Map showing the positions of farms in relation to weather stations.

Spectra selection

Soyeurt (2024) has introduced an innovative approach to creating a globally representative spectral database (WRSD) to tackle the challenges associated with spectral extrapolation in predicting milk yield traits for new samples. This method is efficient and space-efficient, employing a two-stage selection process. Initially, a decomposition matrix is generated through principal component analysis using a dataset of approximately 2,137,394 records. Subsequently, an iterative spectral selection is conducted based on a sample location index derived from the principal components (PC). The frequency of spectra occurrence for each location index is calculated to influence the subsequent barycentre calculations. Despite 10 PCs explaining 95% of spectral variability, the barycentre pattern of selected spectra accurately represents the entire dataset, showcasing the effectiveness of a location index based on only 3 PCs. Finally, a WRSD was created using the HoliCow data samples with the same algorithm, selecting around 103,477 spectral data points from a total of 2,137,394 million spectral data collected from 503 farms.

Clustering approach

Hierarchical clustering was employed to identify patterns among futures rather than independent samples based on the predicted spectral dataset. The Ward's agglomerative method with Euclidean distance (Ward, 1963) was selected for its ability to effectively differentiate groups in a multivariate Euclidean space. However, due to the extensive amount of data, only records from April to October and from the years 2018-2023 were included, around 55,759 records used for calculating distances for each observation and cluster analysis. To address the clustering issue, a divide-and-conquer strategy was implemented by dividing the data into 100 subsets, clustering them separately, and then merging the centroids obtained from each subset (Wang *et al*., 2016). This process was repeated five times to ensure the reliability of the final clustering outcomes. The inertia of the dendrogram was utilized to determine the optimal number of groups by identifying a point where there is a significant decrease in inertia gain. This iterative approach enabled efficient analysis of the data despite its magnitude, showcasing a practical solution for managing challenges associated with big data.

Cluster interpretation

Traditional statistical methods such as ANOVA were not appropriate for this analysis due to the high volume of records, which could result in all effects being considered significant. Instead, the emphasis was on interpreting the clusters to understand their implications. To compare the clusters, their distinctions were highlighted by calculating the least squares means (LSM) per group and displaying them in bar graphs on a standardized scale (Franceschini *et al*., 2022).

Results and discussion

The calibration methods were chosen based on the information provided in Table 1, which includes the statistical parameters for the Calibration data of the Weather Station THI3mean equations. A total of 140,618 records were utilized for each model, with a THI value ranging from 25 to 75, a mean of 51, and a standard deviation of 8.03.

The root squared mean ranged from 0.76 to 0.89, with RPDs varying between 2.05 and 3.08. Ultimately, the GLMNET model emerged as the most robust option, primarily due to its ability to eliminate irrelevant or noisy input parameters, reducing the number of variables to the essential minimum required. The model developed by LKV-BW was the first model to incorporate spectral data analysis and weather data from public stations;

Table 1. Statistical parameters for the calibration data of the THI3mean equation

Model	Number of samples	Min	Mean	Max	SD	SEC	R2	RPD
PCR	140,618	25	51	75	8.03	2.51	0.76	2.05
PLS	140,618	25	51	75	8.03	2.47	0.77	2.09
MVR	140,618	25	51	75	8.03	2.47	0.77	2.09
CPPLS	140,618	25	51	75	8.03	2.44	0.77	2.11
GLMNET	140,618	25	51	75	8.03	2.58	0.89	3.08

Table 2. Identification results of the final model – THI3mean based on the spectral model (1st Calibration), animal model (2nd Calibration) and Cross Validation model.

Model	Number of samples	Min	Mean	Max	SD	SEC	R2	RPD
1st Calibration	98.434	25	51	75	7.92	2.58	0.89	3.08
1st Validation	42.184	25	50	75	7.93	2.58	0.89	3.08
2nd Calibration	98.435	25	51	75	7.93	2.58	0.89	3.07
2nd Validation	42.183	25	50	75	7.93	2.57	0.90	3.09
Cross Validation	140.618	25	51	75	8.03	2.58	0.89	3.08

although it does not predict heat stress, it forecasts the temperature and humidity index. The prediction results are promising, with an R² value close to 0.89 based on THI3mean and the predicted CowTHI3mean value. *Table 2* displays the calibration and validation datasets for the GLMNET model. To randomly select different datasets, the Mahalanobis distance was calculated for the first calibration and validation model among all spectral data points. This distance is determined by measuring the test point's distance from the centre of mass divided by the width of the ellipsoid in the direction of the test point (Mahalanobis, 1936). Based on this distance calculation, 70% of spectral data points were chosen for the calibration model and 30% for the validation model. For the second calibration and validation dataset, random selection was performed among all animals. Based on the total number of animals in the dataset, 70% were included in the calibration model and 30% in the validation model. The cross-validation model is based on the K-fold cross-validation process, which can be implemented using the cv.glmnet function.

In addition to the standard glmnet parameters, cv.glmnet introduces its own unique parameters such as nfolds (indicating the number of folds), fold id (allowing for user-supplied folds), and type.measure (specifying the loss metric used for cross-validation): "deviance" or "mse" for squared loss, and "mae" for mean absolute error (Friedman *et al*., 2010).

Additionally, predictions of MIR spectral data for fatty acids and minerals (RobustMilk and OptiMIR), ketone bodies (OptiMIR and OptiKuh), as well as health and immunity markers (HappyMoo, GplusE, and D4Dairy) have been calculated using various MIR models from the afore mentioned projects. These predictions, along with milk components, were used to calculate Pearson correlations based on CowTHI3mean and WSmean3THI (see Figure 2).

Research conducted by Dale *et al*., 2023 and Lemal. P., *et al*., 2023 identified a positive correlation between the CowTHI3mean indicator and WSmean3THI in Germany and Belgium. This suggests that utilizing this indicator may be more effective than traditional traits such as milk yield, protein, or fatty acids.

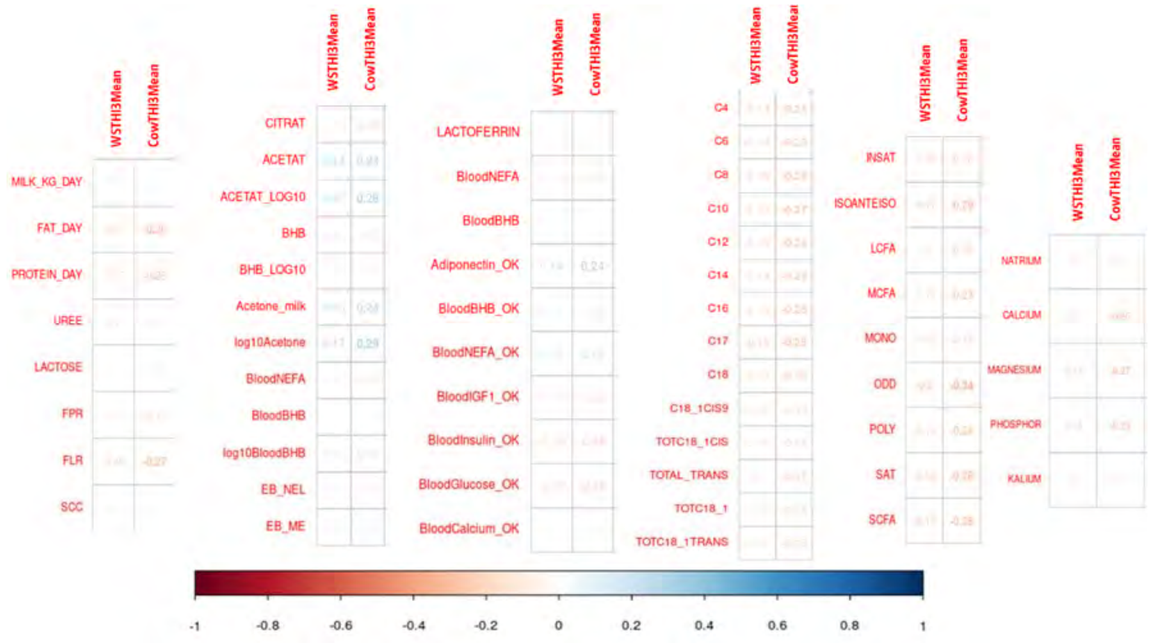

Figure 2. Pearson correlations between milk parameters and WSTHI3Mean and CowTHI3mean values.

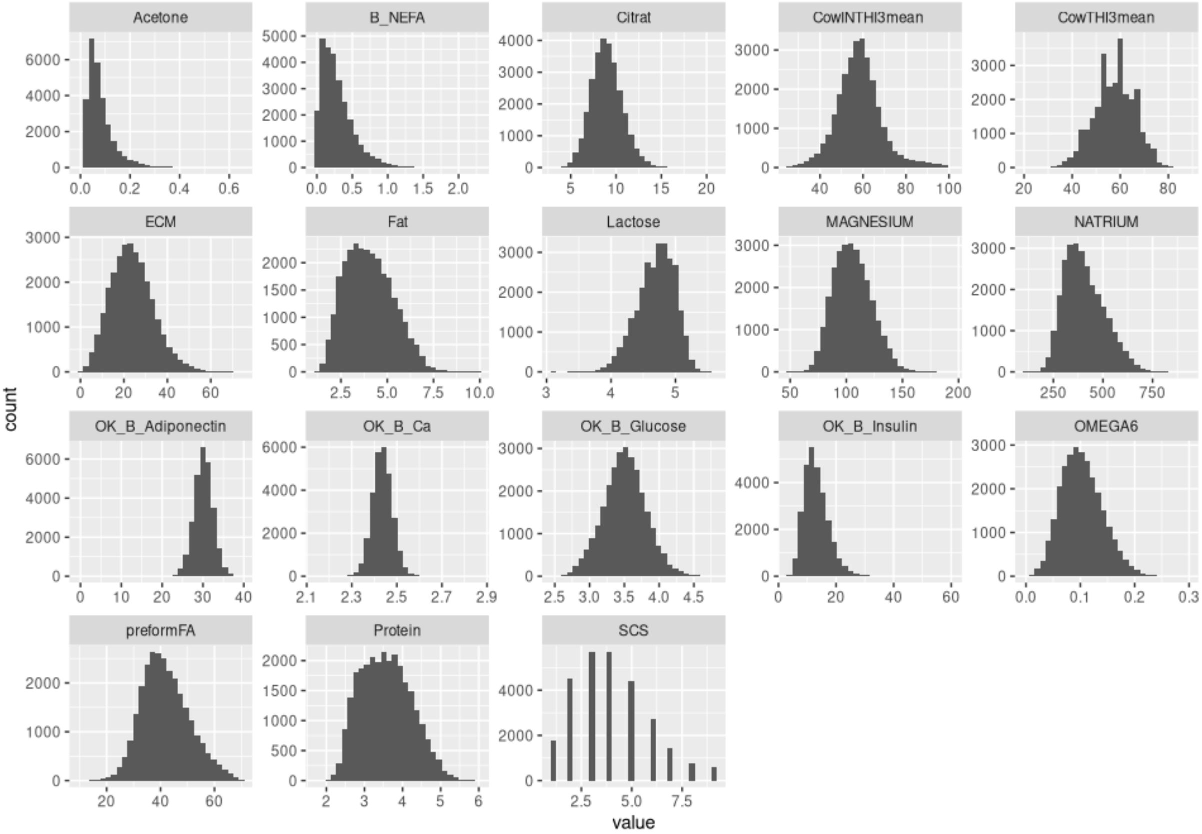

Figure 3. Data used in unsupervised clustering.

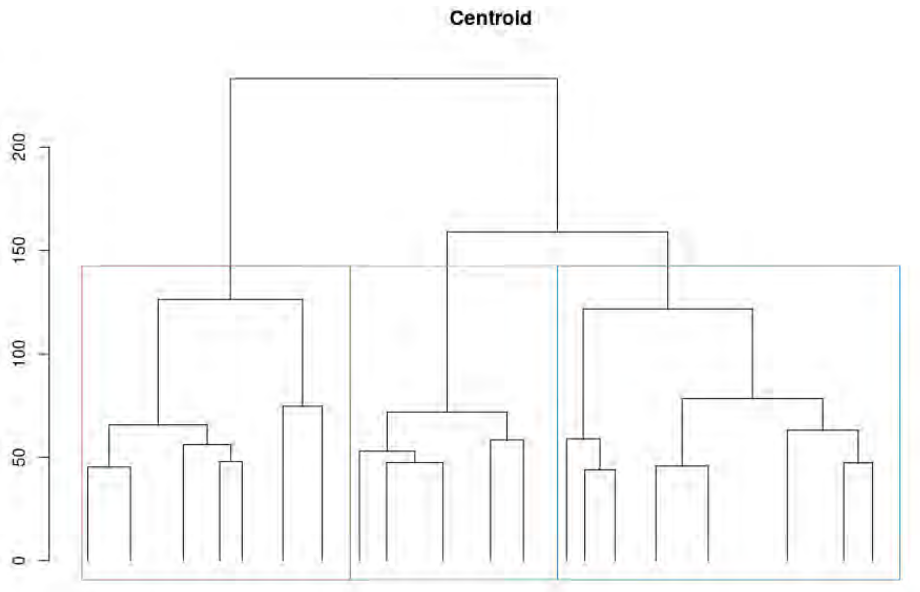

Figure 4. Cluster Analysis: Hierarchical clustering – LKVBW data set

For the unsupervised cluster analysis, the following mainly milk MIR based prediction variables were utilized: acetone in milk, blood NEFA (B_NEFE), citrate, CowTHI3mean, model for inside barns THI prediction based on Pessl and MobiMets devices (CowINTHI3mean), energy-corrected milk (ECM), fat, lactose, magnesium, sodium (natrium), blood adiponectin (O_B_Adiponenctin), blood calcium (OK_B_Ca), blood glucose (OK_B_Glucose), blood insulin (OK_B_Insulin),as well as omega-6 fatty acid, preformed fatty acid, protein, and somatic cell score (SCS). The distribution of the dataset used can be observed in Figure 3.

As accurately identifying heat stress based solely on CowTHI3mean values is challenging, cluster analysis was conducted in the HoliCow predictions dataset using these predictions as variables specifically between April and October, as well as from 2018 to 2023. The results revealed three distinct groups (refer to Figure 4). To further comprehend the cluster analysis, Classification and Regression Trees (CART) were utilized. It was observed that the trees' classification depended on whether the dependent variable was a numeric value.

In the CART analysis, the variables utilized for classification into the three groups were acetone in milk, blood NEFA, CowTHI3mean, lactose, blood glucose, omega-6 fatty acid, preformed fatty acid, and protein. These specific variables were chosen based on their significance in determining heat stress levels within the dataset. The inclusion of these factors allowed for a more comprehensive and low accurate classification of the data into distinct groups based on their respective values. As shown in Figure 5, class 1 was only predicted well at 54%, with 30% of the entire dataset classified as class 1. Class 2 was classified between 53% and 77% with a balanced accuracy of 63%, while class 3 was classified between 48% and 76% with a balanced accuracy of 71%. The cluster with the highest accuracy from the CART model was class 3, which also exhibited higher mean standard prediction (msp) for all components considered in the modelling process (Figure 6). Protein, magnesium, blood insulin, blood calcium, fat, and blood adiponectin had a positive msp higher than 0.75, while acetone, CowTHI3mean, ECM, preformed FA, and lactose had a negative msp lower than -0.35.

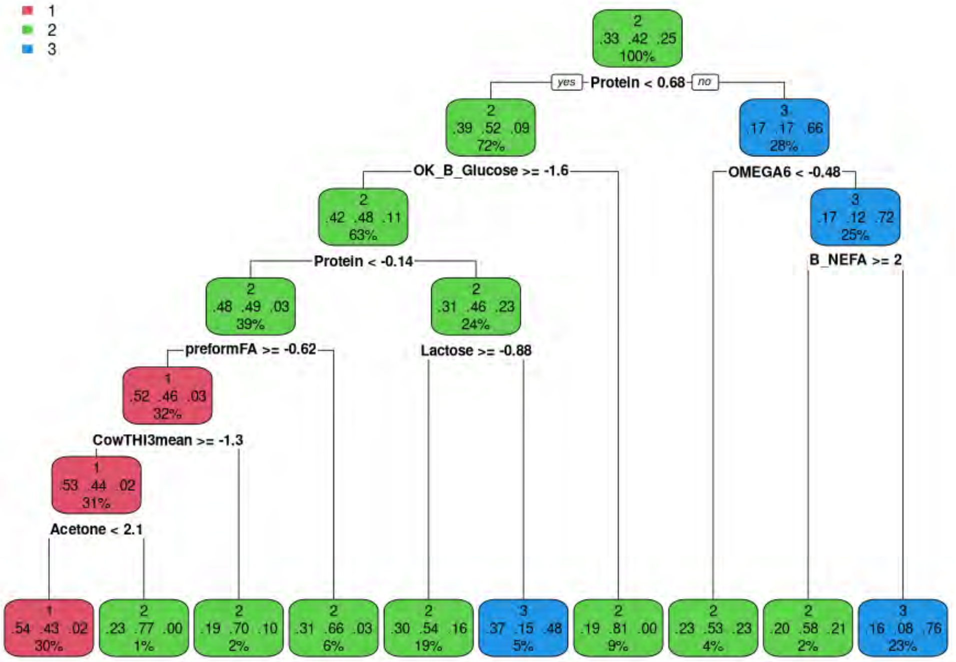

Figure 5. Classification and regression trees based on the cluster analysis.

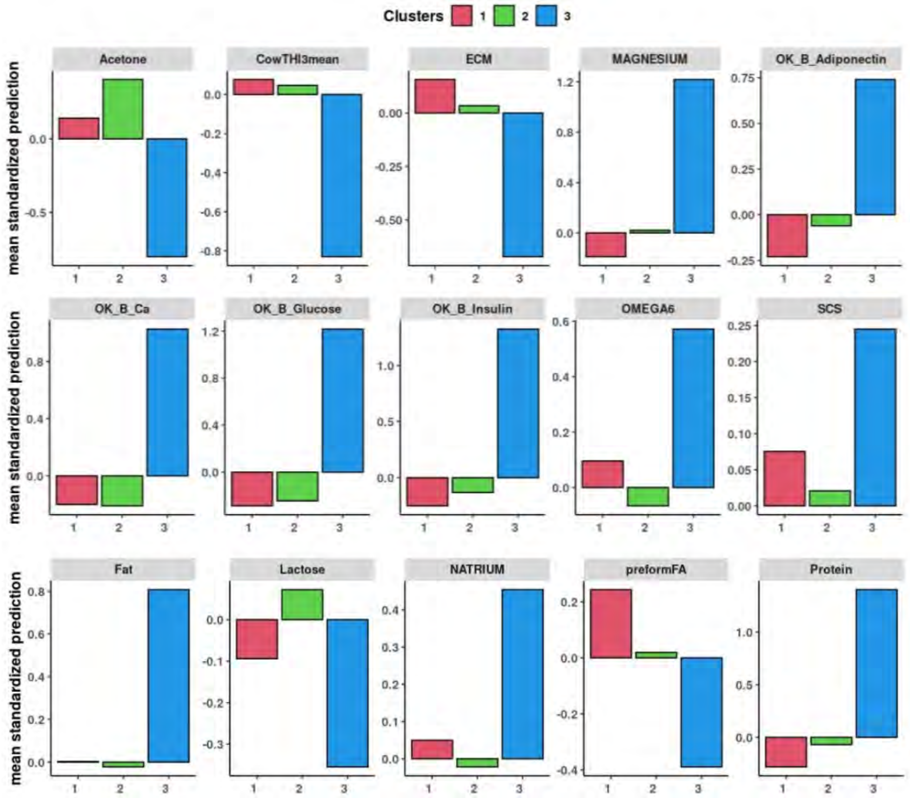

Figure 6. Correlation between cluster response and milk recording data and MIR spectra predictions used for clustering.

Conclusions

In the initial phase of the HoliCow project, significant progress and promising results have been noted. The project has shown great potential in achieving its objectives and goals, indicating a positive trajectory for future developments. The early findings suggest that the project is on track to deliver valuable insights and advancements in the field, setting a strong foundation for further research and innovation. The next steps involve validating the predictions against heat stress or health problems situations by utilizing the same animals in the time series analysis and closely monitoring the development of the clusters. The challenge of selecting the most suitable clustering algorithm arose due to the array of options available, including K-means, hierarchical clustering, and DBSCAN. The decision-making process considered our data and research objectives, with valuable input from the HoliCow research team and insights from previous studies (Franceschini *et al*., 2022). Cluster analysis is an iterative procedure, and plans are underway to interpret the results effectively to gain a deeper understanding of each cluster's characteristics in the upcoming phase of the project. It is crucial to refine the outcomes and improve the quality of clustering for practical implementation in pilot farming scenarios.

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Investigating the impact of heat stress and subsequent recovery on fatty acid profiles in bovine milk

P.A. Tu[1], M.K. Yang[1], Y.H. Yeh[1], J.W. Shiau[1], J.F. Huang[2] and Y.H. Chen[1]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture,
No.207-5, Pitoumian, Xihu Township, Miaoli 368003, Taiwan
[2]Taiwan Livestock Research Institute, No.112,
Farm Road, Hsinhua, Tainan 71246, Taiwan
Corresponding Author: hsuan@mail.tlri.gov.tw

Abstract

Heat stress (HS), particularly prevalent in tropical regions such as Taiwan, poses significant threats to animal health, dairy production, and the composition of milk. This stress affects not only the welfare and productivity of cows but also increases the costs of herd management, thereby impacting the profitability of dairy farming. Under the ongoing climate change, Taiwan is expected to experience more frequent high-temperature days, emphasizing the need to evaluate and mitigate the adverse effects of HS on dairy production. HS induces various physiological changes in dairy cattle, including increased respiration and heart rates, along with a rise in core body temperature. The most profound impacts of HS are observed in the form of reduced dry matter intake and a decline in milk yield. These changes are attributed to energy-intensive metabolic adaptations that cattle undergo for heat dissipation, which in turn contribute to the decrease in milk production. An experimental study of HS was conducted over 4 consecutive days, with a daily average temperature-humidity index (THI) exceeding 74, followed by a recovery period with an average daily THI below 68. Milk samples were collected bi-daily during this period, which included a baseline phase (days 1-3), the HS phase (days 5 and 7), and the recovery phase (days 9 and 11). These samples were analyzed for their fatty acid (FA) profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total de novo FA, mixed FA, and preformed FA, using MilkoScan FT$^+$ 300 equipped with Fourier-transform infrared spectra. The results from this experiment showed that HS caused a significant reduction in the relative percentage of SFA, de novo FA, mixed FA, MCFA, C14:0, and C16:0 FAs, accompanied by an increase of that in UFA, preformed FA, LCFA, C18:0, and C18:1 FAs. These changes in the FA profile are expected to alter the physical properties and nutritional value of milk fat. While some FA levels partially returned to normal during the recovery phase, they did not fully revert after short periods of recovery. This study highlights the metabolic adaptations of lactating cattle in response to acute HS. There was a noticeable shift in the milk FA profile, characterized by a decrease in FAs predominantly containing SCFA to MCFA, and an increase in those primarily consisting of LCFA. These alterations in FAs could potentially serve as biomarkers for HS in dairy cattle, providing a valuable tool for daily monitoring and management. In conclusion, HS profoundly influences the FA profile of bovine milk, signifying a metabolic shift towards increased LCFA. This alteration, not completely reversible even in a short-term recovery phase, strengthens the critical need for effective HS management and abatement strategies in dairy farming. This is particularly urgent given the rising global temperatures, which could exacerbate the HS challenges to the dairy industry in Taiwan.

Keywords: milk, fatty acid, heat stress, recovery.
Presented at: Session 2: SC Milk Analysis – New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

HS is particularly prevalent in tropical regions such as Taiwan and poses significant threats to animal health, dairy production, and the composition of milk (Liu *et al*, 2019). This stress not only affects the welfare and productivity of cows but also increases the costs of herd management, thereby impacting the profitability of dairy farming (Kadzere *et al*, 2002). Under ongoing climate change, it is expected that Taiwan will experience more frequent high-temperature days (Ho *et al*, 2016) which emphasizes the need to evaluate and mitigate the adverse effects of HS on dairy production (Summer *et al*, 2019). Physiological changes induced by HS in dairy cattle include increased respiration and heart rates along with a rise in core body temperature (Polsky and Keyserlingk, 2017). The most profound impacts are observed in reduced dry matter intake and decline in milk yield - attributed to energy-intensive metabolic adaptations for heat dissipation contributing to decreased milk production.

The influence of HS on milk composition, beyond milk yield, has been the subject of extensive research. Several studies have reported a decrease in total protein and total fat content in milk under HS conditions (Bernabucci *et al*, 2015; Hill and Wall, 2015). However, conflicting findings exist, with some studies indicating no significant decrease in fat percentage in heat-stressed cows (Hammami *et al*, 2015; Lacetera *et al*, 2003). Additionally, there is evidence suggesting that an increase in the THI is associated with a decrease in the content of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA), and an increase in long-chain fatty acids (LCFA) (Lacetera *et al*, 2003). Despite these findings, there is a lack of detailed information regarding the impact of HS and subsequent recovery on milk FA profiles. Therefore, our study aims to investigate the effects of HS and subsequent recovery on milk FA profiles.

Material and methods

Cows and experimental design

- The study was conducted using lactating Holstein cows in a commercial dairy farm in Taiwan. A total of 77 Holstein dairy cows in early lactation were subjected to a heat challenge for over 3 consecutive stages. First with a baseline phase with a temperature-humidity index (THI) below 68. Then, an HS phase with a daily average THI exceeding 74, followed by a recovery period with an average daily THI below 68. Milk samples were collected bi-daily during this period, which includedBaseline phase (day 1-3).
- HS phase (day 5 and day 7), and the
- Recovery phase (day 9 and day 11).

Milk FA profile analysis

Milk samples were thoroughly analyzed for a variety of fatty acid profiles, which included saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), *de novo* FA/newly synthesized FA, mixed FA, C14:0 fatty acid (FA), C16:0 FA, C18:0 FA, and C18:1 FA . The analysis was performed using the MilkoScan FT$^+$ 300 equipped with Fourier-transform infrared spectra.

Statistical analysis

Statistical analysis included the use of ANOVA and Tukey's post hoc test to assess differences in FA profiles among the different days in baseline, HS, and recovery phases.

Results

Effects of HS on FA profiles in milk

The results from the study showed that HS caused a significant reduction in the relative percentage of SFA, de novo FA, mixed FA, MCFA, C14:0, and C16:0 FAs, accompanied by an increase in USFA, preformed FA, LCFA, C18:0, and C18:1 FAs (Figure 1 and 2). These changes in FA profiles indicate that HS leads to alterations in milk composition, with a shift towards higher levels of unsaturated and long-chain fatty acids. The metabolic shifts observed during HS, particularly the decrease in FAs predominantly containing SC FA to MCFA, and an increase in those primarily consisting of LCFA, demonstrate the profound influence of HS on the metabolic adaptations of lactating cattle (Table 1).

Recovery of FA Profiles following HS

The recovery phase showed some restoration in the FA profiles, with a partial reversal of the changes observed during HS. While some FA levels partially returned to normal during the recovery phase, they did not fully revert after short periods of recovery (Figure 1 and 2). The *de novo* FA, mixed FA, SCFA, C14:0 FA, C16:0 and MCFAs' levels showed a partial recovery but remained lower than baseline levels during the recovery phase. For UFA, PUFA, LCFAs', C18:0 and C18:1 FAs didn't show significant reduction during the recovery phase. During this period MUFA and preformed FAs significantly decreased (P < 0.05), suggesting that HS impact on bovine milk's FA profiles is not completely reversible in the short term (Table 1).

Table 1. Effect of heat stress on fatty acid (FA) composition of milk fat.

Milk FA	**mg/g of total FA**					
	Baseline	**D5 heat stress**	**D7 heat stress**	**D9 Recovery**	**D11 Recovery**	**P value**
C14:0	96.9^{a}	93.2^{a}	86.1^{b}	78.4^{c}	83.1^{bc}	< 0.0001
C16:0	347.8^{a}	301.2^{d}	309.9^{cd}	316.7^{c}	334.4^{b}	< 0.0001
C18:0	84.1^{b}	83.8^{b}	89.5^{a}	90.4^{a}	88.4^{ab}	< 0.0001
C18:1	198.7^{d}	223.8^{bc}	232.6^{ab}	236.1^{a}	220.8^{c}	< 0.0001
Saturated FA	681.1^{a}	619.7^{bc}	611.2^{c}	606.1^{c}	633.2^{b}	< 0.0001
Unsaturated FA	234.7^{c}	262.8^{b}	278.4^{a}	275.2^{a}	254.1^{b}	< 0.0001
Mono-unsaturated FA	213.1^{d}	242.5^{b}	253.5^{a}	249.6^{ab}	229.3^{c}	< 0.0001
Poly-unsaturated FA	21.7^{b}	20.3^{b}	24.9^{a}	25.6^{a}	24.8^{a}	< 0.0001
SCFA	83.2^{a}	72.5^{b}	71.7^{b}	71.7^{b}	76.4^{b}	< 0.0001
MCFA	525.7^{a}	452.6^{c}	453.9^{c}	457.5^{c}	488.9^{b}	< 0.0001
LCFA	299.9^{c}	322.7^{b}	337.9^{a}	346.5^{a}	319.6^{b}	< 0.0001
Total de novo FA	216.1^{a}	169.0^{c}	165.2^{c}	172.0^{c}	190.8^{b}	< 0.0001
Mixed FA	385.4^{a}	357.1^{c}	366.3^{bc}	366.9^{bc}	378.5^{ab}	0.008
Preformed FA	331.9^{c}	384.6^{a}	387.9^{a}	386.5^{a}	355.8^{b}	< 0.0001

Each value is the mean of 77 samples.
Total de novo FA = sum of C4:0 to C14:1 fatty acids; total mixed FA = sum of C16:0 and C16:1 fatty acids; total preformed = sum of all fatty acids with more than 15 carbon atoms.
$^{a\text{-}d}$ Least squares means with different superscripts within a row are significantly different (P < 0.05).

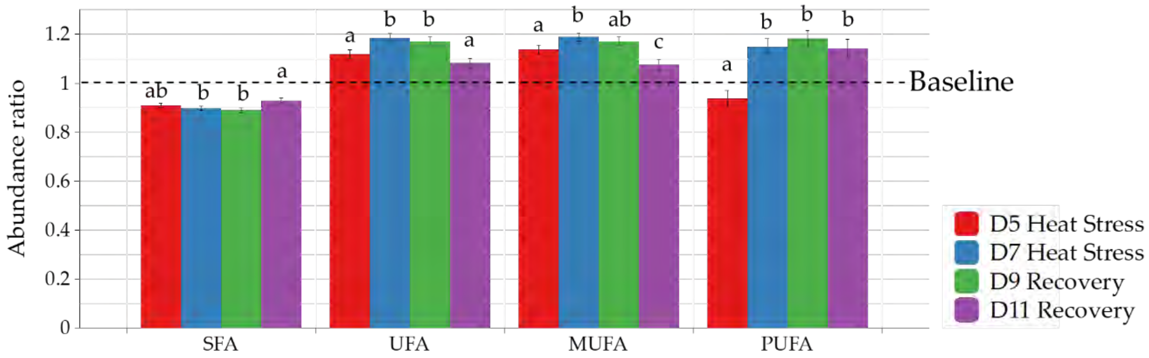

Figure 1. Effect of heat stress on the relative abundance of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and unsaturated fatty acids (UFA) in milk. Abundance ratios for SFA, MUFA, PUFA, and UFA during days 5 and 7 of heat stress (HS) and days 9 and 11 of recovery are presented in comparison to the baseline levels observed on days 1 to 3. Error bars represent standard error (n = 77). Statistically significant differences among different HS and recovery phases are indicated by different superscripts (P < 0.05).

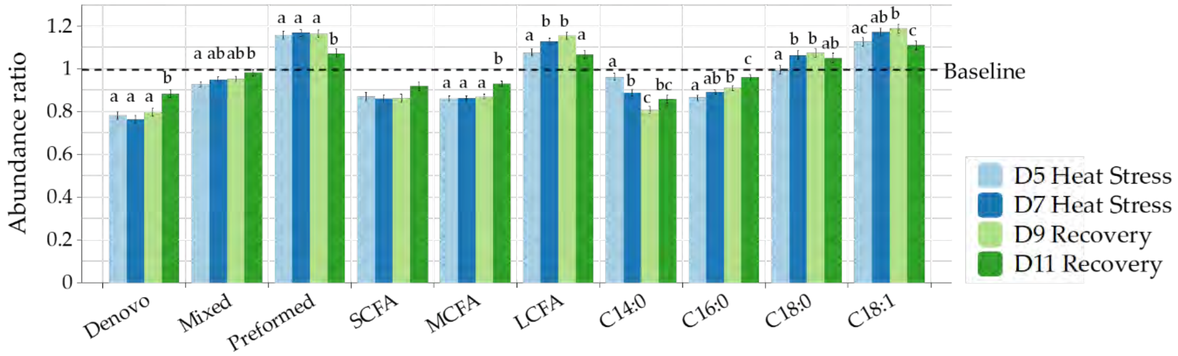

Figure 2. Effect of heat stress on the relative abundance of de-novo fatty acids (de-novo FA), mixed fatty acids (mixed FA), preformed fatty acid (preformed FA), short chain fatty acid (SCFA), medium chain fatty acid (MCFA), long chain fatty acid (LCFA), C14:0 fatty acid (FA), C16:0 FA, C18:0 FA, and C18:1 FA during days 5 and 7 of heat stress (HS) and days 9 and 11 of recovery are presented in comparison to the baseline levels observed on days 1 to 3. Error bars represent standard error (n = 77). Statistically significant differences among different HS and recovery phases are indicated by different superscripts (P < 0.05).

Discussion

It is well established that most of the C4:0 to C14:0 and almost half of the C16:0 FA in milk are synthesized de novo in the mammary gland, whereas the rest of the C16:0 and approximately all LCFA originate from blood lipids (Knudsen *et al*, 1986). HS can significantly alter the synthesis and composition of these FAs in bovine milk (Li *et al*, 2016). Our study's findings highlight the metabolic adaptations of lactating cattle in response to HS. The significant alterations in the FA profiles, particularly the shift towards increased LCFA, are indicative of the physiological changes that occur in dairy cows during HS and subsequent recovery periods.

Understanding these changes in the FA profile is crucial for evaluating the impact of HS on the nutritional composition of milk and its potential implications for human consumption. Furthermore, the results suggest that the altered FA profiles observed during HS may have implications for dairy product quality (Jenkins and McGuire, 2006). These findings have important implications for the dairy industry, as they demonstrate that HS can significantly impact the FA composition of milk. HS affects the quality of dairy products primarily by altering the composition of FAs in cow's milk, which can have implications for the nutritional value, taste, and processing properties of the products (Liu *et al*, 2017). Research indicates that HS causes changes in the triacylglycerol (TAG) profile of milk, with a reduction in TAG groups containing SCFA and MCFA and an increase in those containing LCFA. This change in TAG composition could modify the physical properties of milk fat. Additionally, HS was shown to significantly reduce the levels of certain polar lipid classes, which are main structural constituents of the milk fat globule membrane and play a critical role in stabilizing the milk emulsion system (McManaman, 2014).

The reduction in SCFA and the increase in LCFA during HS is a clear indication of the metabolic adaptations of lactating cattle to high-temperature conditions. Hammami *et al* (2015) also showed similar results, indicating that the rise in THI between seasons correlated with a reduction in the content of SCFA and MCFA, and an increase in LCFA. HS might also lead to a decline in total protein and total fat content in milk, which can affect the texture and flavor of dairy products (Chandan, 1997). Furthermore, the reduction in milk fat globule membrane polar lipids, such as sphingomyelin which has beneficial effects on human health, could have implications for the nutritional value of milk and its health benefits (Lopez *et al*, 2008). Consequently, HS not only presents challenges for maintaining the welfare and productivity of dairy cattle but also for preserving the quality of milk and dairy-related foods.

The findings of this study underscore the need for effective HS management strategies in dairy farming, especially in regions like Taiwan that are projected to experience more frequent high-temperature days due to climate change. Adaptive measures such as improved ventilation, access to shade, and cooling systems can help mitigate the adverse effects of HS on dairy cattle, thereby preserving milk quality and quantity. Furthermore, the observed partial recovery of FA profiles during the recovery phase suggests that interventions to support lactating cows during and after HS periods can aid in restoring milk composition to some extent. This highlights the potential for targeted nutritional and management interventions to minimize the long-term impact of HS on milk FA profiles and overall dairy production.

The study also underscores the potential use of the observed changes in FA profiles as biomarkers for HS in dairy cattle. The relatively rapid change of these markers made it possible for routine monitoring and early detection of heat stress in dairy cows. This suggests that monitoring the FA profiles of milk could provide a valuable tool for daily assessment and management of HS in dairy farming. Identifying and implementing effective heat alleviation strategies based on these biomarkers could help minimize the impact of HS on dairy production and product quality.

Conclusion

The study provides important insights into how heat stress affects lactating cattle's metabolism and milk composition. It highlights the increase in long-chain fatty acids in bovine milk and emphasizes the need for proactive measures to protect dairy production from environmental challenges. Understanding these biochemical changes can help dairy farmers implement strategies to maintain animal well-being and milk quality in changing climate conditions, while also serving as valuable biomarkers for monitoring heat stress effects on milk production.

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From milk recorder to bulk tank: understanding factors affecting consistency in fat and protein reporting

M. Thompson[1] and D.P Berry[2]

[1]*The Irish Cattle Breeding Federation (icbf), ICBF, Link Road, Ballincollig,*
P31 D452, Co. Cork, Ireland
[2]*Animal and Grassland research and Innovation Centre, Teagasc,*
Moorepark, Fermoy, Co. Cork, Ireland
Corresponding Author: mthompson@icbf.com

Abstract

Irish dairy producers milk record their cows, on average, 4 times annually; results reported include milk fat and protein percent (as well as milk yield). Farmers receive a mean herd (i.e, bulk tank) fat and protein percent generally every two days. Discrepancies often exist between both measures taken at approximately the same day.

The mean of the reported fat and protein percent of all cows milk recorded on a given day in a herd was compared to the bulk milk results taken on the same day; also compared was the mean of the three bulk milk collections taken before and after a milk recording event.

The dataset comprised of 4,660 test day milk recordings from 1,784 herds in the year 2023. Fat and protein percentages were, on average, underestimated for the milk recording sample compared to the bulk samples. The discrepancy between test day milk recordings and bulk collections were greater for fat percentage then protein percentage.

On average, milk recorded fat and protein percentages both underestimated relative to the bulk collections. The mean of the three bulk collections taken after a milk recording deviated, on average, more from the milk recording values, while those taken on the same day and before the recording were better aligned.

The Pearson correlations between milk recorded and bulk tank fat percent taken on the same day was 0.857 whilst the mean of the three bulk collections before and after a given milk recording had respective Pearson correlation of 0.841 and 0.828.

The root mean square error (RMSE) of the residuals between milk recorded and bulk collected fat on the same day were 0.347, whereas the RMSE for the mean of the three bulks collections before and after a milk recording were 0.353 and 0.424, respectively. This analysis also highlighted the influence of factors such as the type milk recording device, herd average cow yields and seasonal effects on the values reported in both milk recordings and bulk collections. However, they only scratch the surface when trying to gain a true understanding of the reasons discrepancies, particularly in fat % occur.

Keywords: milk recording; Irish dairy.
Presented at the ICAR Anual Conference 2024 in Bled at the Session Session 3: Factors Affecting the Accuracy of the Recording Day

Introduction

Milk recording (MR) is a crucial management tool for Irish dairy farmers, providing valuable insights into herd health and performance. Since the abolition of milk quotas in 2015, the practice of milk recording has grown steadily on Irish dairy herds. According to the latest figures from the ICBF database, over 1.1 million cows are milk recording across 9,100 herds. Typically, Irish dairy producers milk record their cows' four times annually, receiving data on milk fat, protein percentages, and yield.

Farmers also receive bulk tank (BT) results, which report the mean herd fat and protein percentages approximately every two days. However, discrepancies often arise between the results obtained during milk recordings and the bulk milk samples, even when both are measured on the same day.

The primary objective of this study is to explore and quantify the discrepancies between milk recording and bulk milk collection results on Irish dairy farms. Furthermore, the study aims to investigate factors that may contribute to inconsistencies in the reported fat and protein percentages, thereby improving the understanding of why differences may occur.

Material and methods

Data

The data used in this study were sourced from a pre-existing database managed by the Irish Cattle Breeding Federation (ICBF). Test day milk recording (MR) and bulk tank (BT) herd averages were extracted from this database. MR samples were recorded using the alternative AM-PM recording scheme, as approved by the International Committee for Animal Recording (ICAR, 2021). BT samples were collected and reported by dairy processors during on-farm bulk milk collections.

The dataset included 4,660 test day MR records from 1,784 herds, each of which had a corresponding bulk tank recording taken on the same day. All herds had a minimum of four milk recordings in 2023 and were contracted to supply milk to dairy processors.

Analysis

The mean fat and protein percent of all cows recorded in a herd on a given test day were compared to the corresponding bulk milk results collected on the same day. Additionally, comparisons were made to the mean fat and protein percent of the three bulk milk collections taken both before and after a milk recording event. Accuracy was assessed using Pearson correlations and the root mean squared error (RMSE) of the residuals between MR and BT results.

Results

Summary statistics

Table 1 presents the summary statistics for fat and protein percentages recorded during MR and BT collections, including the mean, standard deviation (SD), and minimum/ maximum values.

On average , MR fat and protein percent is underestimated compared to the BT with the largest discrepancies occurring in fat percent. These discrepancies are reflected by Pearson correlations of 0.853 and 0.929 for fat and protein percent, respectively, with corresponding RMSE values of 0.352 for fat and 0.124 for protein percent.

The mean of the three bulk milk collections taken after a milk recording deviated more from the milk recording values than those taken on the same day or before the recording. Pearson correlations between MR and BT fat percent taken on the same day

Table 1. Summary statistics of MR & DB milk fat and protein percentage.

Trait		Mean	SD	Min/Max
MR	Fat %	4.33	0.56	2.87/7.12
	Protein %	3.67	0.29	2.92/5.07
BULK	Fat %	4.52	0.52	3.36/6.88
	Protein %	3.75	0.29	2.97/4.91

Table 2. Pearson correlation and RMSE between MR fat percent and the bulk fat percent taken on the same day, the mean of the 3 bulks before and after.

Fat %	Pearson Correlation	RMSE
Same Day MR-Bulk	0.857	0.347
Mean of Three Bulks Before	0.841	0.353
Mean of Three Bulks After	0.828	0.424

Table 3. The Impact of recording service type on Pearson Correlations and RMSE of fat and protein percent between MR and bulk samples.

Recording type	Number of herds	Fat % correlation	Fat % RMSE	Protein % correlation	Protein % RMSE
EDIY	2,036	0.89	0.36	0.94	0.13
Manual	2,624	0.82	0.35	0.92	0.12

were 0.857, while the correlations for the mean of the three bulk collections before and after the MR were 0.841 and 0.828, respectively. The RMSE of the residuals between MR and BT fat percent was 0.347 for same-day comparisons, and 0.353 and 0.424 for the bulk collections before and after, respectively.

Impact of recording type

Table 3 shows the impact of milk recording device type on the Pearson correlations and RMSE for fat and protein percent between MR and BT samples. The EDIY recording method had higher correlations and RMSE values compared to the manual recording method.

Impact of test day yield and season

Average test day cow yields ranged from less than 10L to 40L across all test day MR events. Herds were categorized based on average test day cow yield, and Pearson correlations and RMSE were calculated within each category. A downward trend was observed in fat percent correlations as test day yields increased, suggesting that discrepancies in fat percent grow with higher yields. However, the differences in RMSE across yield categories were less pronounced. Similarly, correlations for fat percent were lower during the peak milk season, when yields were higher, compared to the off-peak season when yields were lower. The RMSE across seasons showed only minor differences, suggesting that discrepancies in fat percent are less sensitive to seasonal variations than yield levels.

Discussion

This comparative analysis highlights some of the challenges in aligning milk recording (MR) results with bulk tank (BT) samples. The discrepancies observed are likely due to a combination of factors beyond just milk recording type, yield, and seasonality. These potential contributors include:

- **Multiple Milkings in Bulk Tanks**: Milk composition and yield can vary significantly from one milking to the next (Quist et al., 2008). Consequently, aligning the results of a bulk tank sample, which often contains milk from several milkings, with a single milk recording sample is inherently difficult.
- **Assumption of Gold Standard**: While this analysis focuses on identifying factors affecting MR results, it is important to acknowledge that we lack insight into the factors influencing BT sampling. Bulk tank sampling procedures are generally well-documented; however, limited information is available on specific factors impacting fat and protein percentages during bulk collections. Given the sensitivity of MR samples, particularly in determining fat percentages (Fouz et al., 2009), various sources of error may exist during BT collection, potentially affecting the consistency of results when comparing BT to MR sample.
- **Variety of Milking Meters**: Irish dairy farmers use a wide variety of milking meters. Around 50% of herds use electronic DIY (EDIY) systems, such as Tru-Test meters, while the remaining herds rely on various manual meters, including ICAR-approved, non-ICAR-approved, jar meters, and others. The frequency of servicing and calibration of these meters to both manufacturer and ICAR standards is largely unknown and is often left to the discretion of farmers, introducing another layer of potential error that may contribute to inconsistency in reporting.
- **Inconsistent milking machine performance**: Milking machine performance metrics, such as vacuum level and milk flow rate, have been shown to significantly impact overall milking performance (Besier and Bruckmaier, 2016). Air leaks in liners or sub optimal liner type can lead to fluctuations in vacuum and flow rates and result in under- or over-milking, which may have a considerable impact on a given MR sampling (O'Callaghan and Gleeson, 2004).

A follow-up study has been commissioned to investigate the relationship between milking machine performance and MR reporting. This study will provide a more comprehensive understanding of the factors contributing to MR discrepancies at the farm level.

Conclusion

On average, fat and protein percent reported in milk recordings are underestimated compared to bulk tank results, with the largest discrepancies observed in fat percent reporting. While factors such as recording method, yield, and seasonality influence these discrepancies, they do not fully explain them. The underlying issues likely stem from the milk recording infrastructure, including milking machine performance, calibration, and certification. Additional research is necessary to understand the causes behind the misalignment of MR and BT fat and protein percentages.

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Analysis of factors affecting daily milk yields: an initial case study in an automatic thrice-milking farm

X.-L. Wu[1,2]**, M.J. Caputo**[1]**, G.R. Wiggans**[1]**, H.D. Norman**[1]**, A.M. Miles**[3]**, C.P. Van Tassell**[3]**, R.L. Baldwin VI**[3]**, S. Sievert**[4]**, J. Mattison**[4]**, J. Burchard**[1] **and J. Dürr**[1]*

[1] Council on Dairy Cattle Breeding, Bowie, MD, USA
[2] Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, USA
[3] USDA Animal Genomics and Improvement Laboratory, Beltsville, MD, USA
[4] National Dairy Herd Information Association, Verona, WI, USA

Abstract

The methodologies and parameters for estimating daily milk yields in the United States were mainly developed from the 1960s through the 1990s. A recent initiative by the Council on Dairy Cattle Breeding, USDA-AGIL, and the National DHIA aims to update these methods and parameters for estimating daily yields by collecting and analyzing milking data from dairy farms. This study, serving as an initial case study, examined the factors influencing daily milk yield estimation at a dairy farm in New York State and compared the performance of the existing method with a recently proposed one. In total, 63,562 milking data were extracted from approximately 2,200 cows milked thrice daily in this farm. Data cleaning eliminated incomplete or missing records, retaining 47,670 entries from 1,869 cows for subsequent analyses. The average partial yields in kilograms (milking interval time in hours) of the three milkings were 14.6, 16.5, and 13.8 (7.88, 8.79, and 7.25), respectively. Analysis of variance revealed significant effects of milking interval time and months in milk on proportional daily milk yields. The lactation effects on proportional daily yields were significant for the first two milkings but not for the third milking. Nevertheless, the relative importance of milking interval time and lactations was very low. Omitting these two variables resulted in the Wiggans (1986) model. The polynomial-interaction-regression model analysis showed significant effects from partial yields and significant interactions between partial yields and milking interval times on daily yields. The new model gave more accurate estimates than the Wiggans (1986) model. Regarding the relative predictability of the three milkings, the 2nd milkings, having the longest average milking interval time, gave more accurate estimates than the 1st and 3rd milkings. The calculated MCFs in this farm increased slightly for the 1st milkings and remained roughly comparable (or slightly decreased) for the 2nd and 3rd milkings compared to the Wiggans (1986) assessment. These results suggest only minor changes in daily yield correction factors over the past four decades.

Keywords: Accuracy, dairy cattle, milking interval time, interactions, lactation, test-day
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Introduction

The 1960s witnessed a significant shift in milk testing in the United States. Previously, farms followed a rigorous schedule of twice-daily milk tests conducted under supervision every month. This system then shifted towards more economical sampling methods to reduce the costs associated with supervisory visits by the Dairy Herd Improvement Association (**DHIA**). Test frequencies are often adopted to align with varied herd management practices. On a test day, a cow may be milked several times, but not all milkings contribute to the recorded yield. One prevalent technique is the morning and evening (**AM-PM**) method, which alternates between sampling either the morning or evening milking throughout the lactation period (Porzio, 1953). Initially, the total daily milk yield (**DMY**) was estimated by doubling the yield of a single milking, assuming equal length and rate of milk production across both sessions, each lasting precisely 12 hours. Yet, this assumption often does not hold. Morning milking intervals tend to be longer than afternoon milking intervals. Hence, AM milk yields are usually higher than PM milk yields (Puttnam and Gilmore, 1970).

Various statistical approaches have been developed to estimate daily milk yields from incomplete milking data (reviewed by Wu *et al*., 2023a,b). The methodologies and parameters for estimating DMY in the United States were primarily developed from the 1960s through the 1990s. A recent initiative by the Council on Dairy Cattle Breeding, USDA-AGIL, and the National DHIA seeks to update these methods and parameters for estimating DMY by collecting and analyzing milking data from dairy farms. This study examined the factors influencing DMY estimation at a specific site, Farm 1 in New York State, and compared the performance of the existing method with a recently proposed one for estimating daily DMY. It represented an initial case study amid ongoing or planned data collection at other locations.

Materials and methods

Milking data

We extracted 63,562 milking data from Farm 1, representing thrice-milkings daily for around 2,200 Holstein cows. Milkings were collected and weighed at all three milkings for 18 weeks, starting May 5 and ending September 1, 2023. After that, three-day monthly milking data collections were carried out up to 305 days of milk and beyond. Milking times are 4am-12pm (1st milking), 12pm-8pm (2nd milking), and 8pm-4am (3d milking). Milk yields and timestamps were extracted from BouMatic parlor software (https://boumatic.com/us_en/). Records with incomplete and missing data were removed. Milking records with prolonged lactation beyond 305d for up to one more month were retained. Records with days in milk greater than 335 days, approximately accounted for 0.6% of the milking records, were excluded. After data cleaning, we retained 47,670 milking records representing 1,869 cows. The cleaned data represented up to nine lactations (Figure 1), with 64.0% from the first two lactations and 97.1% from the first five lactations. Milking records from lactation six and beyond, accounting for 2.9%, were pooled. Around 74.1% of the cleaned milking records were collected before 156 days in milk, and around 95.5% were collected before 250 days.

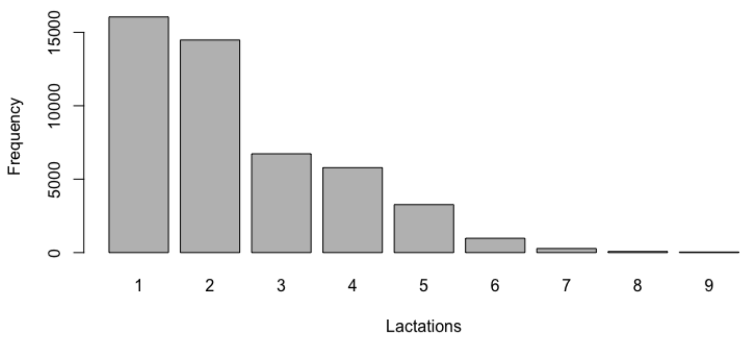

Figure 1. Distribution of milking records by lactation.

Two statistical models are defined. Firstly, for the *i*-th animal, a proportional DMY ($\frac{x_{ijl}}{y_{ijl}}$) is assumed to be a linear function of milking interval time (t_{ijl}), months in milk (m_j), lactations (γ_l), and a residual term (ε_{ijl}).

$$\frac{x_{ijl}}{y_{ijl}} = \alpha + \beta t_{ijl} + m_j + \gamma_l + \varepsilon_{ijl} \qquad (1)$$

The above model expands the Wiggans (1986) model by additionally including the lactations effects and replacing the linear days in milk effect with a categorical variable, months in milk.

MCF are derived for milking interval classes, each spanning 30 minutes while accounting for the average months in milk and lactation effects:

$$F_k = \frac{1}{\hat{\alpha} + \hat{\beta}\bar{t}^{(k)} + \bar{m} + \bar{\gamma}} \qquad (2)$$

where $\bar{t}^{(k)}$ is the average milking interval time for the k-th milking interval class, and $\bar{m}$ and $\bar{\gamma}$ are weighted averages for estimated months in milk and lactation effects, respectively. Omitting these two effects in (1) results in the Wiggans (1986) model, with MCF calculated as follows:

$$F_k = \frac{1}{\hat{\alpha} + \hat{\beta}\bar{t}^{(k)}} \qquad (3)$$

Hence, a DMY is estimated as follows:

$$\hat{y}_{ijl(k)} = F_k x_{ijl(k)} \qquad (4)$$

The second model, which we refer to as polynomial-interaction-regression, accounts for the interactions between partial yields and milking interval time, both linear and quadratic, as follows:

$$y_{ijl} = \left(b_0 + b_1 t_{ijl} + b_2 t_{ijl}^2\right) x_{ijl} + m_j + \gamma_l + \epsilon_{ijl}$$

$$= b_0 x_{ijl} + b_1\left(t_{ijl} x_{ijl}\right) + b_2\left(t_{ijl}^2 x_{ijl}\right)$$

$$+ m_j + \gamma_l + \epsilon_{ijl} \qquad (5)$$

MCF are derived pertaining to a specific milking interval time *t*,

$$F_t = \hat{b}_0 + \hat{b}_1 t + \hat{b}_2 t^2 \qquad (6)$$

In the above, the MCF at time t can be viewed as a baseline MCF, $F_0 = \hat{b}_0$ and adjusted according to the milking interval time, $\Delta_t = \hat{b}_1 t + \hat{b}_2 t^2$.

Then, a DMY is estimated as follows:

$$\hat{y}_{ijl} = F_{t=t_{ijl}} x_{ijl} + \hat{m}_j + \hat{\gamma}_l \qquad (7)$$

Here, $F_{t=t_{ijl}}$ stands for a MCF on specific milking interval time t, assigned to all animals satisfying $t_{ijl} = t$.

Accuracy measures

The accuracy of estimated DMY was evaluated based on two criteria: correlation and R^2 accuracy. The former is the correlation between estimated and actual DMY. The R^2 accuracy is the following:

$$R^2 accuracy = \frac{Var(y)}{Var(y)+MSE} \qquad (8)$$

where $V_{ar(y)}$ is actual phenotypic variance, and *MSE* stands for mean squared errors.

Analysis of variance (ANOVA) was conducted based on each of the two models separately. The importance of predictor variables was assessed by the Lindeman, Merenda, and Gold (LMG) metric of R squared (Lindeman *et al*., 1980). It measures the contribution of each predictor to the R-squared value, which is averaged over all possible orders of entering the predictors into the regression model. The confidence intervals for relative importances were obtained via 1000 bootstrap samples of the LMG R^2.

Results and discussions

Milking data summary statistics

Overall, the mean (95% Confidence interval) of test-day milk yields was 45.0 kg (28.6 ~ 62.8 kg). Across lactations, the average test-day milk yield increased from 38.1 kg on the first lactation to 47.2 kg on the second lactation 2, peaked (49.9 kg) on lactation 3, and then began to drop on lactation four and beyond, from 49.7 kg (lactation 4) to 48.8 kg (lactation 6+) (Figure 2; upper).

Average proportional daily yields showed slight variations between lactations, except lactation 1 (Figure 2; middle). Possibly, this was because the average milking interval times for the three milkings were also consistent across the lactations except the first lactation. Thus, it would be reasonable to compute common daily yield correction factors for later lactations, but arguable for the first lactation.

Overall, average proportional daily milk yields varied substantially between the three milkings (Figure 2; middle). The first milkings had the largest average proportional daily milking yield across lactations (0.35 – 0.38), followed by the third milkings (0.32 – 0.34); the second milkings had the least average proportional daily milk yield (0.31). The substantial differences in proportional daily yields were attributed to varied milking interval times for the three milkings (Figure 3; bottom). The average (95% confidence interval) of milking interval time was 8.79 (7.84-9.75) hours, 7.25 (6.39-8.07) hours, and 7.88 (7.06-8.81) hours, respectively, for the three milkings. On average, the first milking interval time was approximately 1 hour longer than the third and 1.5 hours longer than the second. Nevertheless, the average milking interval time varied very slightly between lactations, except for lactation one. Approximately the first milking interval time was 8.6 hours for lactation 1 and 8.9 hours for lactations 2 through 6+; the second milking interval time was 8.1 hours for lactation 1 and 7.8 hours for lactations 2 through 6+; the third milking interval time was 7.3 hours for lactation 1 and 7.3 hours (Figure 3; bottom). In accordance with the lengths of milking interval time, the first milkings had the largest average DMY (16.5 kg), followed by the third milkings (14.6 kg); the third milkings had the lowest average DMY (13.8 kg).

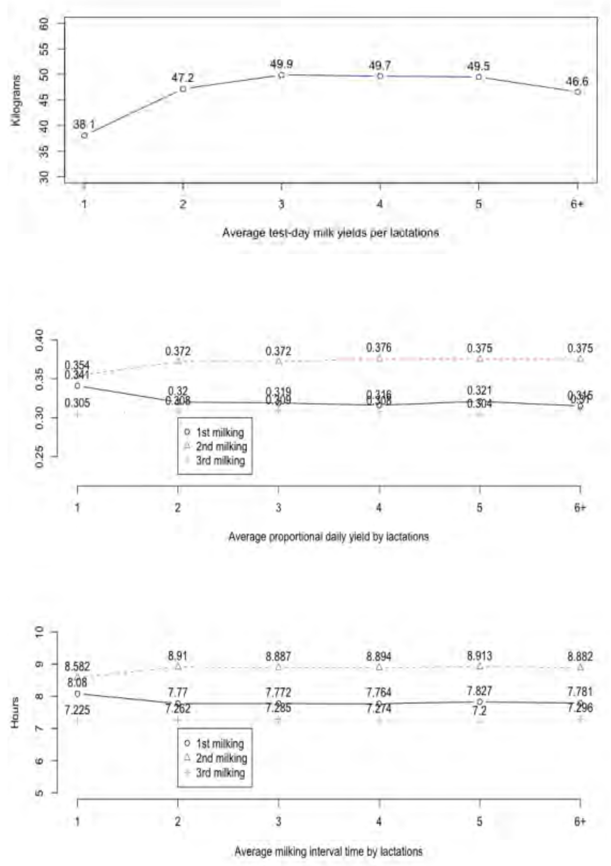

Figure 2. Trends of changes by lactations in average test-day milk yield (upper), average proportional daily yields (middle), and average milking interval time (bottom).

Relative importance of predictor variables

Analysis of variance based on the proportional daily DMY model (1) showed significant effects of milking interval time (Pr <2.20e-16 for all three milkings), months in lactation (Pr = 0.0008 for 1st milkings; Pr = 2.52e-10 for 2nd milkings; Pr = 0.0001 for 3rd milkings), parities (Pr <2.20E-16) on proportional DMY. ANOVA based on the PIR model (5) revealed significant effects from partial milk yields (Pr < 2.20e-16), months in milk (Pr < 2.20e-16), and parities (Pr < 2.20e-16) on DMY. The results also showed significant interactions between partial yields and linear milking interval times (Pr < 2.20e-16) on DMY and significant interaction effects between partial yields and quadratic milking interval time for 1st milkings (Pr = 9.42e-08) and 3rd milkings (Pr = 1.03e-11) but not significant for the 2nd milkings (Pr = 0.1785) on DMY. These significant interaction effects justified using PIR models in the present study.

Table 1 presents the relative importance of predictor variables for two models in estimating daily milk yields across three different milkings (1st, 2nd, and 3rd). The values provided are the means and 95% confidence intervals of the LMG R², which measure the proportion of variance explained by each predictor. For the proportional DMY Model (1), milking interval time was the most significant predictor, with relatively high mean importance values across all milkings (0.157, 0.135, 0.159); months in milk had very low importance, indicating it contributes minimally to explaining the variance in

DMY (0.002, 0.004, 0.002); Lactations also had a minor contributor, with slightly higher values than months in milk but still low (0.040, 0.032, 0.001). The low importance of months in milk and lactations agrees with the Wiggans (1986) model, which ignores these variables. Nevertheless, the total relative importance sums to around 0.199 for the 1st milking, 0.170 for the 2nd milking, and 0.148 for the 3rd milking, suggesting that the predictors in this model together explain only a low to modest portion of the variance in daily milk yields. There may be other significant variables influencing proportional DMY that have not yet been identified.

For the PIR model, partial yields were the most significant predictor, with consistently high importance across all milkings (0.285, 0.280, 0.274). The interactions between partial yields and linear and quadratic milking interval time also had a major contributor, with substantial mean importance values (0.226, 0.244, 0.225) for the interaction with a linear milking interval time and also notable mean importance values (0.158, 0.199, 0.172) for the interaction with quadratic milking interval time. Months in milk showed higher importance in the PIR Model (5) compared to the proportional DMY Model (1), but still relatively low (0.022, 0.021, 0.020). The relative importance of lactations varies more across milkings, with higher values in the 1st and 3rd milkings compared to the 2nd (0.129, 0.083, 0.101). The total relative importance sums to 0.820 for the 1st milking, 0.830 for the 2nd milking, and 0.790 for the 3rd milking, indicating that the PIR Model predictors together explain a much larger portion of the variance in daily milk yields compared to the proportional DMY model. However, both results are not directly comparable because they modeled different quantities. The dependent variable in the former model was proportional DMY, whereas it was DMY in the latter model.

Table 1. Relative importance (mean and 95% of IMG R^2) of predictor variables in two models [1]

Predictors	**1st milking**			**2nd milking**			**3rd milking**		
	Mean	**Q2.5%**	**Q97.5%**	**Mean**	**Q2.5%**	**Q97.5%**	**Mean**	**Q2.5%**	**Q97.5%**
Model 1									
MIT	0.157	0.145	0.171	0.135	0.121	0.149	0.159	0.146	0.172
MIM	0.002	0.001	0.004	0.004	0.003	0.007	0.002	0.002	0.005
LACT	0.040	0.035	0.046	0.032	0.026	0.037	0.001	0.001	0.003
Sum	0.199			0.170				0.148	
Model 2									
PY	0.285	0.280	0.290	0.280	0.276	0.284	0.274	0.269	0.279
TAR1	0.226	0.222	0.230	0.244	0.240	0.247	0.225	0.222	0.229
TAR2	0.158	0.154	0.162	0.199	0.196	0.202	0.172	0.168	0.175
MIM	0.022	0.020	0.025	0.021	0.019	0.024	0.020	0.018	0.023
LACT	0.129	0.124	0.133	0.083	0.080	0.086	0.101	0.096	0.106
SUM	0.82			0.83			0.79		

[1] MIT = milking interval time; MIM = months in milk; LACT = lactations; PY = partial yields (1st, 2nd, or 3rd); TAR1 = interaction between PY and linear MIT; TAR2 = interaction between PY and qudratic MIT.

Accuracy of estimated daily milk yields

Table 2 compares the accuracy of estimated daily milking yields using two models, each under two scenarios. The scenarios differed based on whether the effects of months in milk and lactation were accounted for. GW1 and PIR1 did not include the variables for months in milk and lactations, whereas GW2 and PIR2 accounted for their effects. The accuracy is measured by the correlation between estimated and actual daily milk yields, the R^2 accuracy, and the K value, which is the ratio of the estimated daily milk yields over the variance of actual daily milk yields.

The Wiggans (1986) models, GW1 and GW2, showed roughly similar performance with slight differences in correlations, R^2 accuracies, and K values. Both models tend to overestimate the variance ($K > 1$). The PIR1 and PIR2 models generally had a higher correlation and R^2 accuracies than GW1 and GW2, indicating they provide more accurate estimates of daily milk yields than the current method. Compared to the GW models, PIR1 had around 1-2% increase in R^2 accuracy, and PIR2 had approximately 4-6% increase in R^2 accuracy. However, these two PIR models performed differently on the variance of estimated DMY. PIR1 gave an overestimated variance of estimated DMY, whereas PIR2 led to a smaller variance of DMY than the actual daily milk yield variance. Generally speaking, the estimates from a linear regression tend to have a smaller estimate variance than the actual variance because the residuals are excluded. However, PIR1 was a model without intercept. When fitting linear regression models, the inclusion or exclusion of an intercept has a significant impact on the variance of the predicted values. The intercept in a regression model captures the average expected value of the dependent variable when all predictor variables are at zero (assuming zero is within the range of normal values for these predictors).

Including an intercept typically reduces the sensitivity of the model to fluctuations in the data by adjusting the baseline level of the response. This often leads to smaller coefficients for the predictors because the intercept absorbs much of the average outcome, reducing the variability that each predictor needs to explain. Hence, the variance of the predicted values generally reflects more closely the natural variability in the data centered around the mean.

Without an intercept, each predictor variable must account not only for the variability related to its specific influence on the dependent variable but also for its overall mean. This often requires larger coefficients, as each predictor must scale more significantly to fit the data points. Because the model without an intercept is overly sensitive to changes in the predictor variables and tends to have larger coefficients, the range of predicted

Table 2. Accuracy metrics of estimated daily milking yields using two the Wiggans (1986) (GW) method and a polynomial-interaction-regression (PIR) model, respectively [1,2].

Methods	**1st milking**			**2nd milking**			**3rd milking**		
	Corr	**R^2**	**K**	**Corr**	**R^2**	**K**	**Corr**	**R^2**	**K**
Before variance rescaling									
GW1	0.880	0.781	1.237	0.901	0.809	1.253	0.875	0.769	1.285
GW2	0.879	0.791	1.152	0.902	0.801	1.3207	0.875	0.769	1.283
PIR1	0.883	**0.800**	1.205	0.903	**0.815**	1.2277	0.877	**0.777**	1.249
After variance rescaling									
GW1	0.880	0.806	1.000	0.901	0.835	1.000	0.875	0.800	1.000
GW2	0.879	0.806	1.000	0.902	0.836	1.000	0.875	0.800	1.000
PIR1	0.883	**0.811**	1.000	0.903	**0.837**	1.000	0.877	**0.803**	1.000
PIR2	0.906	**0.841**	1.000	0.909	**0.847**	1.000	0.889	**0.819**	1.000

[1] Corr = correlation; R2 = R2 accuracy; K = ratio of estimated versus actual daily milk yield variance.

[2] GW1, PIR1 = Omitting months in milk and lactations; GW2, PIR2 = These models included the effects of months in milk and lactations.

values can be significantly wider. This amplifies the variance of the predictions because the model tries to compensate for the lack of a baseline adjustment by stretching the effect of the predictors to cover all data points. Table 3 shows model parameters for the polynomial-interaction-regression with (PIR2). Without accounting for the effects of months in milk and lactations (PIR1), the regression coefficients for partial yields were between 5.19 and 8.36. In contrast, the regression coefficients were substantially smaller (2.78 – 5.97) with the PIR2 model when accounting for the effects due to months in milk and lactations.

PIR2 had a higher R2 accuracy than PIR1 because PIR2 accounted for the effects of months in milk and lactation. This is often the case when one or more secondary variables are not randomized in the experimental design, such that deviates due to these differences are not zero. Otherwise, PIR and PIR2 would perform similarly. In contrast, GW1 and GW2 performed similarly, which may suggest that simply accounting for secondary variables by their averages in the Wiggans (1986) is inefficient.

It should be noted that, in PIR2, the months in milk effects were expressed as inherently related to the overall mean. In other words, though the overall mean was not present in the PIR2 model equation, it was presented via the months in milk effects. Therefore, PIR2 gave a smaller estimate variance than the actual variance. Variance rescaling brought all K values to 1, indicating that the variance of estimated daily milk yields now matches the actual yields perfectly. Thus, variance rescaling effectively adjusted the variance of estimated yields to match the actual yields, improving the overall accuracy of the models except for PIR2. For PIR2, because the estimated daily yield variance was smaller than the actual variance and because the months in milk and lactation effects were adjusted additively, variance rescaling led to a slight decrease in the accuracy.

In Table 4, multiplicative correction factors (MCF) for three milkings were derived from a historical reference (Wiggans, 1986), and compared to the current results derived by two models (GW and PIR) across milking intervals between 5.75 and 10.25 hours. For the 1st Milkings, the GW and PIR models consistently show higher MCF values than the historical reference across all intervals. For the 2nd and 3rd milkings, MCF derived from the GW and PIR models are slightly lower than the reference. These results indicate minor changes in MCF over the past decades. The PIR model shows a trend towards slightly lower MCF values across all milkings compared to the GW model. The

Table 4. Comparison of 3X multiplicative correction factors (MCF) obtained for every 30 minutes based on the present milking dataset and the reference (Ref) MCF for trice-milkings[1,2].

Milking interval time, hrs	1st milking			2nd milking			3rd milking		
	Ref.	GW	PIR	Ref.	GW	PIR	Ref.	GW	PIR
5.75	3.76	4.11	3.98	3.89	3.74	3.48	3.92	3.94	3.83
6.25	3.54	3.81	3.73	3.65	3.53	3.34	3.68	3.69	3.60
6.75	3.34	3.55	3.50	3.45	3.33	3.21	3.47	3.46	3.40
7.25	3.17	3.32	3.29	3.26	3.16	3.08	3.28	3.26	3.20
7.75	3.01	3.11	3.11	3.10	3.01	2.96	3.12	3.08	3.03
8.25	2.87	2.94	2.94	2.95	2.87	2.84	2.96	2.92	2.87
8.75	2.74	2.78	2.80	2.81	2.74	2.72	2.83	2.78	2.72
9.25	2.62	2.63	2.67	2.69	2.62	2.60	2.70	2.64	2.60
9.75	2.51	2.51	2.57	2.57	2.51	2.48	2.59	2.53	2.48
10.25	2.41	2.39	2.49	2.47	2.42	2.37	2.48	2.42	2.39

[1] GW = MCF according to Wiggans (1986); PIR = polynomial-interaction-regression; both models did not accou for the effects due to months in milk and lactations.

[2] Reference MCF (Wiggans, 1986): $F_{1st} = \frac{1}{0.077+0.0329t}$; $F_{2nd} = \frac{1}{0.068+0.0329t}$; $F_{3rd} = \frac{1}{0.066+0.0329t}$

average (range) of the reference MCF (Wiggans, 1986) was 3.00 (2.41 – 3.76) for the 1st milking, 3.08 (2.47 – 3.89) for the 2nd milking, and 3.10 (2.48 – 3.92) for the 3rd milking. Based on the recent milking dataset analyzed by the Wiggans (1986) model, the average (range) of MCF was 3.11 (2.39 – 3.98) for the 1st milking, 2.99 (2.42 – 3.74) for the second milking, and 3.07 (2.39 – 3.83) for the 3rd milking.

Conclusions

In conclusion, the initial case study demonstrated that modeling proportional DMY as a linear function of milking interval time is a valid strategy. The milking interval was the primary variable influencing proportional DMY where the effects of months in milk and lactations were minor. Still, other major variables that have not yet been discovered can influence proportional DMY. The polynomial-interaction-regression model provided more accurate yield estimates than the Wiggans (1986) model. The new model captures the linear and quadratic interactions between partial yields and milking interval times. The study also revealed that the second milking, with the longest interval, offered the most precise estimates. The calculated MCFs showed only minor deviations over the past four decades despite the significant genetic improvement in daily and lactation yields in the past decades. This result suggests that the proportional daily yields, reciprocal to MCF, remained relatively stable over the past decades. Finally, this study represents an initial case study, and all the conclusions are subject to large-scale validation.

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Assessing the impact of automatic milking systems on milk free fatty acid content in Taiwan

Y.H. Yeh[1], M.K. Yang[1], Y.H. Chen[1], J.F. Huang[2], J.W. Shiau[1] and P.A. Tu[1]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture,
No.207-5, Pitoumian, Xihu Township, Miaoli 36843, Taiwan
[2]Taiwan Livestock Research Institute, No.112, Farm Road, Hsinhua,
Tainan 71246, Taiwan
Corresponding Author: tpa@mail.tlri.gov.tw

Abstract

Using the Automatic Milking System (AMS) can increase milk yield and reduce labor, but the impact of AMS on the milking behaviour of cows in tropical regions like Taiwan, as well as its effect on the quality of farm raw milk, has not been thoroughly explored. Free Fatty Acids (FFAs) in milk are considered one of the indicators of milk quality, and an increase in FFAs can lead to off-flavors and spoilage in dairy products. This study examines the FFA content in individual cow's milk from different milking systems, and investigates the influence of lactation stages and milking frequency on FFAs in raw milk. The experiment monitored milk samples collected from the Conventional Milking Parlour (CMP) and the AMS from 2021 to 2022, totaling 2,936 and 1,726 samples respectively. The FFA content in these samples was measured using Fourier Transform Infrared Spectroscopy (FTIR). The results show that the milk from cows using the AMS had significantly higher FFAs (1.17 mmol/100g milk fat, $P < 0.01$) compared to those using CMP (0.88 mmol/100g milk fat). The FFA levels in the early stage of lactation (0.82 mmol/100g milk fat) were significantly lower ($P < 0.01$) than in the mid (1.10 mmol/100g milk fat) and late stages (1.17 mmol/100g milk fat) of lactation. When comparing different milking frequencies, cows milked 2, 3, and more than 4 times a day in the AMS had FFAs of 0.89, 1.09, and 1.15 mmol/100g milk fat respectively, with the FFAs in milk from cows milked twice a day significantly lower ($P < 0.01$) than those milked 3 times or more. This study indicates that the difference in FFAs between AMS and CMP in Taiwanese farms is particularly evident in the early stages of lactation, which helps in further investigating the physiological changes in cows during this period. The study confirms that the use of AMS in Taiwanese farms affects milk quality, including cow-related factors and other management aspects. Although the introduction of AMS may initially impact parameters related to milk quality, these effects may reduce or disappear as the lactation stage progresses, the cows adapt, and milk volume increases. Additionally, farm managers and dairy farmers need to pay special attention to the cleaning and maintenance of AMS, as well as the proper cooling of raw milk, to maintain high-quality milk.

Keywords: free fatty acids, milk quality, automatic milking system, conventional milking parlour.
Presented at the ICAR Anual Conference 2024 in Bled at the Session Session 3: Factors Affecting the Accuracy of the Recording Day

Introduction

Using the Automatic Milking System (AMS) can increase milk yield and reduce labor, but the impact of AMS on the milking behaviour of cows in tropical regions like Taiwan, as well as its effect on the quality of farm raw milk, has not been thoroughly explored. Free Fatty Acids (FFAs) in milk are considered one of the indicators of milk quality, and an increase in FFAs can lead to off-flavors and spoilage in dairy products. Therefore, it is important to assess the impact of AMS on the FFA content in milk in order to understand its effect on milk quality in Taiwanese farms.

In Taiwan, dairy farms have increased their herd size, but labor shortages and aging are causing problems. These issues could be resolved by using an automatic milking system (AMS). Mechanization and automation will be the future management model for dairy farms. This study examines the FFA content in individual cow's milk from different milking systems, and investigates the influence of lactation stages and milking frequency on FFAs in raw milk.

Material and methods

The experiment monitored milk samples collected from the Conventional Milking Parlour (CMP) and the AMS from 2021 to 2022, totaling 2,936 and 1,726 samples respectively. The FFA content in these samples was measured using Fourier Transform Infrared Spectroscopy (FTIR). Recording each day's milk production, milking equipment used, stage of lactation, and milking process.

Statistical analysis was conducted using a mixed-effects model to assess the impact of milking system, lactation stage, and milking frequency on the free fatty acid content in milk. The milking system, lactation stage (early, mid, and late), and milking frequency (2 times, 3 times, and more than 4 times a day) were considered as fixed effects. Individual cows were included as random effects to account for the repeated measures on the same cow.

The FFA content in milk samples was analyzed as the response variable, and the differences in FFA levels between the milking systems and across lactation stages and milking frequencies were evaluated using ANOVA. Post-hoc pairwise comparisons were conducted to assess specific differences between the levels of each factor. All analyses were performed using the SAS statistical software, and the significance level was set at $\alpha = 0.05$.

Results

The difference between AMS and CMP for FFAs across days in milk is presented in Figure 1. Cows milked with AMS produced milk with greater FFAs content across lactation. The greatest difference between AMS and CMP was detected in 140 days after calving, whereas the smallest differences were observed after 200 days in milk. The milk from cows using the AMS had significantly higher FFAs (1.17 mmol/100g milk fat, $P < 0.01$) compared to those using CMP (0.88 mmol/100g milk fat) (Table 1). The FFA levels in the early stage of lactation (0.82 mmol/100g milk fat) were significantly lower ($P < 0.01$) than in the mid (1.10 mmol/100g milk fat) and late stages (1.17 mmol/100g milk fat) of lactation.

When the milking frequencies of cows were compared, it was observed that cows milked twice a day had lower levels of FFAs in their milk fat (0.89 mmol/100g) compared to those milked three times per day (1.09 mmol/100g) and more than four times per day (1.15 mmol/100g) in an AMS system. This difference was found to be statistically significant with a p-value of less than 0.01. The results of the study indicate that the use of an automatic milking system can have a significant impact on the FFA.

Table 1. Least squares means and P-values of fixed effects in the statistical analysis of free fatty acid (FFA) concentration in milk.

Item	Group	FFA (mmol/100g milk fat)	P value
Milking system	AMS	1.17 ± 0.04^{a}	<0.0001
	CMP	0.88 ± 0.05^{b}	
Lactation stage	Early	0.82 ± 0.05^{a}	<0.0001
	Middle	1.10 ± 0.04^{b}	
	Late	1.17 ± 0.05^{b}	
Milkings	Control	0.97 ± 0.03^{ab}	<0.01
	2	0.89 ± 0.04^{b}	
	3	1.09 ± 0.05^{a}	
	4+	1.15 ± 0.08^{a}	
Milking system × Lactation stage	AMS × Early	1.08 ± 0.07^{ab}	<0.05
	AMS × Middle	1.23 ± 0.06^{a}	
	AMS × Late	1.20 ± 0.06^{ab}	
	CMP × Early	0.55 ± 0.11^{c}	
	CMP × Middle	0.97 ± 0.06^{b}	
	CMP × Late	1.13 ± 0.06^{ab}	
Lactation stage × Milkings	Early × Control	0.99 ± 0.08^{bcd}	<0.05
	Early × 2	0.61 ± 0.09^{d}	
	Early × 3	0.77 ± 0.10^{cd}	
	Early × 4+	0.89 ± 0.14^{bcd}	
	Middle× Control	0.97 ± 0.05^{bcd}	
	Middle× 2	1.03 ± 0.07^{abc}	
	Middle× 3	1.26 ± 0.08^{ab}	
	Middle× 4+	1.16 ± 0.12^{abc}	
	Late × Control	0.95 ± 0.05^{bcd}	
	Late × 2	1.05 ± 0.05^{abc}	
	Late × 3	1.26 ± 0.08^{ab}	
	Late × 4+	1.41 ± 0.16^{a}	

$^{a–d}$ Groups that do not share a common superscript letter are significantly different in their FFA levels (P<0.05).
AMS: automatic milking system; CMP: conventional milking parlour.
Early, Middle, and Late corresponds to 7 to 100 DIM (days in milk), 101 to 200 DIM, and 201 to 305 DIM, respectively. The 2, 3, and 4+ represent daily milking frequencies with AMS, while Control refers to twice daily milking using CMP.

Discussion

In previous study (Marchi *et al*., 2017), cows milked with AMS produced milk with greater FFA content across lactation. The greatest difference between AMS and CMP was detected within the first 80 days after calving, whereas the smallest differences were observed after 260 days in milk. This result is similar to our study, the smallest differences were observed during the late lactation in both experiments.

In this study, FFAs content was greater (+0.29 mmol/100 g milk fat) in milk from cows milked in AMS than CMP. The results show a similar trend to previous studies. Marchi *et al*. (2017) indicates the FFAs content in milk in AMS is higher than in CMP by 0.16 mmol/100 g milk fat. FFAs are produced through the degradation of milk fat into glycerol and FFAs via lipolysis reactions. Cooling and mechanical treatments of milk can disrupt the membrane of fat globules, leading to an increase in FFAs levels. This increase in FFAs is primarily associated with higher milking frequency or shorter milking intervals (Klei *et al*., 1997; Justesen and Rasmussen, 2000). Wiking *et al*. (2019) indicated that when the milking interval was less than 585 minutes, FFA levels increase as the milking interval shortens. More frequent milking leads to lower milk yield per milking. This is because the increased frequency of milking results in a higher

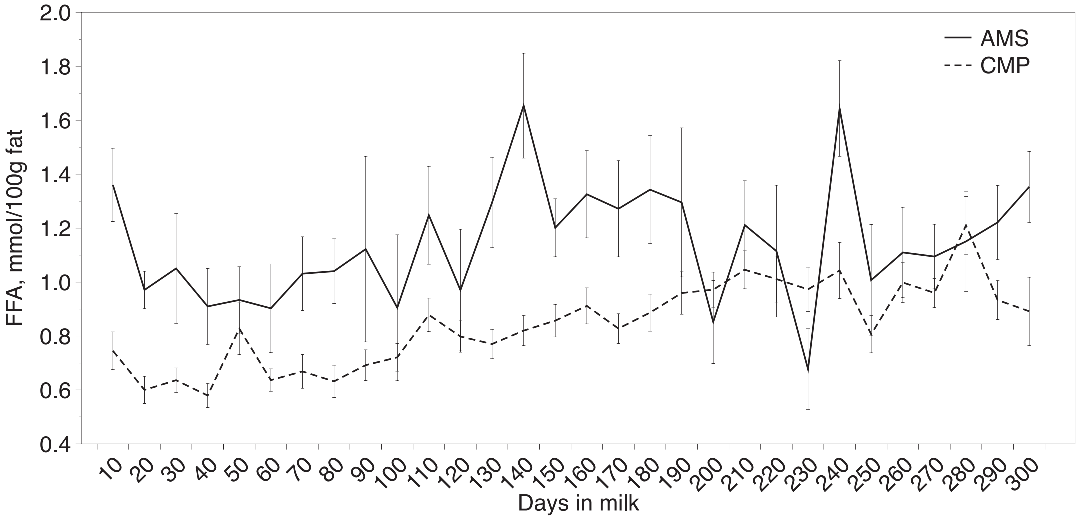

Figure 1. Free fatty acid (FFA) levels (mean ± standard error, mmol/100g fat) for automatic milking system (AMS, solid line) and conventional milking parlour (CMP, dashed line) over 15 days intervals through the entire 305 days of lactation.

air-to-milk ratio in the pipeline, reducing the stability of milk fat globules (MFG). When milk is pumped or agitated, mixing with air occurs, causing MFG rupture upon collision with air bubbles. As a result, membrane material and core fat are released into the milk plasma when air bubbles collapse or merge. Additionally, low quarter milk yields are linked to elevated (FFA) levels (Rasmussen *et al*., 2006). Additionally, milk from cows milked more than twice daily tends to have larger fat globules, which are more susceptible to lipolysis compared to smaller fat globules. Wiking *et al*. (2006) stated that the increase in FFA content at higher milking frequencies in AMS is attributed to both biological and mechanical factors. However, biological factors may have a greater impact, as spontaneous lipolysis is heightened. These findings suggest that the use of an automatic milking system, combined with a higher milking frequency, may contribute to increased levels of FFAs in milk.

This study suggests that there is a noticeable disparity in free fatty acids between automated milking systems and conventional milking parlors in Taiwanese farms, especially during the initial phases of lactation. This finding opens up opportunities for delving deeper into the physiological transformations occurring in cows during this specific period. Furthermore, exploring these differences may provide insights into potential improvements or adjustments to enhance milk quality and overall efficiency within dairy farming operations.

Conclusions

The use of AMS in Taiwanese farms may have a long-lasting impact on milk quality. Certain cow-related factors and management aspects may continue to be affected even as cows adapt and milk volume increases. Furthermore, farm managers and dairy farmers need to pay special attention to the cleaning and maintenance of AMS, as well as the proper cooling of raw milk, to maintain high-quality milk.

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DecisiveDry – Decision support for proper use of Selective Dry Cow Therapy while enhancing dairy sustainability

J.S. Clay and R.H. Fourdraine

Dairy Records Management Systems, 313 Chapanoke Road,
Suite 100, Raleigh, NC 27603, USA

Abstract

To boost adoption of Selective Dry Cow Therapy (SDCT) among its dairy producer customers, Dairy Records Management Systems in the U.S. introduced a web-based software app that can be flexibly applied in herds that are good candidates for this management strategy. The decades-long use of Blanket Dry Cow Therapy (BDCT) has been effective to control mastitis levels in dairy herds but it contributes substantively to the level of antibiotic use and the risk for antibiotic resistant pathogens. DecisiveDry first helps by determining whether the herd is a good candidate for SDCT by assessing for the recent year: number of measurements of SCC, year average SCC and number of testdays that herd average SCC is greater than a level set by the user. Next DecisiveDry identifies which of the soon to turn-dry cows may be eligible for SDCT. Cow eligibility is assessed using SCC levels of the three most recent testdays plus the number of days since the most recent case of clinical mastitis. Of course, it is recommended that all cows receive teat sealant. The app provides various forms of lists of cows to turn dry and whether each cow is eligible for only teat sealant. Lastly, the app provides financial estimates of cost (treated cows) and savings (non-treated cows) of drugs and labor.

Keywords: Clay, mastitis, SDCT, therapy, antibiotics, sustainable.
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Introduction

SDCT is not a new concept and is widely used in some European countries. However in the United States, SDCT has not gained as much traction and, on many dairy farms, dry cow therapies are applied to all cows at dry-off (BDCT). The common perspective is that BDCT minimizes mastitis problems with fresh cows and simplifies staff workflow. BDCT has been the gold standard to prevent intramammary infections (IMI) because it is most effective to methodically treat all quarters of every cow. Also, it is perceived that it is easiest for the milking staff to treat all quarters. However, it has been found that there is no difference in IMI subsequent to either BDCT or SDCT[1]. Additionally, SDCT reduces antimicrobial use by 66%[1] which reduces cost plus is becoming increasingly important to consumers.

The DecisiveDry app provides flexible yet methodical guidance to enable producers to deploy SDCT in their herd. DecisiveDry first assesses the herd's DHI herd average records to determine SDCT appropriateness and then it assesses DHI records for each potential dry cow. By closely following one of the three lists of potential dry cows, producers can be assured of consistent and proper application of the guidelines.

Is the herd a good candidate for SDCT?

By convention, application of SDCT has been targeted for herds with annual herd average SCC of 250,000 cell/mL or less and must have a minimum of 8 DHI testdays with SCC results. Of the 10,000 herds (2.0M cows) serviced by DRMS in 2023, 75% managed their herds to result in annual herd average SCC of less than 250,000 based on 8+ DHI testdays within the year.

Before including SDCT in a management scheme, a herd manager should also consider whether there has been an inordinate number of testdays with herd average SCC greater than 250,000. So – were there any spikes of herd average SCC?

For the 75% of DRMS herds with herd year average SCC less than 250,000 and a minimum of 8 SCC testdays, Table 1 displays percent of testdays with testday herd average SCC spikes (> 250,000) The shaded area in Table 1 portrays percent of herds with high numbers (3 or more) of testday SCC spikes. Clearly, the small herds produced a greater percentage of testday SCC spikes (25% for herds with less than 100 cows) than the larger herds (8% for herds with 1000+ cows). However overall, 80% (of the previous 75%...) of herds would qualify for SDCT.

Is the cow a good candidate for SDCT?

Typically within herds that qualify for SDCT, managers will screen cows to turn dry using multiple metrics to optimize potential for success with SDCT and minimize chances of mastitis in the subsequent lactation. Usual screening is for less than 150,000-200,000 cell/ mL on the last testday and an average SCC on the last three testdays of less than 200,000. Additionally, a cow may be excluded because of a case of clinical mastitis within 90 to 100 days.

Table 1. Percent of DRMS Herds by Herd Size with Year Average SCC < 250,000 with Number of Testdays that Averaged High SCC (≥ 250,000 cell/mL).

Herd			
	Size		
# Cows	0	1 - 2	3+
< 100	39	36	25
100-299	56	27	16
300-999	72	18	10
1000+	79	13	8
All	49	31	20

Table 2. Percent of DRMS Cows that Qualify for SDCT by Herd Size in Qualifying Herds.

	Herd	
Size # Cows	Percent of Herds	Percent of Cows Qualified for SDCT
< 100	52	76
100-299	29	80
300-999	13	82
1000+	6	83
All		79

After filtering for the herds identified in Table 1 with fewer than 3 testday average SCC spikes (80% of the herds with 8 testdays in 2023), the table below depicts the percentage of cows that would qualify for SDCT because SCC on the final testday prior to dry-off was less than 200,000 and the average SCC for the final three testdays was less than 200,000. Seventy-nine percent of cows qualified - and there was little difference in percents of qualifying cows by herd size.

Targeted producers

In its outreach campaigns for DecisiveDry, DRMS primarily targets producers that use its proprietary on-farm Dart herd management software and producers that take advantage of DartSync. DartSync is co-hosted software residing both on-farm and on DRMS servers. This software backs up the on-farm database while also synchronizing with data tables at DRMS. Although most producers send data from on-farm only once per day, there is the flexibility to synchronize multiple times daily. This synchronization facilitates the readiness of the DecisiveDry app for proper management of eligibility for SDCT. Approximately 45% of the cows that are serviced by DRMS are targeted by these systems.

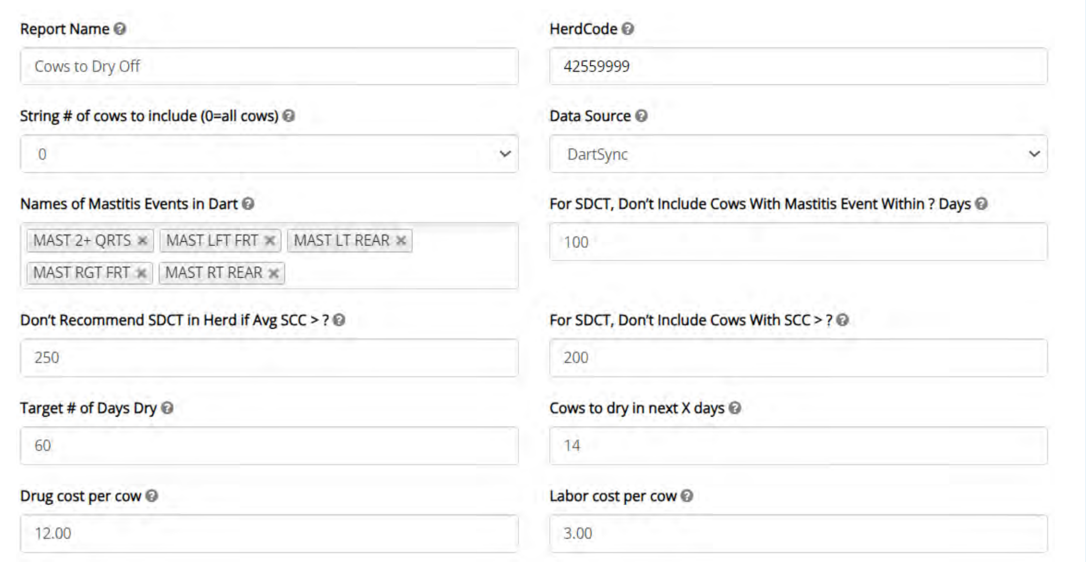
DecisiveDry is a component of HerdHQ suite of apps

The HerdHQ suite of apps provide an array of services for DRMS clients including producers and industry members such as herd consultants. Like all of the apps in the HerdHQ suite, although DecisiveDry will function appropriately using data and information from herds that do not use Dart or DartSync, it operates most effectively for participating herds.

Getting started: herd and cow setup

As depicted in Figure 1, the producer will complete entries to enable DecisiveDry to assess herd records and make recommendations. The interpretation of most fields are apparent but some additional explanations a *String # of cows to include*: enables the producer to subset cows by lot. Many herds set=0 to include all lots.

- **Data Source**: although a Dart producer will most likely choose DartSync, other producers will choose to access dry-off advice immediately after testday.
- **Names of Mastitis Events in Dart**: because of flexibility in Dart for naming health events, the producer will identify the names associated with cases of clinical mastitis events in his herd.
- **Cows to Dry in Next X Days**: enables customization to fit various management schemes for frequency of dry-off.
- **Drug and Labor costs**: per cow estimates will facilitate computation of financial impacts.



Report Name: Cows to Dry Off

HerdCode: 42559999

String # of cows to include (0=all cows): 0

Data Source: DartSync

Names of Mastitis Events in Dart: MAST 2+ QRTS, MAST LFT FRT, MAST LT REAR, MAST RGT FRT, MAST RT REAR

For SDCT, Don't Include Cows With Mastitis Event Within ? Days: 100

Don't Recommend SDCT in Herd if Avg SCC > ?: 250

For SDCT, Don't Include Cows With SCC > ?: 200

Target # of Days Dry: 60

Cows to dry in next X days: 14

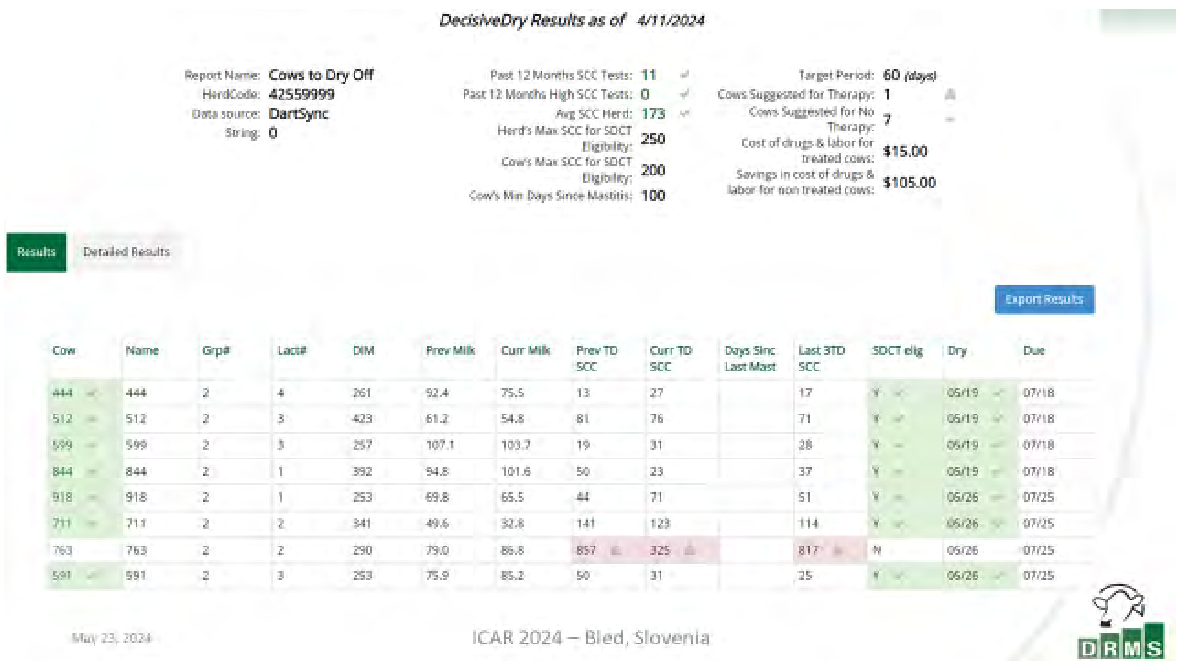
Drug cost per cow: 12.00

Labor cost per cow: 3.00

Figure 1. Input parameter screen for DecisiveDry.

DecisiveDry output

As illustrated in Figure 2, assessment results of the herd's eligibility for SDCT are portrayed in either green (OK to use SDCT for cows) or red (BDCT recommended) using the above criteria. Additionally, the herd assessment provides counts of the numbers of cows in the dry-off cohort to suggest for treatment and no-treatment. Plus, the producer's own estimates of per cow costs are used to predict the cost of treatment and the savings for no-treatment of the dry-off cohort.



DecisiveDry Results as of 4/11/2024

Report Name: Cows to Dry Off
HerdCode: 42559999
Data source: DartSync
String: 0

Past 12 Months SCC Tests: 11
Past 12 Months High SCC Tests: 0
Avg SCC Herd: 173
Herd's Max SCC for SDCT Eligibility: 250
Cow's Max SCC for SDCT Eligibility: 200
Cow's Min Days Since Mastitis: 100

Target Period: 60 *(days)*
Cows Suggested for Therapy: 1
Cows Suggested for No Therapy: 7
Cost of drugs & labor for treated cows: $15.00
Savings in cost of drugs & labor for non treated cows: $105.00

Cow	Name	Grp#	Lact#	DIM	Prev Milk	Curr Milk	Prev TD SCC	Curr TD SCC	Days Sinc Last Mast	Last 3TD SCC	SDCT elig	Dry	Due
444 ✓	444	2	4	261	92.4	75.5	13	27		17	Y ✓	05/19 ✓	07/18
512 ✓	512	2	3	423	61.2	54.8	81	76		71	Y ✓	05/19 ✓	07/18
599 ✓	599	2	3	257	107.1	103.7	19	31		28	Y ✓	05/19 ✓	07/18
844 ✓	844	2	1	392	94.8	101.6	50	23		37	Y ✓	05/19 ✓	07/18
918 ✓	918	2	1	253	69.8	65.5	44	71		51	Y ✓	05/26 ✓	07/25
711 ✓	711	2	2	341	49.6	32.8	141	123		114	Y ✓	05/26 ✓	07/25
763	763	2	2	290	79.0	86.8	857 ⚠	325 ⚠		817 ⚠	N	05/26	07/25
591 ✓	591	2	3	253	75.9	85.2	50	31		25	Y ✓	05/26 ✓	07/25

Figure 2. Output screen for DecisiveDry.

- The lower portion of the output screen lists the dry-off cohort of cows in index order with these additional featuresEligible cows are shaded in green.
- Not-eligible cows are shaded in pink and include highlight symbols.
- Columns are sortable.
- CowID may be clicked to gain access to other information.
- Wide and narrow versions are available.

Cowside management

As illustrated in Figure 3, when the producer uses a phone with the narrow version of output or sends the CSV file to a phone, all necessary information will be available cowside for proper management.

In summary, as U.S. dairy farmers continue to move towards using less antibiotics while also focusing on maintaining and improving overall herd health, DecisiveDry is an easy-to-use decision support tool. It is customizable to the individual farm situation, will speed up the process of sorting through information for many cows, and it can help minimize inappropriate or unnecessary antibiotic use. DecisiveDry can lead to greater profitability while supporting overall sustainability of the dairy farm.

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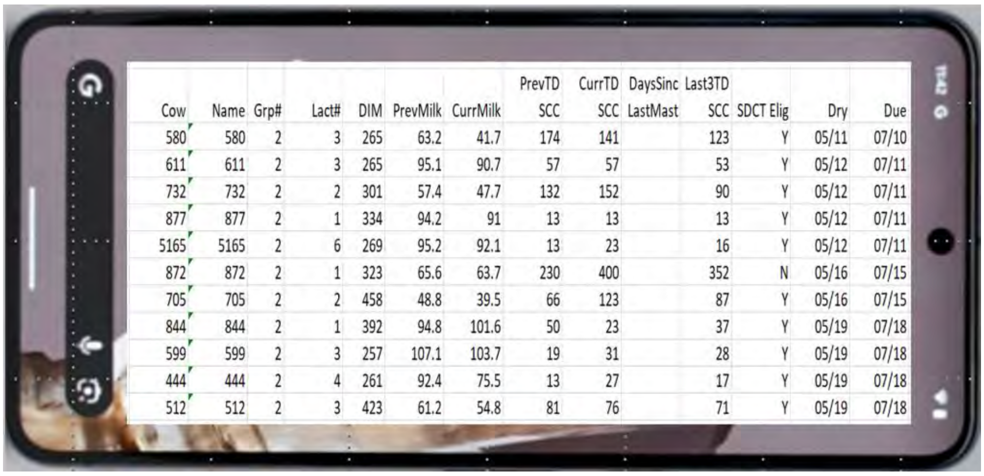

Cow	Name	Grp#	Lact#	DIM	PrevMilk	CurrMilk	PrevTD SCC	CurrTD SCC	DaysSinc LastMast	Last3TD SCC	SDCT Elig	Dry	Due
580	580	2	3	265	63.2	41.7	174	141		123	Y	05/11	07/10
611	611	2	3	265	95.1	90.7	57	57		53	Y	05/12	07/11
732	732	2	2	301	57.4	47.7	132	152		90	Y	05/12	07/11
877	877	2	1	334	94.2	91	13	13		13	Y	05/12	07/11
5165	5165	2	6	269	95.2	92.1	13	23		16	Y	05/12	07/11
872	872	2	1	323	65.6	63.7	230	400		352	N	05/16	07/15
705	705	2	2	458	48.8	39.5	66	123		87	Y	05/16	07/15
844	844	2	1	392	94.8	101.6	50	23		37	Y	05/19	07/18
599	599	2	3	257	107.1	103.7	19	31		28	Y	05/19	07/18
444	444	2	4	261	92.4	75.5	13	27		17	Y	05/19	07/18
512	512	2	3	423	61.2	54.8	81	76		71	Y	05/19	07/18

Figure 3. Excel file from DecisiveDry portrayed on phone.

Dry-off treatment of dairy-cows : methods to guide targeted antimicrobial use

*W. Obritzhauser[1], S. Kuchling[2], O. Alber[2], C.L. Firth[1], C. Schleicher[2],
K. Fuchs[2], K. Linke[3] and C. Egger-Danner[3]*

[1]Unit of Veterinary Public Health and Epidemiology, Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine, 1210 Vienna, Austria
[2]Data, Statistics and Risk Assessment, Austrian Agency for Health and Food Safety (AGES), Graz, Austria
[3]ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria

Abstract

One of the most common indications for the use of antimicrobials in dairy cattle is the administration of long-acting antibiotic products for drying off. Antibiotic dry cow therapy (ADCT) has proven itself over decades to be an efficient tool to cure existing udder infections and to reduce the number of new infections in the following lactation. However, from a One Health perspective, the routine use of antibiotics is controversial. As the use of antibiotics increases the risk of selecting antimicrobial resistant bacteria, the administration of antibiotic dry cow tubes should be restricted to cows with a proven infection with known mastitis pathogens or those at an increased risk of a new infection during the dry period.

As part of the D4Dairy research project, a cohort study was carried out to investigate whether the selective use of ADCT could lead to a reduction in the total antimicrobial use, without negatively influencing the udder health of the dairy herd. To determine the frequency of udder infections prior to dry-off, as well as the frequency of new infections, bacteriological milk cultures were carried out before dry-off and at the beginning of the subsequent lactation.

The results of the bacteriological milk cultures at the time of dry-off collated during the D4Dairy field study were used as 'gold standard' to develop a practical and cost-effective model for an animal-specific decision-making tool for selective ADCT. Therefore, the diagnostic results were combined with the data collected via the national milk monitoring scheme and the Austrian health monitoring program. Two statistical model approaches (Generalized Estimating Equations (GEE), Random Forest) were applied to predict the diagnostic result on animal level. The agreement between the predictions and the observed result of the bacteriological milk culture was evaluated for those two models, as well as for the selective ADCT recommendations carried out on farm in the D4Dairy field study. The best predictive performance was obtained using a random forest model. However, the test set was rather small. To validate the results, a larger amount of data from routine recordings from the Austrian milk performance recording system was used to train and test the random forest.

Selective dry cow therapy (SDCT) based on herd- and cow-specific somatic cell counts have the potential to reduce antibiotic dry-cow treatments without increasing the risk of deterioration of the udder health status of a herd. With a statistical prediction model like random forest, the use of antibiotics could be reduced even further.

Keywords: targeted dry-off treatment, antibiotic use, decision support, random forest, statistical modelling.
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Introduction

The use of antibiotics at the time of drying off dairy cows has been an integral part of udder health programs for decades. Antibiotic dry cow therapy (ADCT) is still a standard procedure of good agricultural practice on dairy farms today.

Concerns exist that uncritical use of antibiotics in livestock will reduce the effectiveness of antibiotics in humans due to the emergence of multi-resistant bacteria, which are increasingly being detected in humans and in the environment. The use of antibiotics as a management measure when drying off dairy cows is therefore subject to growing criticism.

The pattern of pathogens causing mastitis in dairy cattle has changed in recent years. *Staphylococcus aureus* has replaced *Streptococcus agalactiae* as the most common problem species. Antibiotic dry cow treatment can be effective in *Staphylococcus aureus* infected cows, but the cure rates vary considerably (Barkema *et al*., 2006). Udder infections with Enterobacteriales and *Streptococcus uberis* occur predominantly during lactation and require treatments in the acute phase of the disease (Pinzon-Sanchez and Ruegg, 2011). Antibiotic dry cow treatment should be limited to infected cows and to cows at high infection risk. A systematic use of antibiotics for all cows for dry cow treatment (blanket dry cow treatment, BDCT) is rarely justified. The selective use of antibiotics for drying off (selective dry cow treatment, SDCT) is necessary to avoid losses in milk production due to udder diseases (Cameron *et al*. 2015, Niemi *et al*. 2022).

SDCT is based on the selection of infected cows and cows whose udder health is at risk, with the aim of ensuring the highest possible udder health while at the same time keeping the use of antibiotics as low as possible. Various herd-related and cow-related parameters are used for this selection. In addition to high sensitivity and specificity of the selection criteria used for SDCT, the selection methods must also be practical and financially affordable (McCubbin *et al*. 2022). The basic requirements for successfully implementing an SDCT concept are, in addition to a low bulk milk somatic cell count, a low prevalence of infections with udder-associated mastitis pathogens (Cameron *et al*. 2014), a low incidence of clinical mastitis, good hygiene management at the time of drying off, as well as the ongoing monitoring of the udder health status of the herd (Kabera *et al*. 2020, Santman-Berends, I. M. G. A. *et al*. 2020, Rowe *et al*. 2020a).

The aim of the study at hand was to validate an existing expert-knowledge-based method for SDCT (Biggs *et al*. 2016, Bradley *et al*. 2015, Lipkens *et al*. 2019) and to develop a data-based method to routinely identify animals with a high risk of developing an udder disease at the time of drying-off, for which ADCT is then recommended. A good validated dry-off strategy could thus minimize the antibiotic use while maintaining udder health.

Material and methods

Cohort study

As part of the D4Dairy research project “Measures to reduce antibiotic resistance”, a cohort study was carried out to investigate whether the targeted use of antibiotics for dry-off treatment could reduce overall antibiotic consumption without negatively affecting the udder health of dairy herds. The field study was set up in 31 dairy herds which were not randomly selected (Table 1). 16 herds (case herds) got monthly recommendations for each individual cow to use or not to use an antimicrobial dry cow treatment based on the calculated weighted somatic cell count of the total herd, the

Table 1. Herds characteristics of cohort study participants.

		Case	Control
Number of herds		16	15
Number of cows		1206	1056
Type of farming activity	Full-time	15	13
	Part-Time	1	2
Husbandry system	Freestall	16	14
	Tie-stall	0	1
Predominant breed		Simmental	Simmental, Holstein
Milking technique	AMS	3	3
	Heringbone	6	7
	Side-by-side	5	3
Antibiotic dry cow treatment	Blanket	0	7
	Selective	15	7
	Case-by-case basis	1	1

individual cow somatic cell count of the last two milk recordings before drying off, the lactation number (primiparous, multiparous) (Biggs *et al*. 2016). In 15 herds (control herds) the management of dry-off treatments was carried out as usual (blanket DCT, various SDCT methods). Bacteriological culture of milk samples was conducted before dry-off, after calving and for every mastitis case. Data on antimicrobial use with respect to dry-off treatment were collated. Using this data the dry-off strategies were assessed using cure rate, new infection rate and antimicrobial use as outcome parameters.

Antimicrobial use

For the evaluation of the amount of antibiotics, which was used for antimicrobial dry cow treatment, the dosed based indicator TD (treatment days; Sanders *et al*. 2020) for the use of dry cow tubes was applied. The number of unit doses of antibiotics licensed for dry-cow therapy which were sold to the farmers during the study period was summed up and this figure was divided by the sum of days the cows were kept in the study herds during the study period and multiplied by 365. This number of treatment days for ADCT per cow per year was corrected by the calving interval and the replacement rate of heifers of the corresponding herd (Formula 1).

$$\boldsymbol{\# TD_{365} DCT} = \sum_{i=1}^{n} \frac{\# \, UD/udder_{\,(route\ intramam\ -\ DC)}\ in\ period\ P}{\# \, cow \cdot days\ in\ period\ P\ (days)} \times 365 \times \frac{calving\ interval\ in\ period\ P\ (herd,\ days)}{365} \times \left(1 + \frac{\# \, cow\ LN = 1 \cdot days\ in\ period\ P\ (days)}{\# \, cow \cdot days\ in\ period\ P\ (days)}\right)$$

Formula 1: **#TD$_{365}$DCT** (number of treatment days per cow per year for ADCT) = number of unit doses (UD) per udder given to any cow of a population within 1 year (1 UD = 4 injectors of an antibiotic licensed for intramammary use in dry-cow therapy).

Statistical modelling (data-based selection method)

A data-based, cow-specific dry-off recommendation should aim to provide antibiotics at dry-off only for cows that are infected with a mastitis pathogen or that are at increased risk of a new infection. The data collected in the cohort study was used to statistically model the result of bacteriological milk testing at the time of drying off, however only major mastitis pathogens were considered.

The binary diagnostic result (major / negative) was used as target variable for two statistical approaches. On the one hand a GEE (Generalized Estimating Equations) model with a logistic link function and an exchangeable covariance structure, accounting for similarities among cows and farms, was applied to a training data set (85 % of the observations), on the other hand a random forest model (Breiman, 2001) was trained, and both methods were validated on the same test set. Recordings from the national milk monitoring scheme and the Austrian health monitoring program (e.g. somatic cell count of the last three milk records before the date of the diagnostic test, mastitis diagnosis in the current lactation, somatic cell count at herd level) were used as explanatory (feature) variables in the two models.

For the random forest feature selection is implicitly included in building the different trees. A stepwise forward model selection (Hastie *et al*., 2001) procedure was used to select an optimal GEE model. Each feature was added to the model and the variable with the lowest Quasi-Likelihood Information Criterion (Pan, 2001) was selected in each step.

Predictions from those two models were compared with the observed diagnostic results for a test set that contained 15 % of the observations. Predictive performance was assessed by different performance measures: accuracy, sensitivity, specificity, and f1-score.

To compare the two data-based approaches with the method based on expert-knowledge (Biggs *et al*., 2016), the D4Dairy field study recommendations were evaluated for the same test set and the same performance measures were calculated. The data was split to ensure that any given animal was only observed in one of the two data subsets.

All statistical analysis were performed using the statistical software R, version 4.3.2 (R Core Team, 2023) and the packages geepack (Hojsgaard *et al*., 2006), ranger (Wright and Ziegler, 2017) and caret (Kuhn, 2008).

Extended data set

To validate the data-based decision approach, an extended data set, using bacteriological test data, milk performance data and herd health data from the Austrian cattle data network including 18,810 observations was provided.

Again, the data was split into a training set (85 %) and a test set (15 %), ensuring that an individual animal could only occur in one subset, and a random forest was tuned on the training set. Predictions from the random forest and the SDCT method (Biggs *et al*., 2016) were evaluated on the same test set using the same performance measures (accuracy, sensitivity, specificity and f1-score) as for the D4Dairy field study.

Results

Cohort study: Cure, new infections

Of 4,241 quarter milk samples taken before drying off 3,741 (88.2%) tested negative in the microbiological culture. In 240 (5.7%) of quarter milk samples a major pathogen (*Staphylococcus aureus*, Streptocooci, Enterococci, Enterobacteria, Trueperella) was detected. Interestingly Streptococci were the most common pathogen found in these samples, followed by Enterobacteria. The within-herd percentage of tested cows infected with a major pathogen before drying off varied between 0% and 57%.

The SCC at the last milk recording prior to calving in cows, which were infected with a major pathogen was - as expected – significantly higher than the SCC in cows, which were negative (not infected) in the bacteriological culture, but there was a not negligible overlap in individual cow somatic cell count data of major and not infected (negative) cows (Figure 1).

For 694 lactations the infection status before drying off (and within 100 days before the day of calving) and within 100 days after calving was evaluated. There were no significant differences between the groups of cows which were treated with an antibiotic at drying off and cows, which were not treated with an antimicrobial drying-off product regarding new infections after calving and persistent infections. Due to bacteriological testing of all cows without consideration of the udder health status, there were significantly more cows with no infection in the group, which were not treated with an antibiotic as well as significantly more cured cows in the ADCT-group (Table 2).

No significant differences between herds which received an individual cow recommendation for DCT (case herds) and herds which treated cows at drying off as usual (control herds) for any infection status could be proved. Significantly more cows were cured in the groups treated with an antibiotic (ADCT group) regardless of whether case- or control-herds, which is a clear indication for the effectiveness of antimicrobial dry cow therapy (Table 3).

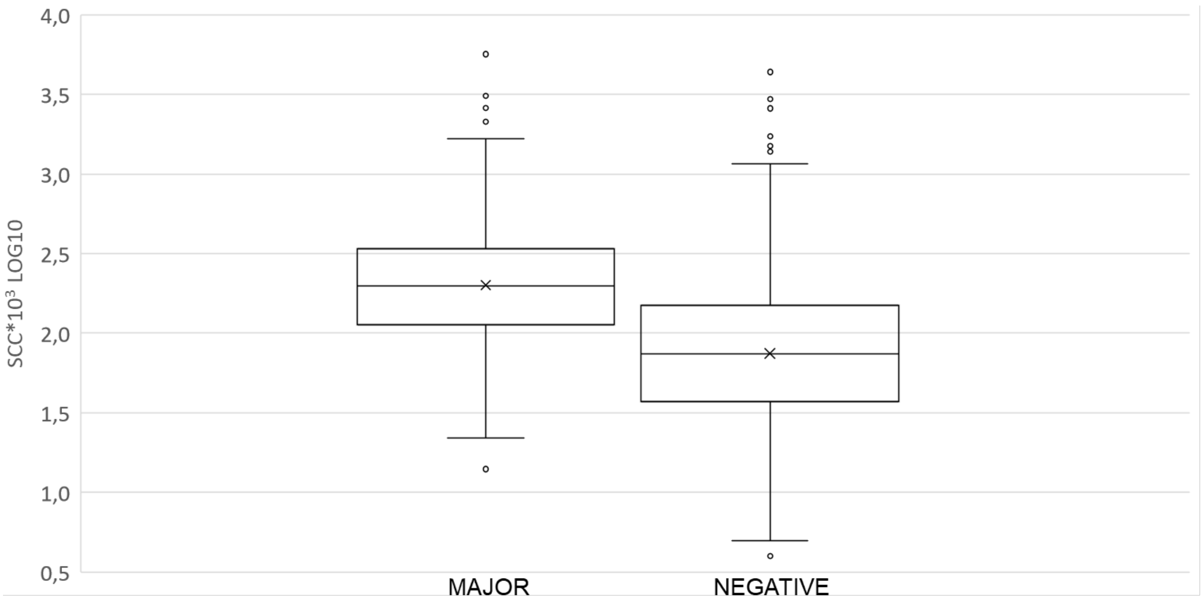

Figure 1. Individual cow somatic cell count (ICSCC) at the last milk recording prior to calving and status of intramammary infection (infected with a major pathogen = MAJOR or not infected = NEGATIVE).

Table 2. Evaluation of the drying-off strategy: cure rates, new infection rates.

			Dry cow therapy			
Status of infection	**Total**		**ADCT**		**no ADCT**	
No infection	404	58%	215	50%	189*	73%
New infection	89	13%	47	11%	42	16%
Cure	171	25%	146*	34%	25	10%
Persistently infected	30	4%	26	6%	4	2%

* significant Pearson´s Chi-squared test

Table 3. Evaluation of the drying-off strategy in case herds (individual cow recommendation for DCT) and control herds (DCT as usual): cure rates, new infection rates.

			Case herds				Control herds			
Status of infection	**Total**		**ADCT**		**No ADCT**		**ADCT**		**No ADCT**	
No infection	404	58%	80	47%	107	75%	135	51%	82	70%
New infection	89	13%	22	13%	24	17%	25	9%	18	15%
Cure	171	25%	58*	34%	10	7%	88*	33%	15	13%
Persistently infected	30	4%	10	6%	2	1%	16	6%	2	2%

* significant Pearson´s Chi-squared test.

Cohort study: Antimicrobial use for dry-cow treatment (ADCT)

The antimicrobial use for ADCT was higher in control herds compared to case herds (mean #TD$_{365}$DCT = 0,783 and 0,585 respectively). The difference of the means was significant (t-Test (mean) p = 0.043), but the medians were not (Mood´s Median-Test (median) p = 0.134). The most likely reasons for this „weak“ association are the relatively small number of study herds and the fact, that some kind of „selective“ dry cow treatment was implemented in most control herds as well.

Data-based selection method

Performance measures were evaluated for the GEE, the random forest and the applied recommendation (Biggs *et al*., 2016) on a test set containing 121 observations, of which 23 had a major pathogen test result in the bacteriological milk culture (Table 4). The GEE had the highest sensitivity, the random forest achieved the highest accuracy, specificity and f1-score. As data was imbalanced regarding the two outcome categories (major /negative) the f1-score provides a more reliable performance measure, especially compared with the accuracy.

Table 4. Comparison of predictions for a positive result of bacteriological milk tests before drying-off for the D4Dairy data set.

	Recommended ADCT (Biggs et al. 2016)	**Generalized Estimating Equations***	**Random Forest**
Accuracy	0.752	0.719	0.876
Sensitivity	0.652	0.739	0.565
Specificity	0.776	0.714	0.949
F1-score	0.500	0.500	0.634

* A cut-off value of 0.19 was used to classify the odds predicted by the GEE model into positive bacteriological milk cultures or negative tests. This was determined using an ROC analysis.

Extended data set

The extended data set contained 59 % negative and 41 % major findings of the bacteriological milk cultures. The test set consisted of 2,838 observations (1,167 with major finding). Performance measures were evaluated for the SDCT method (Biggs *et al*, 2016) and the random forest model (Table 5). Due to the superior predictive performance of random forest, the GEE model predictions for the extended data set were not included.

Table 5. Comparison of predictions for a positive result of bacteriological milk tests before drying-off for the extended data set.

	Recommended ADCT (Biggs et al. 2016)	Random Forest
Accuracy	0.645	0.700
Sensitivity	0.593	0.519
Specificity	0.681	0.827
F1-score	0.579	0.588

Discussion

Udder infections can heal during the dry period. Cure was more common in cows that were treated with an antibiotic at drying-off than in untreated cows, which is in accordance with other authors (Halasa *et al*. 2009a, Halasa *et al*. 2009b).

The selection of cows for ADCT did not lead to more new infections compared to herds that got no recommendation for SDCT. No significant difference in new infections could be proved between the group of cows that received ADCT and the group that did not as well as between case- and control-herds.

The detection of udder infections before drying-off using bacteriological milk testing offers a higher level of reliability for selecting cows for ADCT than indirect selection methods (Rowe *et al*. 2021). However, selection based on bacteriological milk tests involves significantly more effort, time, materials and costs (Rowe *et al*. 2021, Rowe *et al*. 2020b) than selecting cows based on individual milk cell counts.

The diagnosis of udder infections prior to dry-off by bacteriological milk culture provides greater diagnostic certainty than indirect methods to select cows for ADCT based on individual somatic cell counts (SCC), but is associated with significantly higher levels of labour, time, materials, and overall costs.

When setting a somatic cell count threshold for ADCT, it must be noted that with lower limits more cows that are not infected are treated with an antibiotic (Scherpenzeel, C.G.M. *et al*. 2016). In our study different thresholds were used for first-lactating cows (lower threshold value) and for cows in further lactations (higher threshold value) (McCubbin *et al*. 2022, McDougall *et al*. 2021). Additionally different thresholds depending on the calculated weighted somatic cell count of the total herd were used (Biggs *et al*., 2016) to take the increasing risk of new infections due to a high prevalence of chronic udder infections into account. However, the comparison of the prediction models demonstrates, that the selection for ADCT based on herd-, cow- and lactation-specific cell count thresholds alone recommends the use of antibiotics more often than is actually necessary.

The relationship between udder infections, the results of milk performance testing, the lactation age of the cows, and udder health indicators of a herd is complex and could not be well explained with a GEE model because no interactions between individual factors as well as non-linear combinations of the features were taken into account in a first step. With a statistical prediction model such as random forest, an even more precise selection of cows for ADCT could be made, but the data set used was rather small. Model comparisons are more reliable when a broader test data set can be used. Therefore, the random forest model was trained with an extended data set, using bacteriological test data, milk performance data and herd health data from the Austrian cattle data network.

The extended data set resulted mostly from routine recordings without a project setting on the farm. Consequently, in most cases bacteriological milk tests were run only in suspicious cases. Therefore, the results of the bacteriological milk tests were more balanced than in the D4Dairy observations, where each cow had to be examined by bacteriological milk tests. Differences in accuracy and f1-score were smaller for this data set, however they still were better for the random forest. Sensitivity was slightly better for the applied SDCT method, whereas the specificity was clearly higher for the random forest approach. This underlines the conclusion that ADCT was recommended more frequently for the applied SDCT method (Biggs *et al*., 2016), than a bacteriological milk test would have implied. Random forest results would have recommended less antibiotic use. However, due to the marginally lower sensitivity, a few more infections would have been missed compared to the SDCT method (Rowe *et al*. 2021).

All presented methods are based on herd- and cow-specific parameters like somatic cell count and lactation period, whereas the random forest considers much more variables up to three time points before time of drying-off. Consequently, it is possible to get more differentiated recommendations by using the random forest model.

Conclusions

In farms that do not use microbiological milk testing on a regular basis before drying off, the individual somatic cell counts of the last milk recordings before dry-off and the lactation number (first-lactating cows, cows in subsequent lactations) are the key decision-making parameters for the selection of cows for ADCT. The use of antibiotics for drying off can be reduced using a SDCT method based on the weighted somatic cell count of the herd, the cell counts of the individual cows before drying off and the lactation age. The SDCT procedure used in this study did not worsen udder health. With a statistical prediction model like random forest, the use of antibiotics could be reduced even further. The results of this study can be used for the development of a dry cow treatment decision tool that could be integrated into a dairy herd management software. This tool is intended to support farmers and veterinarians in the widespread implementation of SDCT procedures.

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The effect of different brands of automatic milking systems on bulk tank milk bacterial and somatic cell counts in dairy farms in Taiwan

H.H. Liao[1], L.Y. Chen[1], S.H. Wang[1], J.W. Shiau[1] and J.F. Huang[2]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture,
No. 207-5, Pitoumian, Xihu Township, Miaoli 368003, Taiwan
[2]Taiwan Livestock Research Institute, Ministry of Agriculture, No. 112, Farm Road,
Hsinhua, Tainan 71246, Taiwan
Corresponding Author: b604292000@gmail.com

Abstract

The automatic milking system (AMS) is a new type of equipment for the domestic dairy industry in Taiwan. We introduced our first AMS in 2019, and so far a total of 25 AMS are in use. 9 farms used Lely Astronaut (Lely, Rotterdam, Netherlands) and 7 farms used DeLaval VMS (DeLaval, Tumba, Sweden). The milking equipment design and routine procedure may differ depending on the brand of AMS. Among them, the most well-known part is that the robot arms are used in industries with hydraulic drive (DeLaval VMS) or designed for animal milking with pneumatic drive (Lely Astronaut). On the other hand, the teats were individually cleaned, stimulated, and dried by cleaning teat cups with warm air (DeLaval VMS) or cleaned and stimulated by rotating brushes (Lely Astronaut). In this study, the bulk tank milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) records were collected from 3 dairy farms that have used AMS for over 3 years and have become stable in system operation and feeding management for each brand in 2023. The two brands were anonymously represented by brand A and brand B. Differences regarding these milk quality parameters were contrasted using a *t*-test. The results showed that BMTBC in brand B was higher than in brand A, with a highly significant difference ($13.47 \pm 1.39 \times 10^3$ cfu mL^{-1} v.s. $27.06 \pm 3.06 \times 10^3$ cfu mL^{-1}, $P < 0.001$). The difference in BMSCC was also significant between brand A and brand B ($171.40 \pm 7.14 \times 10^3$ cells ml^{-1} v.s. $202.90 \pm 9.65 \times 10^3$ cells ml^{-1}, $P < 0.05$). Significant differences exist among the domestic dairy industry using different brands of AMS in BMTBC and BMSCC. However, the quality of raw milk still complies with the Class A regulations on the standards of purchasing, acceptance, and pricing of raw milk. Preliminary speculation indicates that BMTBC and BMSCC are affected not only by different brands of AMS but also by different feeding management models of dairy farms. The records were collected only from 6 dairy farms. Therefore, it is expected that more dairy farms will be able to use AMS in the future and use them smoothly to provide more information for reference and stabilize the development of the domestic dairy industry in Taiwan.

Keywords: automatic milking system, bacterial counts, somatic cell counts, milk quality, dairy farm.
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Introduction

Automatic milking systems (AMS) are one of the most important technological changes in the domestic dairy industry in Taiwan. AMS can be considered not only as a substitute for milking parlors but also as a new approach to managing dairy farms (Pezzuolo *et al*., 2017). Today, AMS represents a growing reality due to lobbying for labor issues, rising costs, difficulty finding well-trained workers, and difficulty keeping people on farms (Simões Filho *et al*., 2020).

AMS manufacturers estimated that by 2020, approximately 50,000 units had been adopted worldwide (Simões Filho *et al*., 2020), with the initial introduction of commercial AMS in dairy farms occurring in the Netherlands in the early 1990s (Jacobs *et al*., 2012). The majority of these units (90%) were concentrated in Europe, with smaller percentages in Canada (9%) and other countries (1%) (de Koning, 2010). We introduced our first AMS in 2019, and so far a total of 25 AMS are in use, divided into 2 brands. 9 farms used Lely Astronaut (Lely, Rotterdam, Netherlands) and 7 farms used DeLaval VMS (DeLaval, Tumba, Sweden).

The milking equipment design, routine procedure and animal-flow models may differ depending on the brand of AMS. Among them, the most well-known part is that the robot arms are used in industries with hydraulic drive (DeLaval VMS) or designed for animal milking with pneumatic drive (Lely Astronaut). On the other hand, the teats were individually cleaned, stimulated, and dried by cleaning teat cups with warm air (DeLaval VMS) or cleaned and stimulated by rotating brushes (Lely Astronaut) (Castro *et al*., 2018). The flow systems were classified into two categories: guided flow, where dairy cows must pass through the permission gate. If cows have milking permission, they are directed to the milking waiting room; if not, they go to the resting area (DeLaval VMS). Alternatively, there is free flow, where dairy cows have unrestricted access to the milking station, resting area, and feeding area (Lely Astronaut).

This study aimed to investigate the impact of various brands of automatic milking systems on bulk tank milk bacterial and somatic cell counts in dairy farms in Taiwan.

Material and methods

Experimental design and sample collection

This study collected the bulk tank milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) records from the commercial dairy factory for dairy farmer pricing payments. Data were obtained from 6 dairy farms (3 Lely Astronaut and 3 DeLaval VMS) in Taiwan once a month from January to December 2023. These dairy farms have used AMS for over three years and have become stable in system operation and feeding management. The two brands were anonymously represented by brand A and brand B. These farms' primary breed was Holstein, and they used the free-stall barn systems. Each farm fed a different partial mixed ration twice a day, mainly composed of corn silage, alfalfa hay, and concentrate. AMS dispensers provided different amounts of commercial concentrates or feedstuffs during milking time depending on milk production, dry matter intake and days in milk.

Statistical analyses

All data were processed using GraphPad Prism version 6.0. Values of milk quality parameters were used without any transformation: BMTBC in cfu mL-1; BMSCC in cells ml-1. The difference regarding quality parameters between the two brands (Lely Astronaut and DeLaval VMS) was analyzed for statistical significance using an unpaired Student's t-test (two-tailed). *P* values of less than 0.05 (* $P < 0.05$) were used as the level of statistical significance, and *P* values of less than 0.01 (** $P < 0.01$) were indicated highly of statistical significance.

Results and discussion

Table 1 showed that throughout the whole period studied, the mean values of brand B were greater for BMTBC (27.06± 3.06 × 10^3 cfu mL^{-1}) and BMSCC (202.90 ± 9.65 × 10^3 cells ml^{-1}) than brand A (13.47 ± 1.39 × 10^3 cfu mL^{-1} and 171.40±7.14 × 10^3 cells ml^{-1}), moreover, it could be observed from figure 1 that brand A had a highly significant difference in the comparison of BMTBC. From another perspective, Table 2 showed the monthly average of BMTBC and BMSCC from different brands, also represented by a bar chart (Figure 2), it could be found that compared to brand A, the BMTBC of brand B had more significant fluctuations in different months, but in BMSCC, it is relatively stable like brand A. Therefore, it is estimated that there may be other factors affecting the quality of raw milk besides brand differences.

We investigated the application of AMS in six dairy farms and identified several factors influencing the experiment's results. These factors include the number of milking cows, the capacity of each AMS, milk yield, and milking frequency (Table 3). The number of milking cows and the capacity of each AMS directly impact the need for comprehensive care strategies and monitoring. As the number of milking cows or the capacity of each AMS increases, so does the demand for attentive management. Additionally, higher milk production requires more energy input. Increasing milking frequency helps alleviate pressure on cows' udders and decreases somatic cell and bacteria counts in raw milk.

Significant differences exist among domestic dairy farmers who use different brands of AMS in BMTBC and BMSCC. However, the quality of raw milk still complies with the Class A regulations on the standards of purchasing, acceptance, and pricing of raw milk. Preliminary speculation indicates that BMTBC and BMSCC are affected not only by different brands of AMS but also by different feeding management models of dairy farms.

Our research has uncovered a crucial issue: the number of milking cows, milk yield, milking frequency, and milk discard strategy were different between farms. However, the information provided by different AMS brands varies, leading to diverse decisions by dairy farmers regarding the milking process and discarding. This discrepancy can affect the BMTBC and BMSCC values of bulk tank milk. We understand how the technology works, and its operational procedures can assist farmers and technicians in making decisions about adopting new technology. In the future, we will continue to collect data on AMS and integrate the differences in strategies among different brands to find strategies suitable for Taiwan to maintain the high quality of AMS milk.

Table 1. The average of bulk milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) from brand A (n=3) and brand B (n=3) in 2023.

Milk quality parameters	Brand[1]		P-value
	A (n=36)	B (n=36)	
BMTBC (10^3 cfu mL^{-1})	13.47 ± 1.39^b	27.06 ± 3.06^a	<0.01
BMSCC (10^3 cells ml^{-1})	171.40 ± 7.14^b	202.90 ± 9.65^a	<0.05

[1] The data were shown as mean ± SE.
[a,b] Within the same row, values with different superscripts are significantly different ($P < 0.05$).

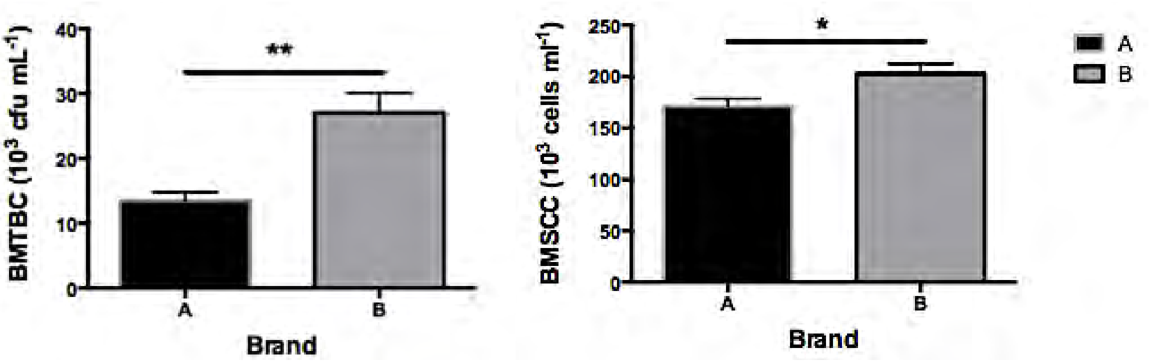

Figure 1. The average of bulk milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) from brand A (n=3) and brand B (n=3) in 2023.

Table 2. The monthly average of bulk milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) from brand A (n=3) and brand B (n=3) in 2023.

Bulk milk total bacterial counts (BMTBC)				
Brand	**A (n=3)**		**B (n=3)**	
month	**Mean**	**SE**	**Mean**	**SE**
1	9.54	1.30	44.74	27.34
2	15.62	5.87	33.04	17.31
3	11.37	2.36	20.37	4.09
4	12.26	2.94	25.20	5.22
5	11.25	5.12	49.00	20.64
6	14.25	7.58	29.32	12.57
7	13.07	5.72	21.54	3.13
8	20.23	10.79	18.36	2.50
9	15.09	3.27	25.14	4.51
10	14.10	4.68	23.36	3.01
11	7.92	2.06	17.81	3.45
12	16.90	4.62	22.79	10.85
Bulk milk somatic cell counts (BMSCC)				
Brand	**A (n=3)**		**B (n=3)**	
month	**Mean**	**SE**	**Mean**	**SE**
1	170.83	7.51	158.18	34.58
2	152.47	24.72	182.22	46.44
3	145.42	27.06	184.99	38.95
4	138.99	13.43	189.55	39.46
5	158.02	24.57	202.25	53.40
6	194.73	37.92	247.74	71.74
7	227.33	25.90	215.74	20.32
8	221.91	11.70	214.18	31.30
9	200.34	13.07	225.71	16.39
10	166.03	3.02	210.28	7.10
11	143.79	1.95	202.65	9.19
12	136.53	20.85	186.54	18.38

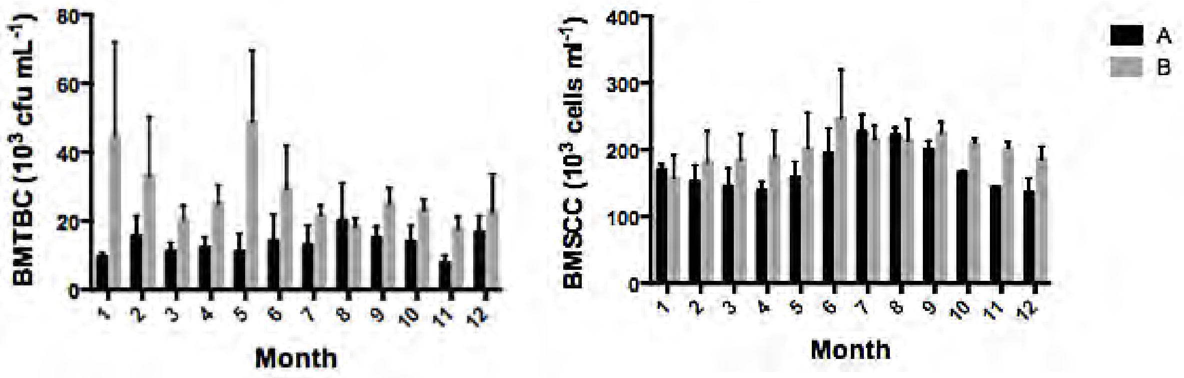

Figure 2. The monthly average of bulk milk total bacterial counts (BMTBC) and somatic cell counts (BMSCC) from brand A (n=3) and brand B (n=3) in 2023

Table 3. The average number of milking cows, number of AMS, cows per AMS, milk yield per cow per day, and milkings per cow per day in six dairy farms in 2023.

Experiment farm	Number of milking cows (no.)	Number of AMS (no.)	Cows per AMS (no.)	Milk yield per cow per day (kg)	Milkings per cow per day (no.)
A1	96	2	48	37.2	3.10
A2	76	2	38	34.3	2.90
A3	120	2	60	33.4	2.70
B1	220	4	55	31.0	2.74
B2	120	2	60	33.5	2.49
B3	48	1	48	37.3	2.25

Conclusions

The information provided by different AMS brands varies, leading to diverse decisions by dairy farmers regarding the milking process and discarding. This discrepancy can affect the BMTBC and BMSCC values of bulk tank milk. Although there is limited data collection from only 6 dairy farms, an increasing number of dairy farms is anticipated to embrace AMS in recent years. This preliminary result allows us to identify the reasons for the unstable quality of AMS milk and provides a direction for improvement in the future.

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Comparative dynamics of milk fatty acids for primiparous and multiparous Holstein cows in early lactation

Y.H. Chen[1], M.K. Yang[1], Y.H. Yeh[1], J.W. Shiau[1], J.F. Huang[2] and PA. Tu[1]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture,
No.207-5, Pitoumian, Xihu Township, Miaoli 368003, Taiwan
[2]Taiwan Livestock Research Institute, No.112, Farm Road, Hsinhua,
Tainan 71246, Taiwan
Corresponding Author: tpa@mail.tlri.gov.tw

Abstract

The milk fatty acid (FA) profile is a valuable indicator of a cow's nutritional and metabolic status, potentially aiding in assessing metabolic status at the individual cow level. However, limited knowledge exists regarding milk fat composition changes with parity. Understanding these changes during early lactation could enhance our understanding of dairy cow physiology. This study aimed to investigate whether milk FA composition differed between Holstein cows of different parities in early lactation. We characterized the milk FA profiles from day 7 to day 60 postpartum in primiparous (PP) and multiparous (MP) cows. A total of 26 Holstein Friesian dairy cows, including 12 PP and 14 MP, were included in the study and divided into two groups based on parity. Milk samples were collected on days 7, 14, 21, 30, and 60 post-calving and analysed for milk FA profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total *de novo* FA, mixed FA, and preformed FA, using MilkoScan FT^{+} 300 equipped with Fourier-transform infrared spectra. Blood samples were collected on days 7, 14, and 21 postpartum for the analysis of non-esterified fatty acids, beta-hydroxybutyrate (BHBA), glucose, and triglycerides, as well as for evaluating body condition scores (BCS). Partial Least Squares Discriminant Analysis (PLS-DA) was used to analyse the changes in milk composition over time in PP and MP. The results of PLS-DA showed changes in milk FA over lactation in both groups. PP had higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP ($P < 0.05$). MP had higher levels of SFA, *de novo* FA, and SCFA compared to PP ($P < 0.05$). PP had higher BHBA levels in milk, suggesting a more severe negative energy balance post-calving compared to MP. However, the average postpartum BCS and preformed FA were higher and BCS loss and *de novo* FA were lower in PP than in MP. In the early lactation stage of dairy cows, the cow mobilizes stored body fat to meet high energy demands, leading to an increase in metabolic products such as ketone in the blood. PP cows may release higher levels of preformed FA during early lactation because their higher BCS. PP cows might also be more inclined to allocate energy to continued growth and milk production in the early stages of lactation. This could lead to a higher release of preformed fatty acids from stored body fat to meet the demands of milk production. These findings could inform nutritional management strategies to better meet the requirements of cows in early lactation.

Keywords: body condition score, energy balance, parity.
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Introduction

The milk fatty acid (FA) profile serves as a crucial indicator of a dairy cow's nutritional and metabolic status, reflecting factors such as dietary intake, ruminal biohydrogenation, and mammary lipogenesis (McFadden and Corl, 2009). Factors like breed, parity, lactation stage, and feeding practices also influence the FA composition of milk (Poulsen *et al*., 2012; O'Callaghan *et al*., 2020). The FA profile of bovine milk is complex, comprising approximately 400 different FAs derived from mammary gland synthesis and circulating plasma (Giannuzzi *et al*., 2022). During early lactation, the mobilization of adipose reserves and diet processing result in changes in milk FA composition (Chilliard *et al*., 2000). These changes can be indicative of the cow's energy status and metabolic health (Giannuzzi *et al*., 2022). Specific FAs, such as c9-18:1, are mobilized from body reserves during negative energy balance (NEB) in early lactation, reflecting the severity of NEB and serving as indicators of energy status (Bastin *et al*., 2011; Gross *et al*., 2011). Conversely, short- and medium-chain FAs (e.g., C14:0) are synthesized *de novo* in the mammary gland and decrease in proportion during NEB (Churakov *et al*., 2021).

Non-esterified fatty acids (NEFA) in circulation reflect body reserve mobilization and dry matter intake (DMI), while beta-hydroxybutyrate (BHBA) reflects fat oxidation completeness in the liver (Adewuyi *et al*., 2005). NEFA released from lipid stores are either taken up by the udder to provide milk triglycerides or are oxidized in the liver as an alternative energy source (Duffield, 2000). The plasma NEFA concentration is therefore an index of lipid mobilization, with a rise in NEFA pre-partum suggestive of an energy deficit at this time. Elevated NEFA and BHBA concentrations indicate an increased risk of fatty liver and ketosis (Leblanc, 2010). These dysfunctions mainly concern the failure of individual animals to cope with complex nutritional and metabolic processes and to adapt to large variations in them during early lactation (Mulligan and Doherty, 2008). Although poor adaptation may start before calving, often it happens without clinical signs (Trevisi and Minuti, 2018; Mezzetti *et al*., 2020). Other markers of EB include changes in body condition score (Thorup *et al*., 2012; Chebel *et al*., 2018).

Parity significantly influences milk FA profile and yield, potentially due to differences in energy requirements and FA synthesis between primiparous (PP) and multiparous (MP) cows (Wilms *et al*., 2022; Bilal *et al*., 2014; Contarini *et al*., 2014; O'Callaghan *et al*., 2020). Limited information exists regarding milk fat composition changes with parity, particularly in early lactation, which could offer insights into dairy cow physiology (Contarini *et al*., 2014). Therefore, this study aims to investigate potential differences in milk FA composition between PP and MP Holstein cows in early lactation, shedding light on the physiological variations associated with parity in dairy cows.

Material and methods

Cows management and experimental design

There were 26 Holstein Friesian dairy cows, including 12 PP and 14 MP, were included in the study and divided into two groups based on parity. The cows entered the study during the first week after calving and stayed until 60 days in milk. All cattle were fed a total mixed ration (TMR) twice a day (at 0500h and 1400h) and had free access to clean water. The TMR formulation followed the NRC (2001) guidelines and included bermudagrass hay, alfalfa hay, corn silage, soybean hulls, brewers' grains, and a concentrate consisting of corn and soybean meal. Cattle were milked twice daily (0500 and 1600). All cows were housed together in a free stall facility equipped with rubber beds and solid concrete floors, which were scraped clean by a tractor six times a day. During the study period, the cows did not have access to pasture.

Blood collection and biochemical profile examination

Blood samples were collected at d 7, 14, and 21 post-calving. Aseptic jugular venipuncture was performed using a 20-G needle and sterile vacutainers (EDTA, heparinized, or clot activators). EDTA vacutainers were employed to collect blood samples for BHBA, glucose, and triglyceride analysis. Clot activator vacutainers were used to obtain serum samples for NEFA analysis, while heparinized vacutainers were utilized for plasma biochemical profile analysis. After collection, the blood samples were centrifuged at 1,500 g for 15 minutes to separate serum and plasma, which were then stored at -20 °C until further analysis.

Blood samples were also analyzed immediately after sampling for BHBA and glucose using test kits (Optium Beta Ketone Test Strips and Optium Blood Glucose Test Strips; FreeStyle, Abbott, USA). Serum samples were utilized for the analysis of NEFA using a Hitachi 704 Analyzer (Hitachi, Japan). Plasma biochemical samples were used to analyze triglyceride using a 7170 Chemistry Analyzer (Hitachi, Japan).

Milk sampling and FA profile analysis

Milk samples were collected from individual cows at 7, 14, 21, 30 and 60days in milk by the AFIMEN management system (Afimilk Ltd., Israel). The DHI (dairy herd improvement) laboratory analyzed and recorded each milk FA profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total *de novo* FA, mixed FA, and preformed FA, using MilkoScan FT+ 300 equipped with Fourier-transform infrared spectra. (FOSS, Denmark).

BCS records

Trained personnel evaluated the body condition score (BCS) of the cows using a 5-point scale (1 = thin, 5 = fat) (Edmonson *et al*., 1998) at 7, 14, 21 days postpartum. The BCS assessment was consistently performed by the same observer. BCS loss refers to the difference in BCS between day 21 and day 7 postpartum.

Statistical analysis

Statistical analysis included the use of ANOVA and Tukey's post hoc test to assess differences in biochemical profile, FA profiles and BCS among the different parities. To analyze the variations in FA profiles among PP and MP dairy cows during experiment period, we used Partial Least Squares Discriminant Analysis (PLS-DA). We used the FA profiles data as the independent variable matrix and the different parities as the dependent variable.

Results

PP had higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP ($P < 0.05$, Table 1). MP had higher levels of SFA, *de novo* FA, and SCFA compared to PP ($P < 0.05$). The BHBA value of PP was higher than 1.2 mmol/L, which is the threshold for subclinical ketosis. The average postpartum BCS and preformed FA were higher and BCS loss and de novo FA were lower in PP than in MP.

PLS-DA was used to analyse the changes in milk composition over time in PP and MP. PLS-DA demonstrated the evolution of the milk fatty acid profile from days 7 to 30 post parturition in PP and MP cows (Figure 1). Using PLS-DA, we analyzed the changes in milk composition from day 7 to day 60 postpartum in primiparous and multiparous cows. The results of the PLS-DA revealed that as lactation days increased, there were changes in the distribution of samples, reflecting the changes in milk fatty acids over the course of lactation in primiparous and multiparous cows. For example, multiparous cows tended to cluster towards the negative direction of Component 2 on days 14 and 21, and towards the positive direction of Component 1 on days 30 and 60. The samples from primiparous cows were more dispersed, tending to cluster towards the negative direction of Component 1 and Component 2 on days 30 and 60.

Discussion

The composition of milk fat is strongly influenced by the stage of lactation (Palmquist *et al*., 1993). Initially, the proportion of SCFA produced via de novo synthesis is low,

Table 1. BCS, biochemical profile, milk fatty acid (FA) composition in primiparous and multiparous cows during experiment period.

Item	Primiparous cows	Multiparous cows	P-Value
BCS loss	0.02	0.19	< 0.05
BCS	3.06	2.88	< 0.05
Blood parameters			
NEFA (mmol/l)	0.48	0.47	0.88
BHBA (mmol/l)	1.25	0.94	0.06
Glucose (mg/dl)	56.88	56.52	0.86
Triglyceride (mg/dl)	18.16	17.54	0.79
Milk parameters			
Fat (%)	4.03	3.90	0.51
Protein (%)	3.21	3.11	0.24
Fatty acid composition (g/100 g of fatty acids)			
Total Saturated FA	63.45	65.97	< 0.05
Total Unsaturated FA	31.81	28.80	< 0.05
Mono Unsaturated FA	29.05	26.04	< 0.05
Poly Unsaturated FA	2.75	2.75	0.94
De novo FA	19.42	21.94	< 0.05
Mixed FA	28.93	30.43	0.06
Preformed FA	43.19	38.88	< 0.05
Trans FA	3.07	3.57	< 0.05
SCFA	7.84	9.03	< 0.05
MCFA	42.75	43.96	0.36
LCFA	42.55	39.55	< 0.05
C14:0	8.62	9.16	0.07
C16:0	27.37	28.02	0.39
C18:0	13.27	12.61	< 0.05
C18:1	27.43	25.12	< 0.05

BCS: body condition score; BCS loss refers to the difference in BCS between day 21 and day 7 postpartum. NEFA: Non-esterified fatty acids; BHBA: beta-hydroxybutyrate; FA: fatty acid; *de novo* FA: C4 to C14; Mixed FA: C16, C16:1, and C:17; Preformed FA: Greater than or equal to C18; SCFA: C4 to C10; MCFA: C12 to C16; LCFA: C18.

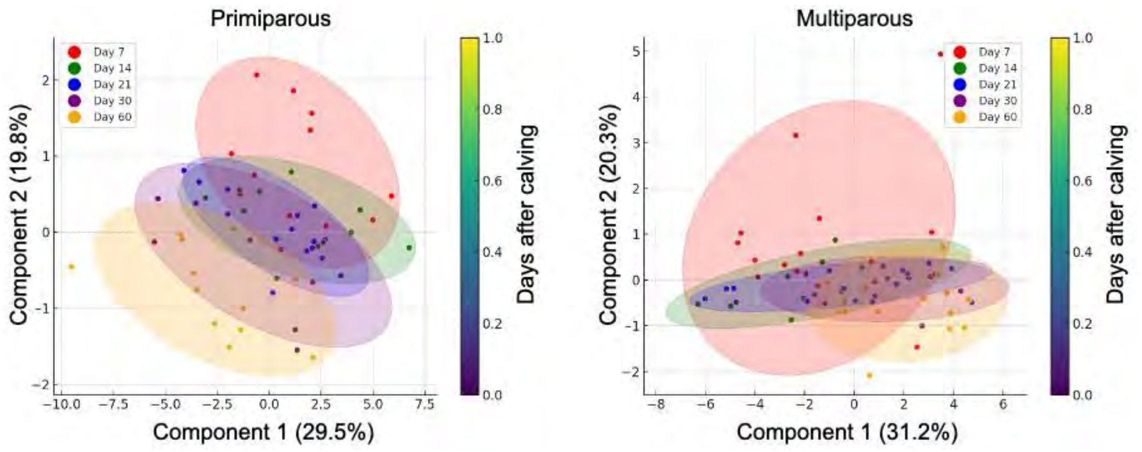

Figure 1. Partial least square discriminant analysis (PLS-DA) depicting the changes in milk fatty acid profile occurring as milk transition from days 7 to 60 post parturition [days 7, 14, 21, 30, 60 post parturition (red, green, blue, violet, yellow, respectively)].

but it increases steadily until at least 8 to 10 weeks into lactation. We used the milk composition data as the independent variable matrix and the different parities as the dependent variable in PLS-DA. By calculating the main components obtained, we were able to identify patterns in milk fatty acid composition among different parities. The results showed that as the days in lactation increased, the distribution of milk FAs from different parities changed, reflecting the trends in milk FA composition over the lactation period.

As lactation progresses, the concentrations of preformed FA decrease, while those of *de novo* FA and mixed-origin FA (e.g., 16:0) increase (Kay *et al*., 2005). The milk FA profiles reflect changes in the cow's energy balance (Churakov *et al*., 2021). PP cows exhibited higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP cows in this study ($P < 0.05$, see Table 1). In contrast, MP cows had higher levels of SFA, *de novo* FA, and SCFA compared to PP cows ($P < 0.05$). These results indicate differences in metabolism between PP and MP cows during the early lactation stage.

During the early lactation stage of dairy cows, the cow mobilizes stored body fat to meet high energy demands, resulting in an increase in metabolic products such as ketones in the blood (Leblanc, 2010). In our study, PP cows had higher blood ketone levels than MP cows, indicating that PP cows experienced higher energy demands. Blood glucose levels are tightly regulated by homeostasis and may not serve as a reliable indicator for monitoring or investigating health status (Herdt, 2000). Therefore, Van *et al*. (2020) and our study did not find differences in blood glucose levels based on parity.

The common NEB is compensated for by the mobilization of fat from body reserves during the first weeks after parturition in dairy cows, leading to the release of preformed FA; C18:1c9 is the predominant UFA in adipocytes and is primarily released through lipolysis during NEB (Rukkwamsuk *et al*., 2000). Subsequently, preformed long-chain non-esterified fatty acids (≥C18) derived from plasma are incorporated into milk fat and inhibit the de novo synthesis of SCFA (C4-C14) by the mammary gland (Bauman and Davis, 1974). Therefore, blood NEFA concentrations are related to milk LCFA concentrations. Additionally, according to Churakov *et al*. (2021), during this experiment, PP cows with C18:1 > 26 (Table 1) have already reached a state of NEB (-30 MJ NEL/d), and blood BHBA > 1.2 mmol/L has reached the threshold for subclinical ketosis, indicating a more severe NEB after calving than MP cows.

BCS is widely recognized as a reliable indicator of a dairy cow's nutritional status, body fat content, and early lactation dry matter intake (Roche *et al*., 2009). Furthermore, the health of dairy cows may be compromised if they lose more than 0.25 BCS points during the first month of lactation (Roche *et al*., 2009). However, in PP cows, the average postpartum BCS was higher and BCS loss was lower than in MP cows, suggesting less mobilization of body fat stores for energy production in milk fat. This indicates significant differences in energy metabolism compared to MP cows in our study.

In our study, PP cows, with their higher BCS, may release higher levels of preformed FA during early lactation. At the start of their first lactation, the competing demands of the mammary gland are superimposed on the requirements for growth (Etherton, 1982). Both insulin and insulin-like growth factor I (IGF-I) have positive growth-promoting effects (Oksbjerg *et al*., 2004), with IGF-I being the primary regulator of postnatal muscle hypertrophy, stimulating protein synthesis, and inhibiting degradation (Etherton, 1982). Studies comparing the metabolic data from PP and MP cows showed consistently higher concentrations of IGF-I throughout the period from −1 to +7 weeks after calving in PP cows (Wathes *et al*., 2007). These results suggest that the differing endocrine background in less mature animals may limit the partitioning of nutrients into milk (Wathes *et al*., 2007; Bilal *et al*., 2014; Contarini *et al*., 2014; O'Callaghan *et al*., 2020). This may be related to differences in energy requirements and partitioning, as well as differences in FA synthesis between PP and MP cows (Miller *et al*., 2006). Our findings could inform nutritional management strategies to better meet the requirements of cows in early lactation.

Conclusion

In our study, PP cows demonstrated higher levels of UFA, MUFA, preformed FA, and LCFA compared to MP cows. Conversely, MP cows exhibited higher levels of SFA, *de novo* FA, and SCFA compared to PP cows. PP cows with BHBA reached the threshold for subclinical ketosis, indicating a NEB after calving compared to MP cows. However, the average BCS postpartum and preformed FA were higher, and BCS loss and *de novo* FA were lower in PP than in MP cows. PP cows also appeared to prioritize energy allocation towards continued growth during the early lactation stages. These observations may be attributed to differences in energy requirements and partitioning, as well as variations in FA synthesis between PP and MP cows. This understanding could inform nutritional management practices during early lactation to better address the distinct needs of cattle in different parity groups.

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Transition Management Index: a new tool to assess the transition period success

D. E. Santschi[1], B. Mion[1], L. Fadul[1], D. Warner[1], A. Fleming[1], R. Lacroix[1], R. Moore[1], and G. Bisson[1]

[1]Lactanet, 555 Boul. Des Anciens-Combattants, Sainte- Anne-de-Bellevue, H9X 3R4, Quebec, Canada
Corresponding Author: dsantschi@lactanet.ca

Abstract

An objective tool to assess the quality of the transition management practices is an important aid in maximizing the performance and profitability of dairy farms. Our objective was to create an index to assess the transition period of dairy cows using Canadian data (Transition Management Index; TMI) and benchmark current practices. The methodology was inspired by the Transition Cow Index® developed by Nordlund (2006). We used over one million DHI cow records from 2017-2020 from all provinces in Canada. included cows from lactation 1 to 7, from 8 breeds (Holstein, Jersey, Ayrshire, Brown Swiss, Guernsey, Canadian, Milking Shorthorn, and others), and 3 milking systems (pipeline, parlour, and robotic systems). Models were developed separately for primi- and multiparous cows using mixed-effect linear regression in R (*lme4* package) with the fixed variables breed, lactation start reason, milk testing scheme, age at first calving (primiparous only), milking frequency, and estimated breeding values for milk, fat, and protein, and a random herd effect. The models for multiparous cows also included previous lactation start reason, DIM, peak DIM, peak milk yield, 305-d milk yield, average somatic cell score (SCS), days dry, and lactation number. The TMI consists of the difference between the expected milk production estimated by the model and the 305-d projection at the first milk test (i.e., between 5-45 DIM), which also included DIM, beta-hydroxybutyrate (BHB), SCS, and 24-h milk yield.

The novelty of the TMI in comparison to the previous index is the inclusion of new traits potentially associated with transition management as well as the calculation of an index for first lactation cows. Results showed that the TMI herd average in 2022 was -27 (range of –1737 to 1334), and 49% of the cows had a negative TMI. Older cows have a lower TMI than younger cows (Lact 1 = 24; Lact 2 = -34; Lact 3+ = -55). Robotic herds had a greater average TMI (39) than pipeline (–54) or parlour systems (–100). There is a positive correlation (R = 0.64) between TMI and milk production. An increase of 100 points in the TMI is equivalent to an increase of 100 kg of milk and 3.5 kg of butterfat per standard lactation. A total of 5,070 Canadian herds were classified in percentiles according to the average TMI and their herd performance and profitability were compared. The top 20% of the herds have a daily milk yield 4.5 kg higher, produce 5.7 kg more milk at peak lactation, and produce 1,584 kg more milk and 62 kg more butterfat per lactation than the average. In addition, the top 20% of the herds have a yearly milk revenue of C$1,307/cow higher and a revenue per day of life of C$0.90/cow higher than the average. Higher herd TMI was associated with greater average milk value (R = 0.61), a lower SCC average (R= -0.35), and a lower percentage of cows with elevated or low MUN levels (R= -0.25). In addition, higher herd TMI was associated (R= -0.26) with shorter calving intervals. We are developing an interactive TMI dashboard to be launched in 2024. The main page features TMI cow records, annual averages, and provincial benchmarks, as well as KPI indicators

and drill-down functions on related transition management aspects such as udder health, energy status, rumen health, and dry period that enable producers and their advisors to assess the transition practices and identify opportunities for improvement.

Keywords: transition period, performance, management .
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Introduction

The transition period is considered the most challenging phase for dairy cows. During this period, nutrient imbalance is observed due to the higher metabolic demands and a decrease in feed intake (Bauman and Currie, 1980; Bell, 1995), consequently leading to an increased risk of oxidative stress (Sordillo and Aitken, 2009), reduced immune function (LeBlanc, 2020), and potentially higher inflammatory response (Contreras et al., 2018). Although those challenges are common to dairy cows during the transition period, a subset of cows may exhibit inadequate adaptation, leading to enduring consequences to performance throughout the whole lactation. Considering the impact of animal productivity on farm profitability, management programs need to be developed aiming to reduce the transition challenges and to quickly identify opportunities for improvement.

The results of the first test in the lactation provide insights into those challenges. A tool called Transition Cow Index™ (TCI) was developed in 2006 (Nordlund, 2006) to objectively evaluate transition management at the herd level. The tool uses fourteen factors from DHI records to project milk yield; and the first test date results to project the 305-d milk yield. Originally developed based on cow records from herds registered on the Wisconsin DHIA, the TCI is currently used in some provinces of Canada as a management tool. Nonetheless, advancements in management practices, along with enhancements in genetics and augmented milk yields, have been observed since 2006. In addition, using a Canadian index may reflect better the intricacies of the Canadian dairy industry. Therefore, our objective was to create an index to assess the transition period of dairy cows using Canadian data and develop an interactive dashboard to facilitate the use of the index.

Materials and methods

Model development

Data was extracted from the Lactanet database for lactations with a calving date between 2017 and 2020. This included animal and herd information, calving and lactation records including 305d milk production, test day records for the first test post-calving occurring between 5-60 DIM, dam deviations for production mature equivalents, and herd level production. Genetic evaluations were also available for all herdbook registered animals. Data included cows from all Canadian provinces and represented the seven dairy breeds in Canada, Holstein, Jersey, Ayrshire, Brown Swiss, Guernsey, Canadienne, and Milking Shorthorn, as well as crossbred or unknown breeds. Only lactations one through eight were retained due to the limited number of records in higher lactations. For first parity animals with a completed first lactation record and first test record in that lactation, further requirements were an age at calving between 18 and 47 months and herdbook registration. The final number of records was 373,297 for first lactations and 688,299 from later lactations.

Models were developed separately for first and later lactations due to the differences in data available for each group, where later lactations will have information on their previous lactation performance. The formulation of the models followed the general methodology described by Nordlund (2006) for the Transition Cow Index™. For each of the first and later parity groups two models were developed, one to estimate the expected 305d milk production without any test day information ("prediction") and one to estimate the 305d milk production with the first test day record ("projection"). The "prediction" milk production minus the "projection" milk production represents the final TMI value of interest.

All models were created using mixed-effect linear regression using the lme4 package in R. Herd at test day was included as a random effect in the training model to account for variation due to the herd environment. Initially, all variables were included as fixed effects in their respective model. Polynomial terms were considered for some traits. Variables were retained if significant (P<0.10) in at least one of the two models following a combination of backward elimination and forward selection. The impact of variable inclusion on the final index values was also considered for retention, such that if variables more complex to implement had little impact, they were removed. A further consideration in variable selection was the degree of missing data for a trait to ensure the model would apply to a large proportion of the population. Final fixed variables included breed, lactation start reason, milk testing scheme, age at first calving (first lactation only), milking frequency, and estimated breeding values for milk, fat, and protein. Later lactation models also included previous lactation start reason, DIM, peak DIM, peak milk yield, 305-d milk yield, average SCS, days dry, and lactation number. The "projection" models included all the same variables as the "prediction" and further included DIM, beta-hydroxybutyrate (BHB), SCS, and 24-h milk yield at the first test.

Results and discussion

Relationship between Transition Cow Index™ and Transition Management Index

At the individual level, there was a strong correlation (R=0.95) between the TCI and TMI results for both lactation 2 and 3+ cows (Figure 1).

Descriptive analysis

At the individual level, the TMI average was -27 and 49% of the cows had an index below zero (Figure 2). The TMI average was slightly higher for Lactation 1 cows (25) than for Lactation 2 (-34) and Lactation 3+ cows (-55). However, the percentage of cows below zero was similar between lactation groups (Lact. 1 = 48; Lact. 2 = 50; Lact. 3+ = 50) (Figure 2). The TMI equation for Lact. 2+ cows incorporate additional variables due to the information about previous lactation, unavailable for Lact. 1 cows. Given this distinction, one might hypothesize that genetic parameters would exert a more significant influence on the TMI of Lact. 1 cows. However, a relative metrics analysis demonstrated that, apart from breed, no variable exhibited greater importance in the Lact. 1 equation.

The TMI average for herds with robotic milking systems (39) was higher than for herds with pipeline (-54) and parlour systems (-100). The higher average is accompanied by a lower percentage of cows with TMI below zero for herds with robotic milking systems (44.1 %) than pipeline (51.4%) and parlour milking systems (53.2%). In 2021, we compared the performance of Canadian herds according to the milking system (Brisson, 2021) and we also find a higher TCI for robotic herds in comparison to pipeline and parlour.

In general, Ayrshire herds had the lower TMI (-150) and the greatest proportion of cows with TMI below zero (58.5%), in comparison to Holstein (TMI = -39, % below 0 =49.9%) and Jersey herds (TMI = -81; % below 0 = 53.8%).

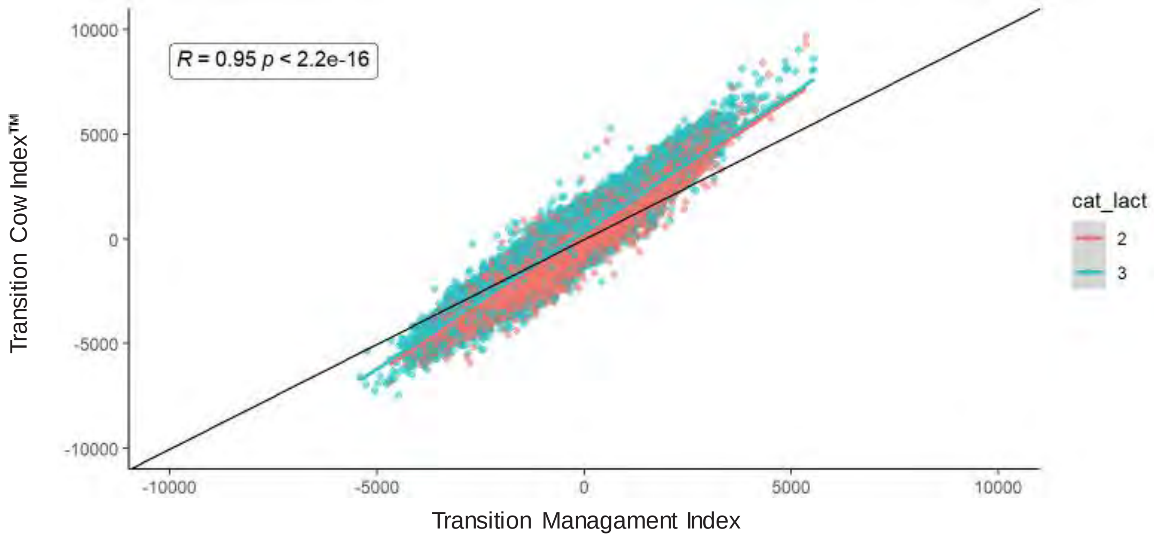


Figure 1. Relationship between the Transition Cow Index™ and the new Transition Management Index for cows from lactation category (cat_lat) 2 and 3+.

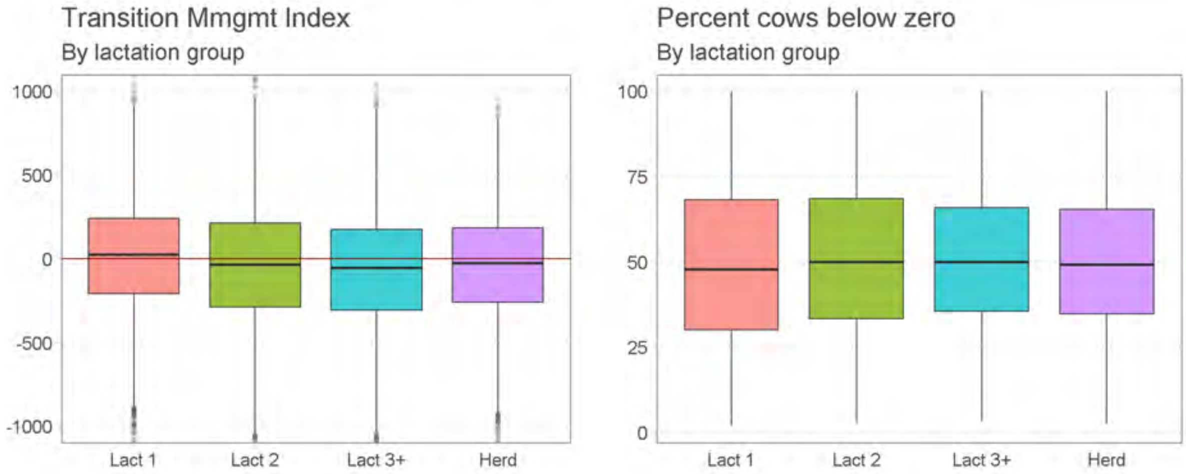


Figure 2. Transition management index average (left) and percentage of cows with a Transition Management Index below zero (right) according to lactation groups.

Relationship between Transition Management Index and performance

The TMI was correlated (R=1) with 305-day milk, and every increase in 100 points in the herd TMI is equivalent to an increase in 100 kg of milk and 3.5 kg of butterfat per standard lactation.

A total of 5,070 Canadian herds were classified in percentiles according to the average herd TMI, and those herds with the top 20% averages had a lower percentage of cows with negative TMI, higher milk and fat yield, had a higher production at peak, and a greater milk revenue than the average (Table 1).

The herd TMI average was negatively correlated with annual SCC average (R=-0.35), calving interval (R=-0.26), and extreme MUN values (< 8 mg/dL or >18 mg/dL; R=-0.25), and positively correlated (R=0.61) with milk value (Figure 3).

Transition Management Index dashboard

We created an interactive dashboard to present the results of the herd Transition Management Index. The dashboard main page contains the TMI herd average, the % of animals with a negative index, the average of the current test, and a graph where is possible to assess individual TMI values. It also contains information about key

Table 1. Production and profitability parameters of 5,070 Canadian herds classified in percentiles according to the average herd Transition Management Index (TMI).

	Herd Percentiles					
	0-20	**20-40**	**40-60**	**60-80**	**80-100**	**Average**
TMI	-534	-188	-10	155	419	-49
Cows with negative TMI, %	78.9	60.7	48.2	36.7	23.3	50.6
Milk yield, kg/day	22.6	26.3	27.7	29.8	32.0	27.5
Fat yield, kg/day	0.94	1.11	1.16	1.25	1.33	1.15
Protein yield, kg/day	0.76	0.89	0.94	1.01	1.07	0.93
Milk yield, kg/year	8,362	9,708	10,226	10,890	11,701	10,117
Fat yield, kg/year	342	400	421	446	477	415
Protein yield, kg/year	279	326	344	365	391	339
Milk at peak lactation, kg	34.6	39.0	40.9	43.2	46.3	40.6
Days to peak lactation	45.6	47.1	47.0	47.6	47.7	47.0
Milk revenue, $/cow/year	4,625	5,679	6,023	6,755	7,289	5,982

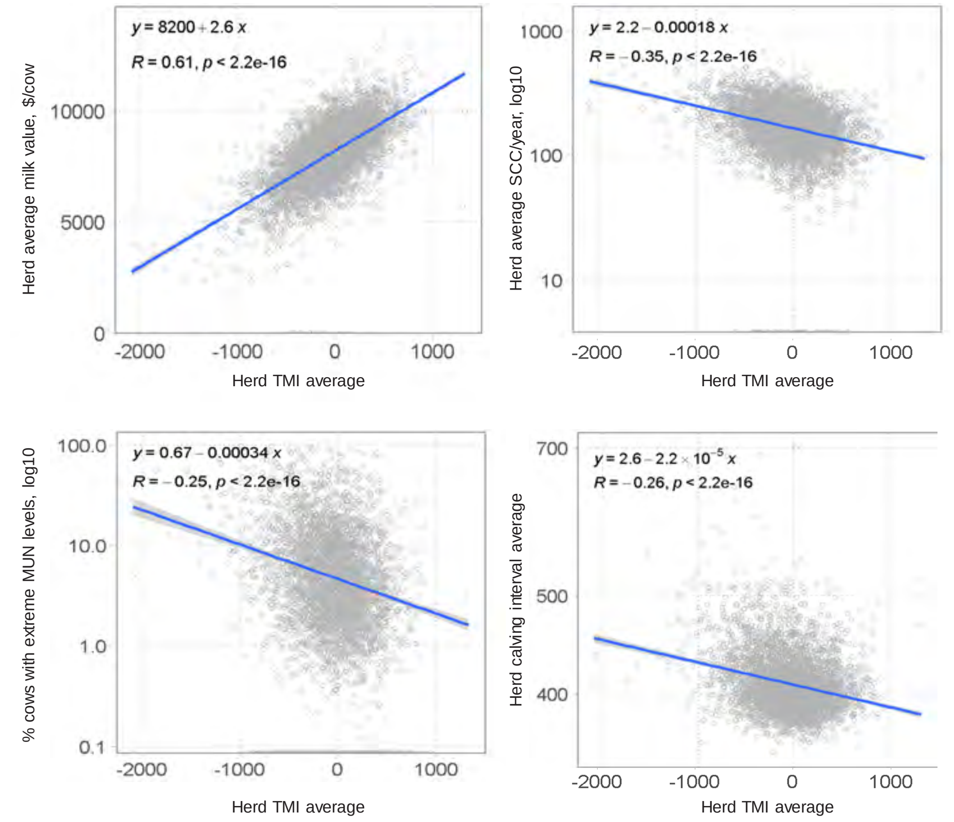
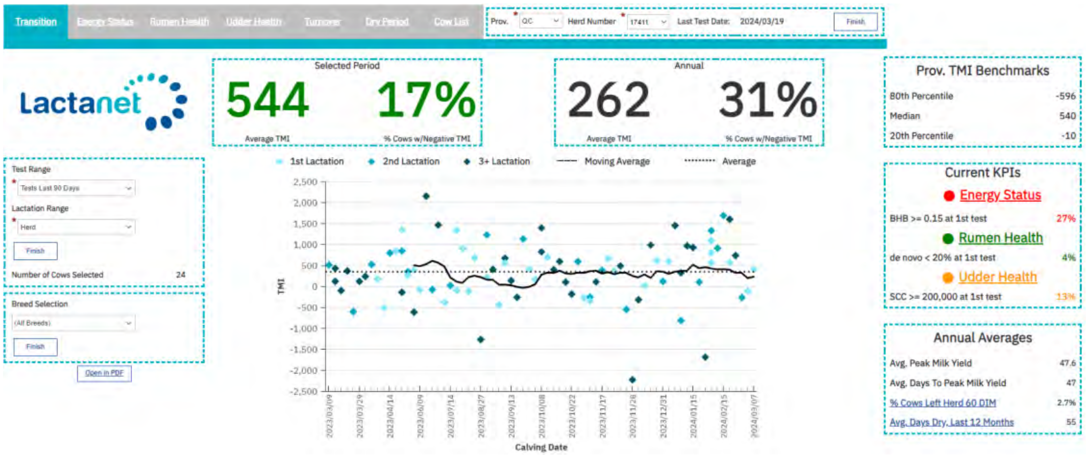


Figure 3. Relationship between herd Transition Management Index and herd average milk value, SCC, % of cows with extreme MUN levels, and calving interval.

performance indicators (KPIs) at transition period, such as udder health, energy status, and ruminal health, dry period length, among other information. Each one of those indicators has its tab, which brings more information about each KPI in early lactation, helping to identify opportunities for improvement (Figure 4).



Prov.: QC
Herd Number: 17411
Last Test Date: 2024/03/19

Test Range: Tests Last 90 Days
Lactation Range: Herd
Number of Cows Selected 24
Breed Selection: (All Breeds)

Selected Period	
544	17%
Average TMI	% Cows w/Negative TMI

Annual	
262	31%
Average TMI	% Cows w/Negative TMI

Prov. TMI Benchmarks	
80th Percentile	-596
Median	540
20th Percentile	-10

Current KPIs

● Energy Status
BHB >= 0.15 at 1st test 27%

● Rumen Health
de novo < 20% at 1st test 4%

● Udder Health
SCC >= 200,000 at 1st test 13%

Annual Averages	
Avg. Peak Milk Yield	47.6
Avg. Days To Peak Milk Yield	47
% Cows Left Herd 60 DIM	2.7%
Avg. Days Dry, Last 12 Months	55

Figure 4. Main page of the Transition Management Index dashboard created by Lactanet Canada.

Conclusion

In conclusion, the Transition Management Index serves as an invaluable objective tool to assess the transition period practices on farm. Its correlation with production, reproduction, and health parameters indicates that the transition success is associated with herd performance and profitability. The use of the TMI and its dashboard will guide producers and advisors to better assess the farm operation, identify opportunities for improvement, and facilitate informed decision-making processes.

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Evolution of mature size, mature production, and the relative maturity and performance during the first two lactations of DHI-registered Holsteins in Canada

R. Molano[1], R.K. Moore[1] and D.E. Santschi[1]

*[1]Lactanet, 555 Boul. Des Anciens-Combattants, Sainte- Anne-de-Bellevue, H9X 3R4,
Quebec, Canada
Corresponding Author: dsantschi@lactanet.ca*

Abstract

Size and milk production of dairy cows have increased over time. However, the magnitude of this increase and its relationship with productivity across lactations has been little studied. The objective of this analysis was to describe the evolution over the last two decades of the mature BW (MBW), mature production and the relative maturity (RMAT) and performance (RPER) during the first two lactations of Holstein cows. Data from first (L1), second (L2) and third and more lactations (L3+) from 2002 to 2021 were extracted from the Quebec DHI data base. Records of age at first calving (AFC, 1,413,772), BW (565,710; 713,668 and 1,152,530) and 305 d-adjusted milk and components yields (1,334,433; 1,0310,24 and 1,538,492) from L1, L2 and L3+ cows, respectively, were averaged per year. The L3+ cows were considered as mature and the reference to evaluate RMAT and RPER of L1 and L2 cows. Data from L3+ cows and AFC were regressed against time while RMAT and RPER were analyzed using a fixed effect model including year, parity and their interaction. The BW and milk (MY), fat (FY) and protein yields (PY) of L3+ cows increased since 2002 ($P<0.01$) at rates of 3.7±0.1, 109±5, 5.7±0.2 and 4.0±0.2 kg per yr, respectively. In 2021 L3+ cows weighed 738±1.2 kg and produced 11,184±56, 447±2.6 and 364±2.8 kg of milk, fat and protein, respectively. The AFC decreased ($P<0.01$) at a rate of 0.15±0.01 mo per year, averaging 24.8±0.13 mo in 2021. L1 cows' RMAT decreased ($P<0.01$) at 0.09±0.01% per year and was 87.4±0.1% of MBW in 2021. Overall, L2 cows' RMAT was 94.46±0.05% of MBW and did not change over time ($P=0.61$). The RPER decreased over time ($P<0.01$) but at a faster rate in L1 than in L2 cows ($P<0.01$) for MY (0.22±0.2 vs 0.06±0.2% per yr), FY (0.17±0.2 vs 0.04±0.02% per yr) and PY (0.24±0.01 vs 0.07±0.02 % per yr). In 2021 MY, FT and PY relative to L3+ cows were 80.3, 81.5 and 81.5% for L1 and 95.0, 95.2 and 96.6 % for L2 cows, respectively. Despite the significant progress in mature lactational performance and AFC, there has been a decline in the RPER of L1 and L2 cows that deserves to be addressed.

Keywords: Maturity, lactation, firts calving, body weight, milk yield.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 6: Evaluation and presentation of new parameters in herd management for dairy farms

Introduction

The average milk production per cow has risen progressively in the past years. In the past 50 years, the average 305-d lactation yield for Holstein cows increased by approximately 5,000 kg (Brito *et al*., 2021). This expressive augment in milk production is driven by intensive genetic selection, along with improvement in nutritional practices, precision management, reproductive technologies, and improvement in health management (Brito *et al*., 2021).

At the same time, the size of dairy cows has increased over the years. Body weight (BW) is a characteristic that impacts nutrient requirements, feed intake, space needs, and medication dosing, among other factors. However, the magnitude of the increase in body weight and its relationship with productivity across lactations has been little studied. Therefore, the objective of this study was to describe the evolution over the last two decades of the MBW, mature production, and relative maturity (RMAT) and performance (RPER) during the first two lactations of Holstein cows.

Material and methods

Data from Lactation 1 (L1), Lactation 2 (L2), and Lactation 3+ (L3+) cows were extracted from Lactanet database, corresponding to a period of 20 years (from 2002 and 2021). A total of 1,413,772 records of age at first calving (AFC), 2,431,870 records of BW (L1 = 565,710; L2 = 713,668; and L3+ = 1,152,530), and 3,903,949 records of 305-d adjusted milk and component yields (L1 = 1,334,433; L2 = 1,0310,24; and L3+ = 1,538,492) were analysed.

An average per year was calculated for the parameters. For calculating RMAT and RPER, data from L3+ cows were considered as mature and used as reference values for L1 and L2 cows.

Data from L3+ cows and AFC were regressed against time; and data from RMAT and RPER were analyzed using a fixed model including the effects of year, parity, and the interaction between year and parity.

Results

Evolution of body weight and milk yield

According to our records, in the past 20 years, there was an increase in the 305-d milk yield of mature cows, and this increase was accompanied by an increase in mature body weight as well (Figure 1). The BW and milk, fat, and protein yields of L3+ cows increased since 2002 at rates of 3.7, 109, 5.7, and 4.0 kg per year, respectively. In 2021, mature cows weighed 738 ± 1.2 kg, and produced 11,184 ± 56, 447 ± 2.6, and 364 ± 2.8 kg of milk, fat, and protein, respectively.

Evolution of age at first calving

The AFC decreased ($P < 0.01$) at a rate of 0.15 ± 0.01 months per year, averaging 24.8 ± 0.13 months in 2021 (Figure 2).

Evolution of relative maturity, and relative milk, fat, and protein yields for first and second lactation cows

Overall, L1 cows RMAT decreased ($P < 0.01$) over time, by a rate of 0.09 ± 0.01% per year and was 87.4 ± 0.1% of MBW in 2021 (Figure 3, L1). The decrease in maturity of L1 cows does not follow the same pattern as that of the age at first calving. Overall, L2 cows’ maturity was 94.46% of MBW and did not change ($P = 0.61$) over time (Figure 3, L2).

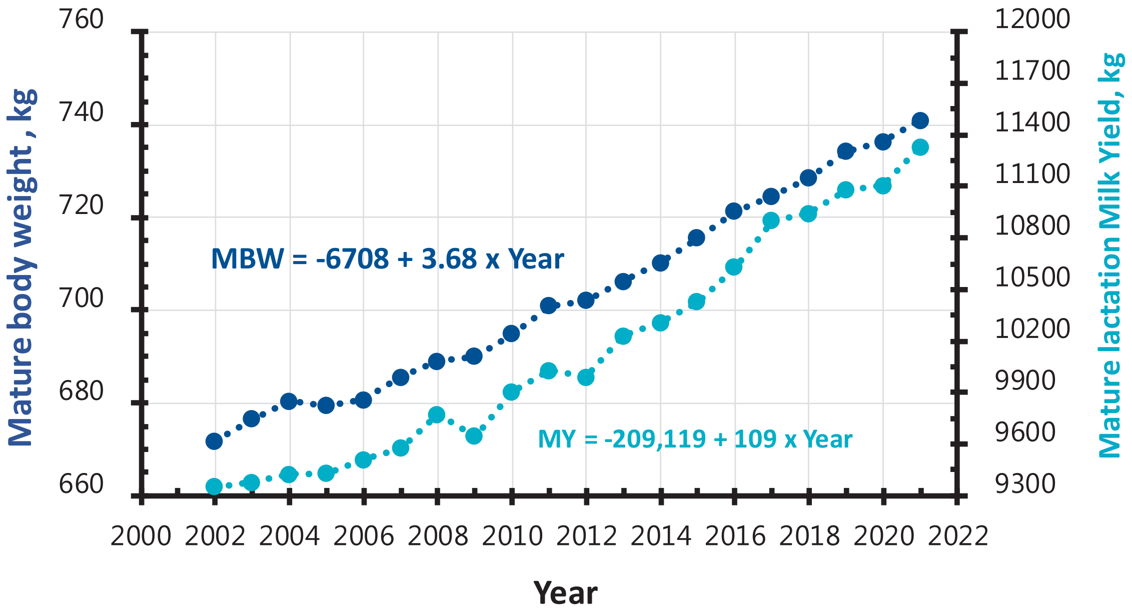

Figure 1. Evolution of body weight and 305-d milk yield of third and more lactation (L3+) cows.

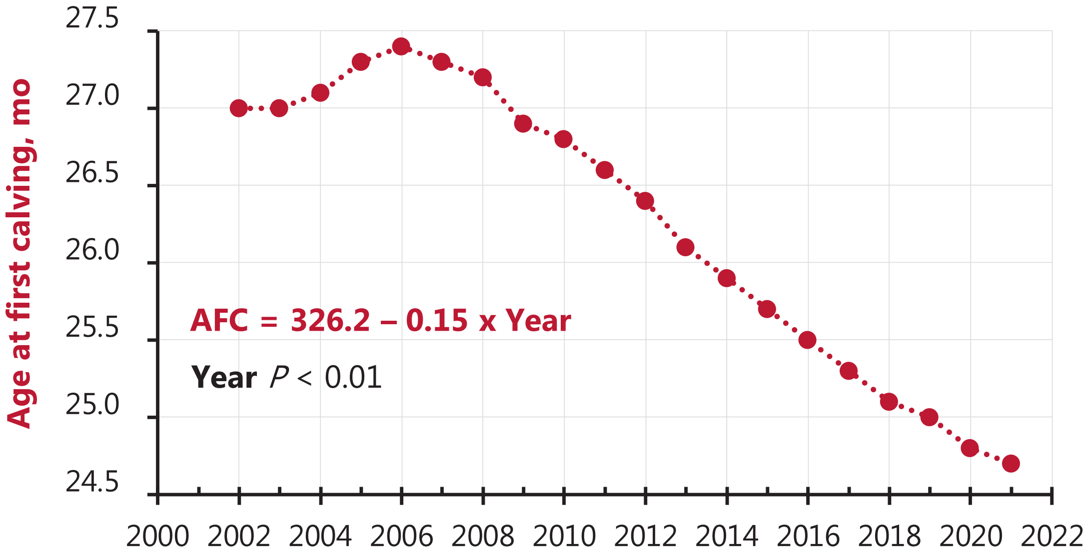

Figure 2. Evolution of age at first calving (AFC).

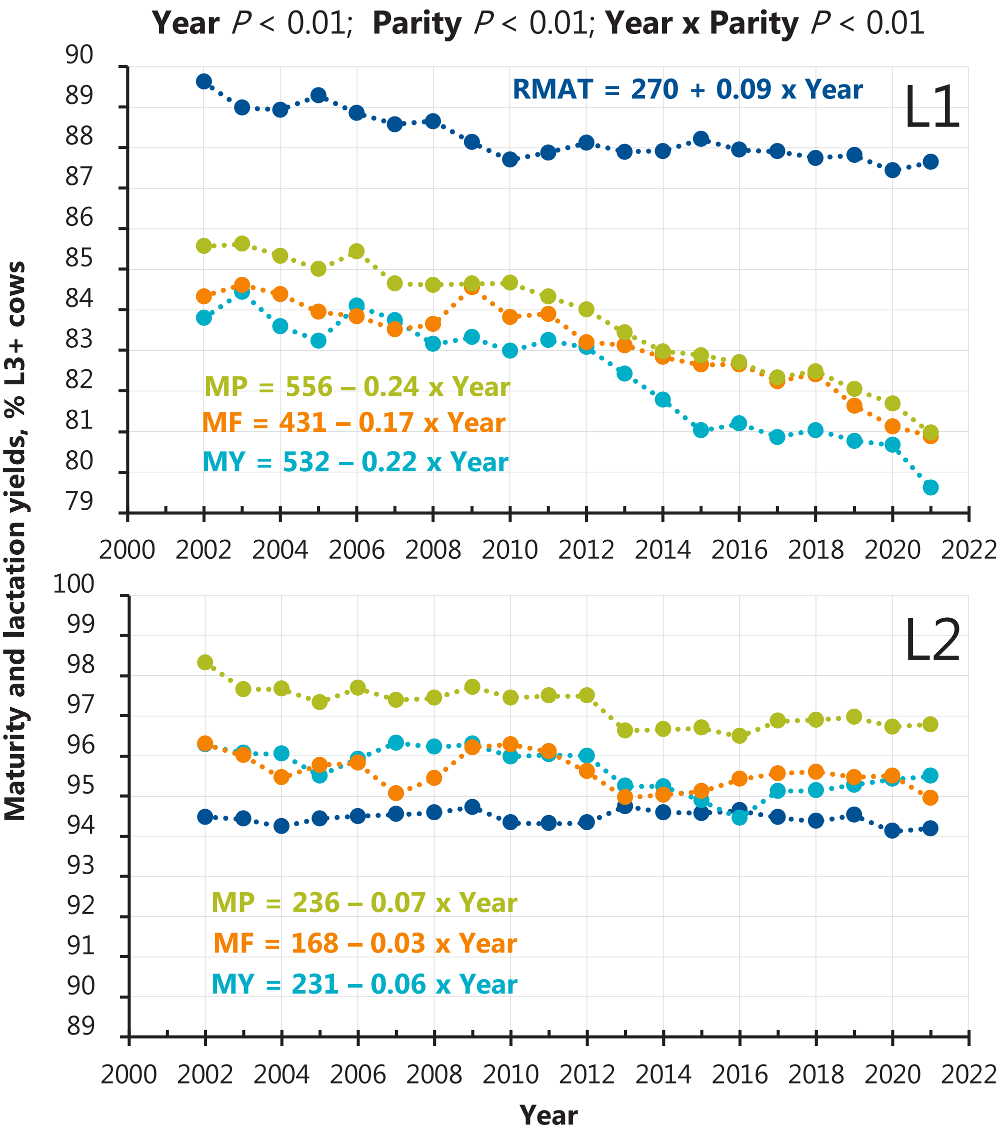

Figure 3. Evolution of the maturity and relative milk (MY), fat (MF), and protein yields (MP) for 1st (L1) and 2nd lactation (L2) Holstein cows.

The relative performance decreased over time but at a faster rate in L1 than in L2 cows and the greatest decline was observed in the protein and milk relative yields. In 2021 milk, fat, and protein yields relative to L3+ cows were 80.3, 81.5, and 81.5% for L1 and 95.0, 95.2, and 96.6 % for L2 cows, respectively. The decline in relative performance of L1 and L2 cows does not correspond to the evolution of their maturity.

Conclusion and implications

In conclusion, significant advancements have been achieved in AFC and the mature lactation performance. The increase in mature performance coincides with an increase in MBW. However, a decline in the relative performance over time has been observed for first and second-lactation cows, particularly for protein and milk yield. Interestingly, this decline in RPER of L1 and L2 cows does not correspond to the evolution of RMAT. Moreover, the decline in RMAT of L1 cows does not follow the same trend as the one observed for AFC. It is imperative to further explore and address the decline in RPER and explore potential management and nutritional factors that could be limiting the performance of L1 and L2 cows.

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Application of machine learning methods to control the milk samples analysis results reliability

I.V. Rukin[1], S.I. Sudarkina[1], M.S. Krutkina[1]and E.V. Kamaldinov[2]

[1]JSC «Agroplem», Moscow, Russian Federation
[2]Novosibirsk State Agrarian University, Novosibirsk, Russian Federation
Corresponding Author: irukin@agroplem.ru

Abstract

Modern animal breeding methods, such as genomic evaluation of breeding values (GEBV), are based on large amounts of phenotypic and genetic data. The reliability of GEBV results and the general selection process depends on the accuracy of the primary phenotypic data. Test day milk samples implied to be collected from unique cows may actually be dispensed from a sampler or milk tank. We will further call such milk samples dispensed or DS. To address this issue, a new DS identification system using clustering algorithm (OPTICS) was developed to improve accuracy in detecting DS in milk samples. Results showed high accuracy in identifying DS in small batches, when samples were not dispensed sequentially or were mixed with unique samples. Large batches with more than 60 DS in each were also accurately detected. However, the algorithm showed low accuracy on batches with low DS proportion. This new method has already been implemented in the milk analysis laboratory and will continue to be refined for better data filtering in breeding value systems.

Introduction

GEBV plays the key role in modern methods of livestock production and selection work. Yearly the number of farms including GEBV in their work raises significantly (Song *et al*., 2023) leading to great increase of data collected and analyzed. While GEBV calculations take into account as much available livestock data as possible, the unreliable data may lead to bias and mistakes in GEBV results and erroneous conclusions in selection work. That is why data quality control is crucial process of data preprocessing before GEBV (Cabrera *et al*., 2020).

One of the primary categories of traits in dairy cattle is milk traits, often assessed through TD (test-day) milk samples analyzed in milk laboratories. Research suggests that one potential factor leading to skewed TD milk results is the collection of samples from tanks, rather than individual cows. It's important to identify samples collected from tanks and exclude them from GEBV (genomic estimated breeding value) analysis. While our laboratory acknowledges batches containing dispensed samples collected from tanks (DS) in sequential order, identifying DS samples mixed with unique samples in a batch is more challenging.

The aim of our work is creation of more accurate recognition of DS system. The main objectives of this study are:

- The recognition of DS in the TD samples batch
- Identification of DS for subsequent data filtering

Material and methods

TD milking data collected from 2019 to 2024 was used to generate test datasets. These datasets included batches with varying amounts of dispensed samples (DS), ranging from 0 to 121 DS per batch. We generated a total of three datasets:

- **Dataset 1:** Comprised of 1019 batches, 994 of which contained between 15 to 100 DS each.
- **Dataset 2:** Comprised of 4298 batches, 4289 of which contained between 15 to 100 DS each.
- **Dataset 3:** Comprised of 1000 batches, 997 of which contained between 15 to 121 DS each.

The generation of DS was done as follows:

- **Dataset 1:** In each batch, one sample was chosen randomly. Its fat and protein content were used to generate 15 to 100 points with mean values equal to the fat and protein content of the chosen sample, and a standard deviation of 0.1. These generated points were added to the batch data file in random strings, mixing DS with unique samples. Consequently, the samples in Dataset 1 are generated as if dispensed from one tank.
- **Dataset 2:** The generation method was similar to Dataset 1, but with a variation in the number of samples chosen to generate DS. Here, the number of samples used to generate DS varied randomly from 1 to 10. Thus, Dataset 2 represents batches with DS obtained from multiple tanks.
- **Dataset 3:** This dataset consists of batches with or without DS from one tank, similar to Dataset 1. However, the number of DS per batch varied depending on batch size: 15-61 DS in small batches, 29-101 DS in medium batches, and 59-121 DS in large batches.

A summary of the generated datasets is shown in Table 1.

To improve the quality control algorithm and recognize DS mixed with unique samples in a batch, we applied unsupervised machine learning. We developed an algorithm based on clustering, utilizing the density-based method OPTICS (Ordering Points To Identify the Clustering Structure) (Ankerst *et al*., 1999), available in the Python scikit-learn module (Pedregosa *et al*., 2011). The core idea of the algorithm is to identify clusters of high-density points in the space of milk sample parameters.

We focused on two milk sample parameters obtained from Fossomatic: fat and protein content. The OPTICS clustering algorithm takes the data to be clustered and the

Table 1. Description of generated datasets.

Dataset	Number of batches	Number of batches with DS	Number of tanks in batch	Number of samples in one tank
1	1019	994	0 or 1	15-100
2	4298	4289	0 or 1-10	15-100
3	1000	997	0 or 1	15-61[1] 29-101[2] 59-121[3]

[1] Small size batches, batch size < 150 samples
[2] Medium size batches, 150 < batch size < 800 samples
[3] Large size batches, batch size > 800 samples

clustering parameters: min_samples (the minimum number of points, MinPts) and max_eps (the maximum distance for clustering).

For identifying batches containing DS, we used max_eps = 0.2. The min_samples value varied based on the number of samples in a batch:

- For small batches (fewer than 150 samples), we used min_samples = 15.
- For medium batches (150 to 800 samples), we used min_samples = 30.
- For large batches (more than 800 samples), we used min_samples = 60.

Results and discussion

We tested the clustering algorithm on the three datasets described above and calculated metrics to evaluate the quality of clustering. First, we assessed the algorithm's ability to recognize batches containing DS. According to the algorithm, a batch is considered to contain DS if more than one cluster is found. The calculated performance statistics and metrics are shown in Table 2 and Table 3, respectively.

The metrics used to evaluate the algorithm's performance quality included:

- Rand Index (RI).
- Adjusted Rand Index (ARI).
- Mutual Information (MI).
- Adjusted Mutual Information (AMI).
- V-measure.
- Homogeneity.
- Completeness.

The results shown in Table 2 display fine algorithm performance on small batches. However, the performance on Datasets 1 and 2 decreases with an increase in batch size, a trend not observed in Dataset 3. This performance decline is presumably associated with the proportion of DS in a batch. As the batch size increases, more samples have similar fat and protein content values, making it harder for the algorithm to determine if a small collection of points is DS. With increasing batch size, the min_samples parameter (the number of samples in a neighbourhood for a point to be considered a core point) also increases. As a result, small clusters of DS cannot be properly detected with this method. Not increasing the min_samples parameter with

Table 2. Clustering performance statistics.

		Dataset 1		**Dataset 2**		**Dataset 3**	
		True	**False**	**True**	**False**	**True**	**False**
All batches	Positive	881	4	4225	1	982	3
	Negative	21	113	4	168	15	0
Small batches	Positive	60	0	305	0	63	1
	Negative	2	1	2	6	3	0
Medium batches	Positive	551	3	2509	1	595	0
	Negative	13	52	2	34	9	0
Large batches	Positive	269	1	1411	0	324	2
	Negative	6	60	0	128	3	0

Table 3. Clustering performance metrics.

		V-measure	RI	ARI	MI	AMI	Homogeneity	Completeness
Dataset 1	All batches	0.13	0.80	0.20	0.03	0.13	0.29	0.08
	Small batches	0.66	0.97	0.78	0.11	0.66	0.78	0.58
	Medium batches	0.16	0.84	0.26	0.04	0.16	0.31	0.11
	Large batches	0.07	0.70	0.10	0.02	0.07	0.22	0.04
Dataset 2	All batches	0.03	0.93	0.04	0.002	0.03	0.27	0.01
	Small batches	0.31	0.96	0.38	0.02	0.30	0.63	0.20
	Medium batches	0.06	0.97	0.10	0.002	0.06	0.29	0.04
	Large batches	0.00	0.85	0.00	0.00	0.00	1.00	0.00
Dataset 3	All batches	0.83	0.99	0.90	0.06	0.83	0.77	0.90
	Small batches	0.73	0.97	0.84	0.15	0.72	0.66	0.82
	Medium batches	1.00	1.00	1.00	0.07	1.00	1.00	1.00
	Large batches	0.64	0.99	0.74	0.04	0.64	0.53	0.80

increasing batch size would lead to a rapid growth in the false positive rate by extracting false, occasional clusters.

Due to the apparent dependence of the algorithm's performance on the proportion of DS in a batch, we decided to generate and analyse a dataset with an increasing number of DS corresponding to the increasing batch size (Dataset 3). The performance of the algorithm on Dataset 3 shows zero false-negative results with a quite low false positive rate, effectively avoiding Type II errors.

Regarding the accuracy of the tests carried out, our algorithm can detect batches with DS if the batch is small or if the DS tank is big enough (more than 30 and 60 samples in medium and large batch respectively). The identification of small number of samples in large batches is still difficult. For further development of the algorithm, we plan to aim our work at:

- Development of method to detect small DS clusters in large batches properly,
- Development of method to choose the proper clustering parameters to detect every serial number of DS properly.
- Development of an algorithm to choose clustering parameters for accurate identification of DS within a batch.

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ResKuh: development of tools, diagnostics and recommendations for better herd management

J. Bieger[1], E.J.P. Strang[1], A. Al Baqain[1], C. Eck[2], M. Aresi[2], L. Clarys[2], K. Drössler[1] and L.M. Dale[1]

[1]Regional association for performance testing in livestock breeding of Baden-Wuerttemberg, Heinrich Baumann Str.1-3, 70190 Stuttgart, Germany
[2]Alsace Chamber of Agriculture, 2 Rue de Rome, 67300 Schiltigheim, France
Corresponding Author: estrang@lkvbw.de

Abstract

Energy transition, limited resources and climate change are setting new priorities for local food security and call for a plan for sustainable food production in particularly affected areas. This is why the Alsace Chamber of Agriculture and the Regional association for performance testing in livestock breeding of Baden-Württemberg have jointly launched a new project in October 2023 to continue the innovative and strong partnership of recent years. As the name “ResKuh” suggests (“Kuh” being the German translation of cow), the project focuses on improving resilience in bovine milk and meat production. The aim of the project is to support farmers in improving the sustainability of their production systems and in optimizing the use of scarce resources in times of climate change. The project area covers the Upper Rhine region with Alsace in France, Baden in Germany and the High Jura in Switzerland. An important topic that is being addressed is the improvement of water management in dairy farming by reducing water consumption and optimizing the use of resources. Another goal is the development of innovative meadow and pasture management to preserve grassland, particularly with regard to dry periods and more efficient use of self-produced feed. Furthermore, experts from the ResKuh project are working on the sustainability of farms, in particular with regard to animal welfare, the greenhouse gas emissions and the energy transition. By bringing together experts from research, training and consultancy fields from the three countries, ResKuh aims to provide suitable tools to support farmers in overcoming the above-mentioned challenges of climate change and to offer technical aids and training for farmers on the topics mentioned. Cooperation across national borders benefits of skills, tools and methods available on both sides of the Rhine and at the same time promotes exchanges between farmers who are affected by the same problems in the three countries involved.

Keywords: herd management, production, dairy cow, dairy farming, MIR, spectral data. Presented at the ICAR Anual Conference 2024 in Bled at the Session 6: Evaluation and presentation of new parameters in herd management for dairy farms

Introduction

Climate change presents numerous challenges across various sectors of human activity in the present day. The increasing frequency and duration of extreme weather events, such as droughts, coupled with rising global temperatures, elevate the risk for farms in terms of water scarcity and animal well-being (Huber and Gulledge, 2011). Livestock systems are particularly vulnerable to the impacts of climate change (Kuczynski *et al*., 2011) and contribute significantly to greenhouse gas (GHG) emissions, including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Lesschen *et al*., 2011).

Addressing the effects of climate change on agriculture and animal production, as well as mitigating these sectors' impact on the environment is crucial to ensure a sustainable food supply for a growing global population (Bauer *et al*., 2016). Research initiatives like the project ResKuh aim to provide adaptation options for agricultural farms in the face of future ecological change. ResKuh, officially known as "Resource optimization and development of sustainable livestock systems in the Upper Rhine region," is a European project co-funded through Interreg. Its goal is to enhance the resilience ("Res") of the bovine ("Kuh" in German) sector in the Upper Rhine region concerning resource management and farm sustainability.

The project focuses on five key areas of animal husbandry, each with its unique challenges: pasture and meadow management, energy management, water management, reduction of greenhouse gas emissions, and animal welfare. The project area covers the Upper Rhine region with Alsace in France, Baden in Germany and the High Jura in Switzerland. Five research groups consisting of experts from the three countries and from different institutions are working in the five areas mentioned before. Each group will develop a methodology to perform diagnoses in pilot farms of the three countries, like the monitoring of the grass growth in pastures, a carbon diagnosis of the CO_2 emissions, a diagnosis of the atmosphere in the stables or the monitoring of the heat stress in dairy cows. The collected data will serve as basis to produce various outputs illustrating the challenges of climate change for the bovine sector and the development of relevant solutions to improve the sustainability of the farms. The project ResKuh aims to provide tools and methods to help the farmers in maintaining the resilience of their farms in the face of climate change, as well as in mitigating their own impact on global warming. Sharing skills, knowledge, and methods of experts from research, training and consultancy across the whole Upper Rhin region throughout this project will help to achieve the above-mentioned goals, while also promoting international research and cooperation between regions affected by the same problems.

Material and methods

The aim of the project is to support livestock farms in the Upper Rhine region in switching to a more sustainable way of farming, by using scarce resources more efficiently and preserving the cultural landscape of this area while also achieving climate-neutral food production wherever possible.

The planned measures are divided into two axes:

1. Increasing the resource efficiency of dairy farms as an adaptation to climate change in two packages of measure improved management of water used on meat and milk producing farms (drinking water and water used in production processes), firstly by reducing water consumption, but also by examining water supply options.
    - introducing innovative grassland and pasture management adapted to climate change in practice, both through the selection of suitable resistant grass varieties and adapted fertilization; in both cases taking biodiversity into account. Another point is the planting of hedges and trees to prevent the grassland from drying out quickly due to wind and sunlight, and through more efficient use of local fodder.
2. Increase environmental protection and improve the sustainability of dairy farms. This objective is to be achieved through three packages of measures:

- improving animal welfare and health through better housing conditions (buildings, ventilation) and through innovative data analyses and studies for breeding robust and resilient animals that can better adapt to a more difficult climate;
- supporting livestock farms in improving their carbon footprint by reducing greenhouse gases (GHG) emissions both in production and through the various possibilities of CO_2 storage, as well as by reducing nitrogen in feeding;
- transition to energy self-sufficiency to limit energy consumption and increase energy production on farms, e.g. by utilizing animal excrement or using a photovoltaic (PV) system to generate energy.

The five international research groups are as followed:

Group "Water management"

Extreme heatwaves and the associated water shortages could jeopardise livestock farming (Doreau *et al*., 2012) in the Upper Rhine region. Groundwater levels are falling and the availability of water is becoming increasingly scarce (Brown *et al*., 2019), even though it is an important resource for livestock farming. In mountainous areas, livestock farmers are already facing water supply problems. Therefore, one of the objectives of this working group is to help farms identify and quantify the different consumptions of water in order to develop measures to improve their water management and optimize consumption by carrying out diagnoses and finding common solutions. The diagnoses will be carried out using a common methodology and a common tool for the project area. It is therefore possible to quantify the amount of water used in the commercialized products and to sensitize livestock farmers to consider the issue of water in their production cycles. The issue of water supply will also be analyzed, as this is an important issue for the animals, especially in situations of heat stress, as well as water quality. As part of this working group, literature research on solutions (e.g. use of different water sources) and an inventory of practices already implemented by livestock farmers will be carried out.

Group "Pasture management"

Global warming, with more intense dry spells combined with high temperatures, is affecting pastures by altering grass growth, composition, and yield (Wu *et al*., 2021). It is imperative for farmers to adapt their practices to ensure sustainable management and sufficient forage production for cattle. The aim is to develop recommendations for the management of meadows and pastures regarding drought, biodiversity, and climate protection. This will be achieved through the joint development of a protocol for measuring grass growth, as well as by analysing the quality of the harvest.

Group "Animal welfare"

Global warming has a major impact on animals. For cows, the ideal outside temperature is between -5°C and 18°C. When temperature and humidity become high, cows begin to suffer from heat stress and are no longer able to dissipate their body heat efficiently (Atrian and Shahryar, 2012; Huber and Gulledge, 2011). To ensure the well-being and productivity of the animals and thus the sustainability of the farms, it is important to work on mitigation measures to avoid this heat stress as far as possible and to adapt the herd towards periods of heat in the best possible way. One possibility is to improve the conditions within the stables by adapting them for a better air circulation to reduce the THI level within the building, thus reducing heat stress for the cows. In particular, the ventilation of the stables is a critical point to monitor to achieve the best possible climate in the buildings (West, 2003).

Another approach will be to analyse the heat stress and resistance of the animals for a selection aid.

Group "Energy management"

The use of energy is essential for the smooth running of livestock farms (Benoit and Mottet, 2023). However, given the current economic, political, and climatic context, it is essential to work on reducing energy consumption to lower it. Whether it is to protect the environment or to ensure the continuity of its business, farms should aim at lowering their energy consumption as energy resources are becoming increasingly scarce, limited, and expensive (Kreps, 2020). For this reason, this project plans to work on the creation or acquisition of an energy diagnostic tool that can be shared across the various project areas. Indeed, there is currently no common energy assessment tool adapted to the diagnosis of farms in France, Germany, and Switzerland. This diagnostic tool will make it possible to identify and quantify the different sources of energy consumption and to work on adjustments and recommendations to reduce energy consumption. Possible alternatives for greener and more autonomous energy production will also be analysed.

Group "Reduction of GHG emissions"

Agriculture and even more specifically livestock farming contribute to the global GHG emissions (Reisinger and Clark, 2018), thus livestock farms should try to reduce their carbon emissions. Therefore, it is necessary to use a carbon diagnosis tool to identify greenhouse gas emission and work on recommendations to reduce them. In Alsace, the Chamber of Agriculture uses the CAP'2ER tool, as does AGRIDEA in Switzerland. As part of a previous project KLIMACO, various carbon diagnosis tools were tested and compared. The results show that the CAP'2ER tool is one of the best, although the translation and utilization for Germany and Switzerland is not yet optimal. However, to obtain comparable data for the project area, the use of the CAP'2ER tool is recommended, which is why this tool will be translated in German and optimized for the German and Swiss regions.

Conclusion

Throughout this international cooperation and the work of the five different groups mentioned, the project ResKuh aims at develop tools, methods and references for farmers to adapt their farm management towards the challenges imposed by climate change upon agriculture and more specifically, upon livestock farming in the Upper Rhine region. As mentioned by the sustainable development goals of the United Nations (UN General Assembly, 2015), the resilience of agriculture will be a key point for the improvement of many goals such as "Food security and nutrition", "Sustainable consumption and production" or "Ecosystems and biodiversity", which is why such development projects are paramount.

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Assessing the impact of automatic milking systems on milk free fatty acid content in Taiwan

Y.H. Yeh[1], M.K. Yang[1], Y.H. Chen[1], J F. Huang[2], J.W. Shiau[1] and P.A. Tu[1]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture,
No.207-5, Pitoumian, Xihu Township, Miaoli 36843, Taiwan
[2]Taiwan Livestock Research Institute, No.112, Farm Road,
Hsinhua, Tainan 71246, Taiwan
Corresponding Author: tpa@mail.tlri.gov.tw

Abstract

Using the Automatic Milking System (AMS) can increase milk yield and reduce labor, but the impact of AMS on the milking behaviour of cows in tropical regions like Taiwan, as well as its effect on the quality of farm raw milk, has not been thoroughly explored. Free Fatty Acids (FFAs) in milk are considered one of the indicators of milk quality, and an increase in FFAs can lead to off-flavours and spoilage in dairy products. This study examines the FFA content in individual cow's milk from different milking systems and investigates the influence of lactation stages and milking frequency on FFAs in raw milk. The experiment monitored milk samples collected from the Conventional Milking Parlour (CMP) and the AMS from 2021 to 2022, totalling 2,936 and 1,726 samples respectively. The FFA content in these samples was measured using Fourier Transform Infrared Spectroscopy (FTIR). The results show that the milk from cows using the AMS had significantly higher FFAs (1.17 mmol/100g milk fat, $P < 0.01$) compared to those using CMP (0.88 mmol/100g milk fat). The FFA levels in the early stage of lactation (0.82 mmol/100g milk fat) were significantly lower ($P < 0.01$) than in the mid (1.10 mmol/100g milk fat) and late stages (1.17 mmol/100g milk fat) of lactation. When comparing different milking frequencies, cows milked 2, 3, and more than 4 times a day in the AMS had FFAs of 0.89, 1.09, and 1.15 mmol/100g milk fat respectively, with the FFAs in milk from cows milked twice a day significantly lower ($P < 0.01$) than those milked 3 times or more. This study indicates that the difference in FFAs between AMS and CMP in Taiwanese farms is particularly evident in the early stages of lactation, which helps in further investigating the physiological changes in cows during this period. The study confirms that the use of AMS in Taiwanese farms affects milk quality, including cow-related factors and other management aspects. Although the introduction of AMS may initially impact parameters related to milk quality, these effects may reduce or disappear as the lactation stage progresses, the cows adapt, and milk volume increases. Additionally, farm managers and dairy farmers need to pay special attention to the cleaning and maintenance of AMS, as well as the proper cooling of raw milk, to maintain high-quality milk.

Keywords: free fatty acids, milk quality, automatic milking system, conventional milking parlour.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 6: Evaluation and presentation of new parameters in herd management for dairy farms

Introduction

Using the Automatic Milking System (AMS) can increase milk yield and reduce labour, but the impact of AMS on the milking behaviour of cows in tropical regions like Taiwan, as well as its effect on the quality of farm raw milk, has not been thoroughly explored. Free Fatty Acids (FFAs) in milk are considered one of the indicators of milk quality, and an increase in FFAs can lead to off-flavours and spoilage in dairy products. Therefore, it is important to assess the impact of AMS on the FFA content in milk in order to understand its effect on milk quality in Taiwanese farms.

In Taiwan, dairy farms have increased their herd size, but labour shortages and aging are causing problems. These issues could be resolved by using an automatic milking system (AMS). Mechanization and automation will be the future management model for dairy farms. This study examines the FFA content in individual cow's milk from different milking systems, and investigates the influence of lactation stages and milking frequency on FFAs in raw milk.

Material and methods

The experiment monitored milk samples collected from the Conventional Milking Parlour (CMP) and the AMS from 2021 to 2022, totaling 2,936 and 1,726 samples respectively. The FFA content in these samples was measured using Fourier Transform Infrared Spectroscopy (FTIR). Recording each day's milk production, milking equipment used, stage of lactation, and milking process.

Statistical analysis was conducted using a mixed-effects model to assess the impact of milking system, lactation stage, and milking frequency on the free fatty acid content in milk. The milking system, lactation stage (early, mid, and late), and milking frequency (2 times, 3 times, and more than 4 times a day) were considered as fixed effects. Individual cows were included as random effects to account for the repeated measures on the same cow.

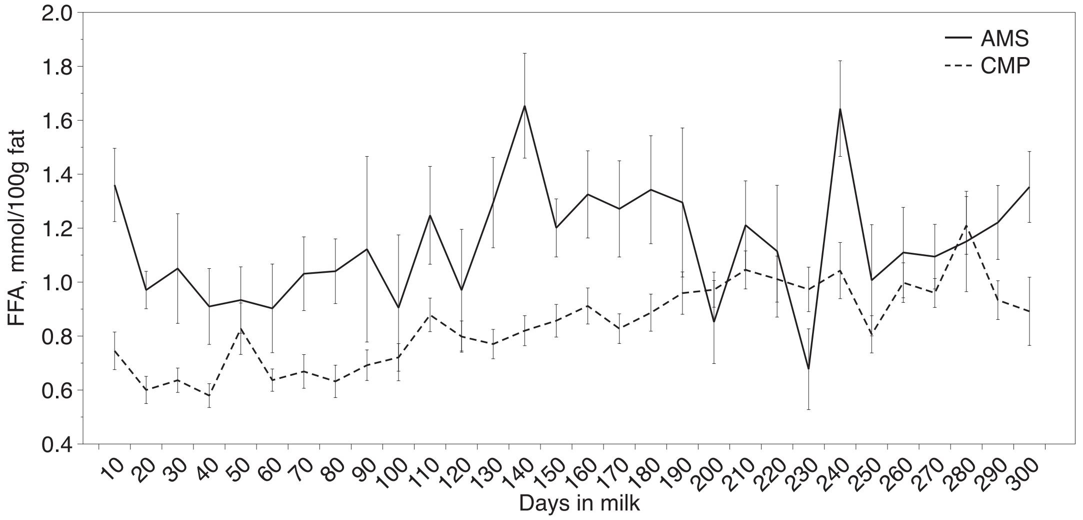

Figure 1. Free fatty acid (FFA) levels (mean ± standard error, mmol/100g fat) for automatic milking system (AMS, solid line) and conventional milking parlour (CMP, dashed line) over 15 days intervals through the entire 305 days of lactation.

The FFA content in milk samples was analyzed as the response variable, and the differences in FFA levels between the milking systems and across lactation stages and milking frequencies were evaluated using ANOVA. Post-hoc pairwise comparisons were conducted to assess specific differences between the levels of each factor. All analyses were performed using the SAS statistical software, and the significance level was set at alfa = 0.05.

Results

The difference between AMS and CMP for FFAs across days in milk is presented in Figure 1. Cows milked with AMS produced milk with greater FFAs content across lactation. The greatest difference between AMS and CMP was detected in 140 days after calving, whereas the smallest differences were observed after 200 days in milk. The milk from cows using the AMS had significantly higher FFAs (1.17 mmol/100g milk fat, $P < 0.01$) compared to those using CMP (0.88 mmol/100g milk fat) (Table 1). The FFA levels in the early stage of lactation (0.82 mmol/100g milk fat) were

Table 1. Least squares means and P-values of fixed effects in the statistical analysis of free fatty acid (FFA) concentration in milk.

Item	Group	FFA (mmol/100g milk fat)	P value
Milking system	AMS	1.17 ± 0.04^{a}	<0.0001
	CMP	0.88 ± 0.05^{b}	
Lactation stage	Early	0.82 ± 0.05^{a}	<0.0001
	Middle	1.10 ± 0.04^{b}	
	Late	1.17 ± 0.05^{b}	
Milkings	Control	0.97 ± 0.03^{ab}	<0.01
	2	0.89 ± 0.04^{b}	
	3	1.09 ± 0.05^{a}	
	4+	1.15 ± 0.08^{a}	
Milking system × Lactation stage	AMS × Early	1.08 ± 0.07^{ab}	<0.05
	AMS × Middle	1.23 ± 0.06^{a}	
	AMS × Late	1.20 ± 0.06^{ab}	
	CMP × Early	0.55 ± 0.11^{c}	
	CMP × Middle	0.97 ± 0.06^{b}	
	CMP × Late	1.13 ± 0.06^{ab}	
Lactation stage × Milkings	Early × Control	0.99 ± 0.08^{bcd}	<0.05
	Early × 2	0.61 ± 0.09^{d}	
	Early × 3	0.77 ± 0.10^{cd}	
	Early × 4+	0.89 ± 0.14^{bcd}	
	Middle× Control	0.97 ± 0.05^{bcd}	
	Middle× 2	1.03 ± 0.07^{abc}	
	Middle× 3	1.26 ± 0.08^{ab}	
	Middle× 4+	1.16 ± 0.12^{abc}	
	Late × Control	0.95 ± 0.05^{bcd}	
	Late × 2	1.05 ± 0.05^{abc}	
	Late × 3	1.26 ± 0.08^{ab}	
	Late × 4+	1.41 ± 0.16^{a}	

$^{a–d}$ Groups that do not share a common superscript letter are significantly different in their FFA levels (P<0.05).
AMS: automatic milking system; CMP: conventional milking parlour.
Early, Middle, and Late corresponds to 7 to 100 DIM (days in milk), 101 to 200 DIM, and 201 to 305 DIM, respectively. The 2, 3, and 4+ represent daily milking frequencies with AMS, while Control refers to twice daily milking using CMP.

significantly lower ($P < 0.01$) than in the mid (1.10 mmol/100g milk fat) and late stages (1.17 mmol/100g milk fat) of lactation.

When the milking frequencies of cows were compared, it was observed that cows milked twice a day had lower levels of FFAs in their milk fat (0.89 mmol/100g) compared to those milked three times per day (1.09 mmol/100g) and more than four times per day (1.15 mmol/100g) in an AMS system. This difference was found to be statistically significant with a p-value of less than 0.01. The results of the study indicate that the use of an automatic milking system can have a significant impact on the FFA.

Discussion

In previous study (Marchi *et al*., 2017), cows milked with AMS produced milk with greater FFA content across lactation. The greatest difference between AMS and CMP was detected within the first 80 days after calving, whereas the smallest differences were observed after 260 days in milk. This result is similar to our study, the smallest differences were observed during the late lactation in both experiments.

In this study, FFAs content was greater (+0.29 mmol/100 g milk fat) in milk from cows milked in AMS than CMP. The results show a similar trend to previous studies. Marchi *et al*. (2017) indicates the FFAs content in milk in AMS is higher than in CMP by 0.16 mmol/100 g milk fat. FFAs are produced through the degradation of milk fat into glycerol and FFAs via lipolysis reactions. Cooling and mechanical treatments of milk can disrupt the membrane of fat globules, leading to an increase in FFAs levels. This increase in FFAs is primarily associated with higher milking frequency or shorter milking intervals (Klei *et al*., 1997; Justesen and Rasmussen, 2000). Wiking *et al*. (2019) indicated that when the milking interval was less than 585 minutes, FFA levels increase as the milking interval shortens. More frequent milking leads to lower milk yield per milking. This is because the increased frequency of milking results in a higher air-to-milk ratio in the pipeline, reducing the stability of milk fat globules (MFG). When milk is pumped or agitated, mixing with air occurs, causing MFG rupture upon collision with air bubbles. As a result, membrane material and core fat are released into the milk plasma when air bubbles collapse or merge. Additionally, low quarter milk yields are linked to elevated (FFA) levels (Rasmussen *et al*., 2006). Additionally, milk from cows milked more than twice daily tends to have larger fat globules, which are more susceptible to lipolysis compared to smaller fat globules. Wiking *et al*. (2006) stated that the increase in FFA content at higher milking frequencies in AMS is attributed to both biological and mechanical factors. However, biological factors may have a greater impact, as spontaneous lipolysis is heightened. These findings suggest that the use of an automatic milking system, combined with a higher milking frequency, may contribute to increased levels of FFAs in milk.

This study suggests that there is a noticeable disparity in free fatty acids between automated milking systems and conventional milking parlors in Taiwanese farms, especially during the initial phases of lactation. This finding opens up opportunities for delving deeper into the physiological transformations occurring in cows during this specific period. Furthermore, exploring these differences may provide insights into potential improvements or adjustments to enhance milk quality and overall efficiency within dairy farming operations.

Conclusions

The use of AMS in Taiwanese farms may have a long-lasting impact on milk quality. Certain cow-related factors and management aspects may continue to be affected even as cows adapt and milk volume increases. Furthermore, farm managers and dairy farmers need to pay special attention to the cleaning and maintenance of AMS, as well as the proper cooling of raw milk, to maintain high-quality milk.

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Environmental impact assessment of milk production: is a simplified tool possible?

M. Zucali[1], G. Gislon[1], V. Ferrari[2], M. Marusi[2], A. Sandrucci[1], A. Tamburini[1], L. Bava[1], R. Finocchiaro[2] and M. Cassandro[2,3]

[1]Università degli Studi di Milano, Dipartimento di Scienze agrarie e ambientali, Via Celoria 2, 20133 Milano, Italy
[2]Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana, Via Bergamo 292, 26100 Cremona (CR), Italy
[3]Dipartimento di Agronomia Animali Alimenti Risorse naturali e Ambiente, Università di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
Corresponding Author: maddalena.zucali@unimi.it

Abstract

The aim of this study was to develop a simplified tool for estimating Climate Change (CC) associated to milk production at farm level that can be easily used by farmers. An accurate environmental impact assessment of milk production is the first step to select the best mitigation strategies to make milk production greener. Most studies in this field use Life Cycle Assessment (LCA) to estimate various environmental impacts of milk production at the farm level. LCA is a robust method, although time consuming. However, the current need of the sector is to start extensive estimation of environmental impact of milk production in dairy farms, at least for the Climate Change (CC) category, to set up a starting point for measuring future improvements. The study was performed on 54 dairy cattle farms located in Northern Italy. A complete LCA analysis was performed, and some performance data were recorded in the last 3 years. The latter were retrieved from the national fertility database managed by the National Breeders Association of Holstein, Brown, and Jersey (ANAFIBJ, Cremona, Italy) and consisted of production, management, and fertility data (i.e. pregnant cows at 120 d, and milk sold per Livestock Unit, LU), and genetic indices (i.e. Health and Economic Index - IES, predicted Methane Emission Index - pCH4). On average, the number of lactating cows in the selected farms was 232.2 (min 56, max 817), Fat and Protein Milk production (FPCM) per lactation was 9591±1357 kg. The inclusion of soybean meal, in the ration of lactating cows, was on average 10.7±5.28%. The CC impact was estimated starting from IPCC 2019 equations for modelling CH_4 and N_20 emissions related to the on-farm processes, while for off farm ones, data from databases were used (Agrifootprint and Ecoinvent). The EF 3.0 method was used for CC estimation. Average CC of the farms in the dataset was 2.00±0.31 kg CO_2 eq/kg FPCM. Subsequently, multivariate analyses were performed using R and SAS software using CC, farm characteristics and performance data. The Principal Component Analysis (PCA) was performed to find a multidimensional relation between variables.

With the aim to find an equation for estimating CC (CC_es) using few variables, easy to be collected at farm level, a linear model with stepwise selection was used. Starting from a collinearity test, variables with high VIF (Variance Inflation Factor) were excluded from the dataset. Stepwise procedure (Ordinary Least Squares, OLS) was used to select the best parameters for CC_es. Variables selected were presence of biogas, percentage of soybean meal in the ration, IES and CH4 indexes, age at first calving, pregnant cows at 120 d, and milk sold per LU. Adjusted R^2 of the equation was 0.63. Validation of the equation was performed by randomly selecting 15 farms from the database 1,000 times to test the equation, and the average correlation coefficient between CC_es and CC was 0.77. From PCA, CC resulted inversely related with biogas presence, percentage of replacement animals on total LU and percentage of cows pregnant at 120 days after calving. The last two parameters are linked with an efficient farm management, characterized by a limited ratio between unproductive (heifers and open cows) and productive animals and by fertility efficiency. The mitigation effect of the presence of biogas was very high. The IES index also showed negative correlation with CC_es, On the other hand, while increasing percentage of soybean meal in the ration resulted an increased value of CC_es. Fertility efficiency parameters i.e. pregnant cows at 120 d, and milk sold per LU were inversely proportional to CC_es.

Keywords: lca, tool, milk, cow, management, environmental impact.
Presented at: Session 11 of ICAR 2024, Methane Emission–Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

Introduction

In literature a generally accepted method for estimating the environmental impact of animal products on a global perspective is the Life cycle assessment (LCA), thanks to its power to include in a holistic assessment the environmental impacts of processes and products (Guerci *et al*., 2013). However, LCA represents a high time-consuming method, making it a method of difficult application in the field, especially for assisting farmers in identifying GHG mitigation strategies to be implemented at farm level. For this reason, a simplified tool for the evaluation of Climate Change (CC), that considers all the farm management aspects, together with genetic and phenotypic parameters, related to animal and farm efficiency, may be useful for improving the environmental sustainability of the milk production sector.

Material and methods

The study was performed on 54 dairy cattle farms located in Northern Italy. A complete LCA analysis was performed, and some performance data were recorded in the last 3 years. The latter were retrieved from the national fertility database managed by the National Breeders Association of Holstein, Brown, and Jersey (ANAFIBJ, Cremona, Italy) and consisted of production, management and fertility data (i.e. pregnant cows at 120 d, and milk sold per Livestock Unit, LU), and genetic indices (i.e. Health and Economic Index - IES, predicted Methane Emission Index - pCH4).

Life Cycle Assessment

The goal of this LCA study was to quantify the CC of 1 kg of fat and protein corrected milk (FPCM), that was used as functional unit. At farm level, the allocation was performed between milk and meat, using a physical method (IDF International Dairy Federation, 2015). System boundaries considered were from cradle to farm gate, and all the inputs and output involved in the productive processes were considered. For the assessment, primary data collected at farm were used as much as possible. Secondary data from databases (Ecoinvent and Agri-footprint databases) and proxy were also used. Emissions of greenhouse gases in air were estimated by using IPCC 2019 guidelines. After classification, characterization was performed through EF 3.0 method. The life cycle impact assessment was performed by using the software SimaPro V 8.3.

Statistical analysis

The complete data set was analysed using SAS 9.4 (2012; SAS Institute Inc., Cary, NC), computing descriptive statistic (Proc MEANS). Multivariate analyses were performed using SAS software 9.4 (2012; SAS Institute Inc., Cary, NC), using CC, farm characteristics and performance data. A Principal Component Analysis (PCA, Proc PRINCOMP) was performed to find a multidimensional relation between variables. With the aim to find an equation for estimating CC (CC_es) using few variables, easy to be collected at farm level, a linear model with stepwise selection was used. Starting from a collinearity test, variables with high VIF (Variance Inflation Factor) were excluded from the dataset. Stepwise procedure (Ordinary Least Squares, OLS) was used to select the best parameters for CC_es. Validation of the equation was performed by randomly selecting 15 farms from the database 1,000 times to test the equation, and the average correlation coefficient between CC_es and CC was calculated.

Results

The results of summary statistics performed on 54 dairy cattle farms of Northern Italy are shown in Table 1.

On the average, the number of lactating cows in the selected farms was 232, Fat and Protein Milk production (FPCM) per lactation was 9591 kg, with an average percentage of fat and protein of 3.83 and 3.40, respectively (Table 1). The inclusion of soybean meal, in the ration of lactating cows, was, on the average 10.7%. Average values collected for IES and CH4 indexes were 161 and 100, respectively. Age at first calving was, on the average, for the 54 farms of the sample, 26.9 months, while percentage of pregnant cows at 120 d was, on the average 58.3%. Average value of milk sold per LU was 6239 kg (Table 1).

Average CC of the farms in the dataset was 2.00±0.31 kg CO_2 eq/kg FPCM.

In Figure 1 results of PCA are shown.

From PCA (Figure 1), CC resulted inversely related with biogas presence, percentage of replacement animals on total LU and percentage of cows pregnant at 120 days after calving. The last two parameters are linked with an efficient farm management, characterized by a limited ratio between unproductive (heifers and open cows) and productive animals and by fertility efficiency. IES index also resulted to be inversely

Table 1. Summary of descriptive statisti

Variable	Unit	Mean	Std	Min	Max
Lactating cows	n	232	186	56.0	817
FPCM[1] per lactation	kg	9591	1357	6754	13284
Fat	%	3.83	0.23	3.28	4.23
Protein	%	3.40	0.12	3.02	3.70
Soybean meal in the ration	%	10.7	5.28	0	22.5
IES index[2]		161	159	-93.6	733
CH4 index[3]		100	1.42	97.1	105
Age at first calving	month	26.9	2.47	23.0	34.7
Pregnant cows at 120 d	%	58.3	9.25	37.0	73.0
Milk sold per LU[4]	kg	6239	827	4494	8093

[1] FPCM, Fat and Protein Corrected Milk
[2] IES index, Health and Economic Index
[3] CH4 index, Methane Emission Index
[4] LU, Livestock Unit

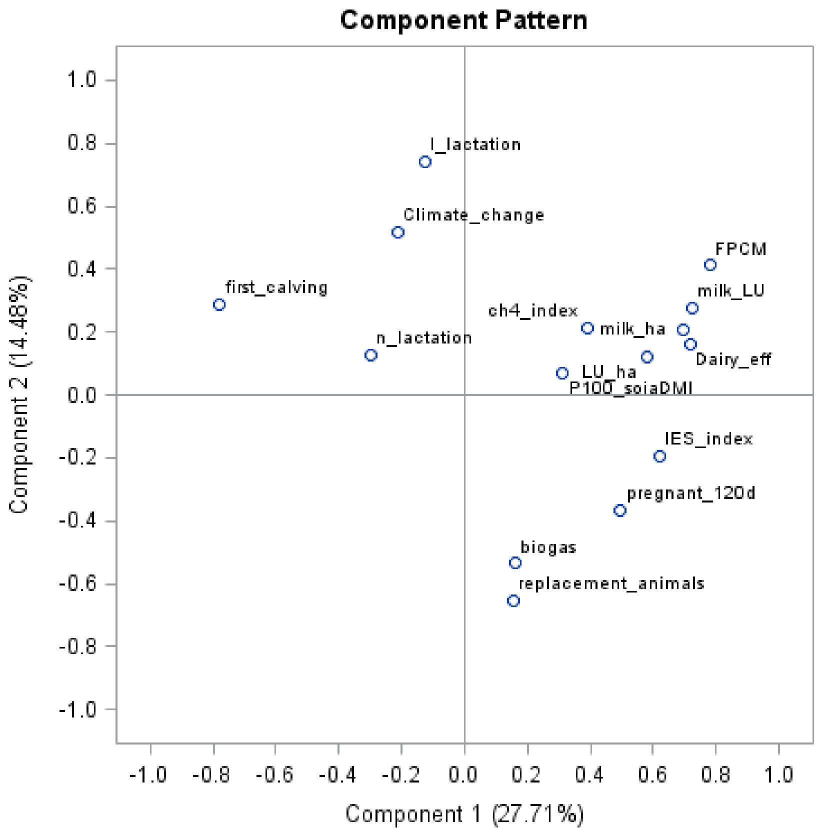

Figure 1. Results of PCA.

related to CC. On the contrary, PCA highlighted a positive correlation between CC and age at first calving, number of lactations and length of lactation (Figure 1).

Variables selected for the estimation of CC (CC_es) (Table 2) were presence of biogas, percentage of soybean meal in the ration, IES and CH_4 indexes, age at first calving, pregnant cows at 120 d, and milk sold per LU. Adjusted R^2 of the equation was 0.63, and the average correlation coefficient between CC_es and CC was 0.77.

The mitigation effect of the presence of biogas was very high. The IES index also showed negative correlation with CC_es, On the other hand, while increasing

Table 2. Variables selected for the estimation of CC.

Variable
Biogas
Percentage of soybean meal in the ration
IES index[1]
CH4 index[2]
Age at first calving
Pregnant cows at 120 d
Milk sold per LU[3]

[1] IES index, Health and Economic Index
[2] CH4 index, Methane Emission Index
[3] LU, Livestock Unit

percentage of soybean meal in the ration resulted an increased value of CC_es. Fertility efficiency parameters i.e. pregnant cows at 120 d, and milk sold per LU were inversely proportional to CC_es.

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Breeding for resistance to gastrointestinal nematodes in French dairy sheep: towards an increase in resilience and sustainability of sheep dairying

J.M. Astruc[1], F. Fidelle[2], C. André[3], C. Grisez[4], L. Bordes[4], S. Jouffroy[4], and P. Jacquiet[4]

[1]IDELE, Castanet-Tolosan, France.
[2]OS ROLP Breed Organisation, Ordiarp, France
[3]CDEO Breeding Company, Ordiarp, France
[4]UMR INRAE ENVT 1225 IHAP and UMT PSR, Toulouse, France
Corresponding Author: Jean-Michel.Astruc@idele.fr

Abstract

Anthelmintic resistance is increasingly present in French sheep flocks. The issue is particularly acute in dairy sheep, as the only anthelmintic drug still available during the lactating period without milk withdrawal time is eprinomectin, a macrocyclic lactone. In the Pyrenean dairy sheep breeds, for which the breeding system is based on pasture grazing under an Atlantic climate, some flocks are in dire straits with no more efficiency of the treatments against the gastrointestinal nematodes (GIN) during the lactating period. Management of GIN in France aims at an integrated control, based on a combination of solutions. It is advocated that treatment should be targeted and selective, grazing should be managed to limit the sources of contamination and resistance of sheep should be increased through genetic selection.

This paper focuses on the genetic lever and describes the process undertaken by the Pyrenean dairy sheep organisations over the last decade to implement a selection for resistance to parasites in the Blond-Faced Manech and Basco-Béarnaise breeds. Phenotyping of resistance to GIN has been achieved since 2008 by experimental infection of the rams entering the insemination centre. The protocol is based on two successive infections with L3 larvae of *Haemonchus contortus*, separated by a 15-day recovery period. Faecal egg count (FEC) one month after each infection and the variation of packed cell volume (ΔPCV) between the time of infection and one month afterwards were measured. FEC and ΔPCV may be considered as indicators of, respectively, the resistance to parasites and the resilience of the animal. 1,826 Blond-Faced Manech rams and 520 Basco-Béarnaise rams have been phenotyped for this trait since 2008. All of them are genotyped. The estimation of genetic parameters of FEC and ΔPCV at each infection shows that FEC is moderately heritable while ΔPCV isplays a lower heritability. The genetic correlations between FEC and ΔPCV are quite high, meaning that the more resistant rams are also the more resilient. The genetic correlations between FEC and ΔPCV on the one side, and the traits currently under selection (milk, fat and protein, somatic cell count, udder morphology) on the other side are low, close to zero. Consequently, the inclusion of resistance to parasites in the breeding objective would not hamper too strongly the efficiency of selection on current traits. A genomic evaluation of FEC and ΔPCV was performed in 2022 and the breeding organisation of the Blond-Faced Manech and the Basco-Béarnaise breeds decided to include resistance and resilience to parasites in the breeding criteria of these breeds. FEC and ΔPCV have been combined in a composite sub-index related to parasite resistance and resilience. This sub-index was then combined with the current selection

index to produce a new Total Merit Index that is now used to select the rams in the breeding program.

Keywords: dairy sheep, resistance to parasites, breeding objective, genetic parameters Presented at the ICAR Anual Conference 2024 in Bled at the Session 7: Breeding for agroecological transition in sheep and goats.

Introduction

Gastrointestinal nematodes (GIN) are ingested by sheep while grazing. They cause significant economic losses (mortality, milk production losses, direct costs of anthelmintics pharmaceuticals). The ecotoxicity of some anthelmintics (e.g. macrocyclic lactones) can provoke undesired effects on non-targeted fauna, coprophagous insects of the pastures mainly (Verdú *et al*, 2018). Moreover, the important adaptation capacity of GIN has made them develop anthelmintic resistances (including multidrug resistance) which has been growingly evidenced over the last few years, leading to a true risk of therapeutic impasse (Jacquiet *et al*, 2024). For all these reasons, breeding for resistance to GIN in sheep is a relevant selection objective.

However, we must have in mind that the genetic selection to increase the resistance/resilience of the host is only one of the pillars of the integrated control of GIN, along with the elimination of the GIN (use of targeted selective treatments, discovery of new molecules) and the drying up of the sources of contamination (e.g. through a better management of pastures and grazing). Use of multiple tools among the integrated parasite management toolbox is the right way to efficiently fight GIN infections (Jacquiet *et al*, 2024).

In France, the Pyrenean dairy sheep breeds (Manech and Basco-Béarnaise breeds), raised mainly outdoors in the western part of the Pyrenean mountains, close to the Atlantic Ocean are subject to severe natural GIN infections due to production systems based on grazing associated to a mild and wet climate. Therefore, the breed organisation of these breeds pioneered in the implementation of genetic selection for resistance and resilience against parasitism in France.

Phenotyping resistance and resilience to gastrointestinal parasites

While many countries and breeds, especially in Australia, New Zealand, UK, Uruguay measure resistance to parasites in situation of natural infections on pastures (Morris *et al*, 2010; Woolaston and Windon, 2001; Ciappesoni *et al*, 2023, Cunha *et al*, 2024), an original design has been set up in France, based on a standardised protocol with controlled infections with *Haemonchus contortus* (Gruner *et al*, 2004a, Jacquiet *et al*, 2015). This protocol is applied to young rams, never exposed to parasites, gathered in breeding/AI centres, which are genetically important because they are the future sires in the selection process. The objective of the protocol is to display differences between rams without hampering their fitness.

The figure 1 summarizes the principles of the standardised protocol and the different measures that are collected. It is based on two successive infections of a given and known dose of L3 of *Haemonchus contortus* (a hematophagous GIN). At the first infection, 3,500 doses of L3 are administered, and 5,000 at the second infection. At each infection, feces are collected 30 days after infection to measure Fecal Egg Count (FEC) through a coprological analysis (Jacquiet *et al*, 2011). A blood sample is also collected the day of the infection and 30 days after to measure packed cell volume (PCV), the purpose being to measure blood loss during infection. Rams are drenched between both infections. When the protocol is applied to meat sheep breeds, L3 doses may

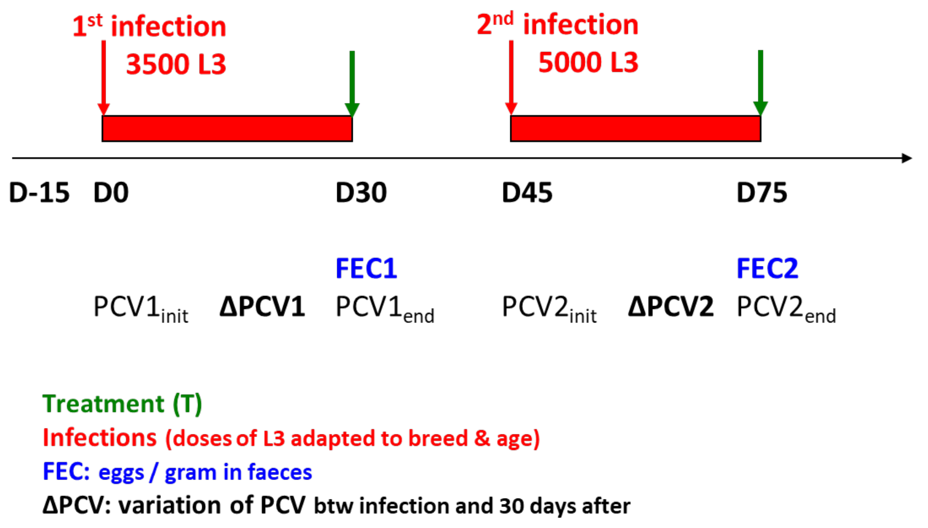

Figure 1. Protocol of experimental infestation (from Jacquiet et al, 2015).

vary depending on age of rams and how they respond to infection. In the case of the Pyrenean dairy sheep breeds (Red-Faced Manech – RFM and Basco-Béarnaise - BB), the protocol occurs when the rams are one-year-old, before their first use as AI rams. When the phenotyping protocol is applied, they have never known the pasture and are therefore naïve regarding parasites.

FEC are an indicator of the resistance to GIN, by assessing the capacity of the sheep to decrease the establishment, the development, the fecundity and the fitness of the worms. The variation of PCV between start and end of infection (ΔPCV) is an indicator of the resilience of the animal regarding the parasitic challenge. Besides, kinetics of PCV also allows to verify the fitness of the sheep after each infection and thus monitor the well-being of the animals.

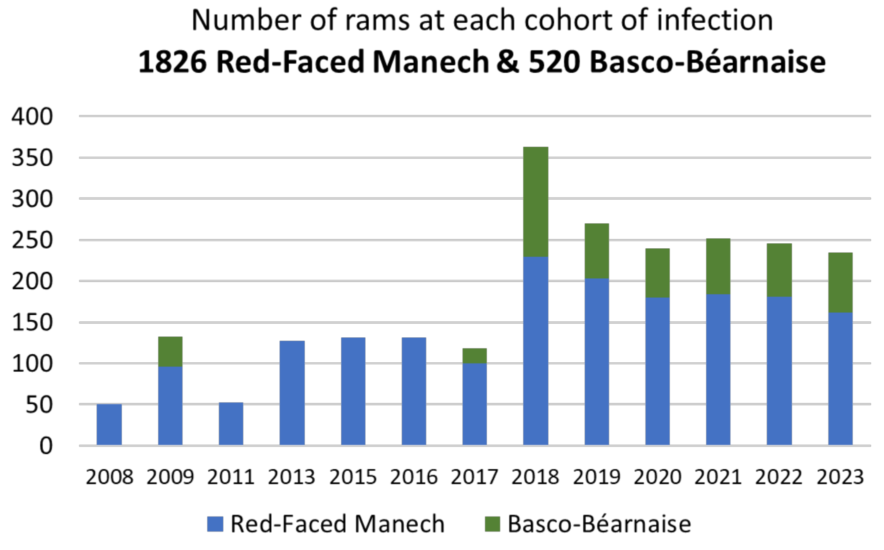

Figure 2. Number of rams submitted to the protocol of phenotyping over the years.

FEC is the commonly used criterion worldwide to measure host resistance to parasites. As it is time-consuming and costly to measure, targeting rams gathered in collective centres makes the selection process more cost-effective, money and time wise. The use of *Haemonchus contortus* is relevant for the following reasons: (i) it is a pathogenic and thermophile GIN; (ii) it is the most prevalent species in south-western France; (iii) most of the time, this species is concerned in case of resistance to drugs. In any case, literature has shown a very high genetic correlation (≈ 1) between resistance to different species of GIN (Gruner *et al*, 2004a). Finally, a very high genetic correlation (≈ 0,9) has been found between natural infections and experimental infections (Gruner *et al*, 2004b).

The first protocol of phenotyping in the Pyrenean breeds was implemented in 2008. Since then, new rams have been phenotyped almost every year. Finally, phenotypes are available for 1,826 RFM and 520 BB rams in 2024. This dataset was used for genetic analyses (Figure 2).

Genetic parameters and genetic evaluation of resistance to gastrointestinal nematodes and resilience traits

Computation of traits

The traits used in genetic analyses are the following:

- The square root of FEC at first and second infection (both infections being considered as a different trait): FEC1 and FEC2. Such a transformation resulted in more symmetrical distributions.
- The variation of PCV between start and end of infections (both infections being considered as a different trait): ΔPCV1 and ΔPCV2 (ΔPCVi=PCVi[init] – PCVi[end]).

Genetic parameters

Genetic parameters of resistance and resilience traits, as well as their standard errors, were estimated using the restricted maximum likelihood estimation with the VCE package. The model used is the following:

$$Y_{ijk} = \mu + P_i + A_j + R_k + e_{ijk}$$

Where Y_{ijk} is the dependent variable (FEC1, FEC2, ΔPCV1 and ΔPCV2), μ is the population mean, P_i is the fixed effect of protocol i (representing the contemporary group, one per year), A_j is the fixed effect of age j of the ram, R_k is the additive genetic random value of the ram k and e_{ijk} is the random residual effect. As the rams were submitted to only one protocol in their lifetime, there were no repetition and therefore no permanent environmental random effect.

The table 1 presents the genetic parameters obtained in Red-Faced Manech.

All heritabilities are significantly different from zero. FEC are moderately heritable, with a higher heritability of FEC at the second infection (0.35 vs 0.20). ΔPCV have a lower heritability (0.12 and 0.14). Genetic correlations across both infections are high (more for FEC than for ΔPCV), but without being the same trait. Genetic correlations are high between resistance and resilience, especially within infections: the most resistant animals are also the most resilient. These parameters show that selection is feasible. The parameters in BB (not shown here) follow the same pattern, yet with some differences (there is no higher heritability of FEC in infection 2 in BB). These results are consistent with those estimated previously (Aguerre *et al*, 2022).

Table 1. Genetic parameters and standard errors of estimation of Fecal Egg Count (FEC) and variation of packed cell volume (ΔPVC) in Red-Faced Manech at infection 1 and 2. Heritabilities are on the diagonal (bold characters), genetic correlations above the diagonal and phenotypic correlation below the diagonal.

	FEC1	FEC2	ΔPCV1	ΔPCV2
FEC1	**0.20 ± 0.04**	+0.82 ± 0.05	+0.96 ± 0.05	+0.47 ± 0.10
FEC2	+0.37	**0.35 ± 0.05**	+0.70 ± 0.07	+0.79 ± 0.10
ΔPCV1	+0.30	+0.14	**0.12 ± 0.03**	+0.40 ± 0.14
ΔPCV2	+0.15	+0.43	+0.06	**0.14 ± 0.01**

Genetic evaluation

The genetic evaluation is performed using the same model as for genetic parameters. Until 2022, the evaluation was polygenic. From 2023 onwards, we have run a genomic evaluation (all the rams phenotyped are also genotyped), enabling the inclusion of resistance and resilience to parasites in the genomic pre-selection step of young rams, simultaneously with other traits.

EBV's of elementary traits (FEC1, FEC2, ΔPCV1 and ΔPCV2) are provided to the breed organisation. In addition, composite indexes are also calculated and provided:

- -a FEC index combining both infections: FECindex = ¼ FEC1 + ¾ FEC2, weights of ¼ and ¾ reflecting the difference of heritability across infections.
- -a ΔPCV index combining both infections: ΔPCVindex = ½ (ΔPCV1 + ΔPCV2), weights of ½ reflecting the same heritability across infections.

-a so-called parasitism index combining resistance and resilience: PARASITISMindex = ¾ FECindex + ¼ ΔPCVindex. The weights were chosen by the breed organisation, with the intention of giving a significant weight to resilience, while keeping a higher (conservative) weight for resistance, arguing that FEC is the most universal indicator worldwide. However, which weights should be given to resistance and resilience is a key question. The answer could be updated in the future, according to new scientific results or following new decisions from the breeding organisation.

Defining a selection objective that accounts for resistance and resilience to parasites and building a selection criterium accordingly in the Pyrenean dairy sheep breeds

To have a quick glance at the background on the selection in the Pyrenean dairy sheep breeds, we must have in mind the following statement:

- the breeding programs of the RFM and BB breeds have been shifted towards genomic selection since 2017, with a reference population reaching 3,300 rams in RFM and 1,100 rams in BB. They are efficient programs, generating in a genetic gain (expressed in genetic standard deviation of the selection index) reaching 0.16 in RFM and 0.15 in BB (Astruc *et al*, 2022).
- the selection criteria include in both breeds fat and protein yield, fat and protein content, somatic cell count, udder morphology traits.
- high level of anthelmintics resistance in the area has made the breeders fully aware of the importance of increasing resistance/resilience to GIN. Yet there is a strong inclination to find the right compromise between currently selected traits and novel

parasite-related traits. Hence, the genetic correlations between all the traits were a key indicator to consider.

Genetic correlations between traits included in the current selection index and traits of the parasite index

Genetic correlations between traits as well as their standard errors, were estimated using the restricted maximum likelihood estimation with the VCE package. The model used is the bivariate model, without repetitions, the fixed effects being those used in the genetic evaluation (described above for FEC and ∆PCV, described in Aguerre *et al*, 2022 for currently selected traits). We present in the table 2 the genetic correlations between FEC1, FEC2, ∆PCV1, ∆PCV2 on the one hand, milk yield (MY), fat and protein content (FC and PC), somatic cell score (SCS), teat angle (TA), udder depth (UD) on the other hand.

Most correlations are close to zero. As the size of the dataset is quite small, half of the correlations are not significantly different from zero. Bearing in mind this limit and looking at the general patterns, whether for resistance or resilience traits, favourable correlations are somewhat balanced with unfavourable correlations. Aguerre *et al* (2022) found correlations slightly different, mainly due to a smaller dataset and the high standard error of estimation. We can state that the genetic correlations are globally low in RFM. Therefore, selecting for resistance and resilience to GIN challenges would not jeopardise the efficacy of the selection on other traits. The smaller size of the dataset in BB does not allow to give consistent results.

In a complementary approach, we calculated the Pearson correlation coefficient between current total merit index (TMI) and the parasitism index combining resistance and resilience, for 663 young rams born in late 2023 and genotyped for the genomic pre-selection. The correlation was at the very low level of 0.04, suggesting neither favourable nor unfavourable relationship, confirming what we observed on elementary traits.

Defining a new Total Merit Index

To define a new TMI including resistance and resilience to GIN, we built a composite index as follows:

TMInew = TMIcurrent + k * PARASITISM, with:

Table 2. Genetic correlations and standard errors of estimation in Red-Faced Manech between Fecal Egg Count (FEC) and variation of packed cell volume (∆PVC) at infection 1 and 2, and 6 major traits included in the current total merit index.

	FEC1	FEC2	∆PCV1	∆PCV2
Milk yield	-0.20 ± 0.11 *	-0.03 ± 0.07	-0.06 ± 0.17	-0.07 ± 0.13
Fat content	0.12 ± 0.13	0.10 ± 0.09 *	0.06 ± 0.17	0.10 ± 0.13
Protein content	-0.26 ± 0.12 *	-0.19 ± 0.08 *	-0.21 ± 0.17 *	-0.02 ± 0.13
Somatic Cell Score	-0.17 ± 0.12 *	-0.12 ± 0.09 *	-0.48 ± 0.23 *	-0.09 ± 0.14
Teat angle	-0.17 ± 0.11 *	0.04 ± 0.08	-0.08 ± 0.19	-0.22 ± 0.12 *
Udder depth	0.12 ± 0.11 *	0.04 ± 0.08	0.41 ± 0.19 *	0.11 ± 0.13

Sign * spots correlations that are significantly different from zero. Correlations are coloured in green when favourable, in orange when unfavourable.

- TMIcurrent is the current TMI including dairy traits (60% in RFM; 65% in BB), SCS (20% in RFM; 17.5% in BB) and udder morphology (20% in RFM; 17.5% in BB)
- PARASITISM defined as above: PARASITISM = ¾ FECindex + ¼ ∆PCVindex

We made k vary from 0 to 1000 and we applied a selection of 25% on a set of young rams submitted to genomic preselection, based on TMInew. We then plot the evolution of TMIcurrent and PARASITISM of the selected rams against k. The results were discussed with the breeding organisation that choose the k value corresponding to their desired compromise between not losing too much on TMIcurrent while sufficiently improving PARASITISM. The re-ranking of the rams was also accounted for. The k chosen resulted in a loss of genetic progress on TMIcurrent of 5% in RFM and 8% in BB, which is weak. The gain on PARASITISM is equivalent to a decrease of 200 eggs per gram in the selected rams of each cohort. In terms of percentage in the TMInew, the weight of PARASITISM is 24% in RFM and 35% in BB.

This new TMI was used for the first time at the genomic preselection step of the young rams born in late 2023. These rams will be used at AI in 2025.

Conclusion and perspectives regarding ICAR guidelines

Breeding for resistance to parasites became necessary in the Western Pyrenean breeds with regards to the situation on anthelmintic resistance in the area. This is in line with the strong willingness of the breeding organisation to select for resilience and generate animals more adapted to agroecological transition (towards less chemical treatments) and to global warming (*Haemonchus contortus* is a thermophile species). Yet the selection will be effective in the mid to long term. For this reason, management of GIN should integrate a combination of solutions. Besides genetic selection to increase resistance of sheep, it is advocated that treatment should be targeted and selective, grazing should be managed to limit the sources of contamination. Beyond the case of Pyrenean breeds, the Lacaune breed, which has also been confronted to anthelmintic resistance these last years, will start phenotyping resistance and resilience to GIN in late 2024, allowing to include these traits in the selection objective within 4 years.

The SMARTER project (Horizon 2020 research and innovation program No. 772787) produced in 2023 a deliverable on recommendations to phenotype resilience. It included resistance/resilience to parasites, with the different ways of phenotyping (traits, proxies, experimental vs natural infection). This deliverable was intended to be translated into ICAR guidelines. To include these recommendations into a new section of ICAR guidelines constitutes a major objective of the Sheep-Goat-Camelid WG.

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Introduction of a genetic evaluation for longevity in Tyrol Mountain sheep

B. Fuerst-Waltl[1] and C. Fuerst[2]

[1]Universität für Bodenkultur Wien (BOKU University),
Gregor-Mendel-Straße 33, 1180 Wien, Austria
[2]ZuchtData EDV-Dienstleistungen GmbH, Dresdner Straße 89/B1/18,
1200 Wien, Austria
Corresponding Author: birgit.fuerst-waltl@boku.ac.at

Abstract

With 15,166 registered ewes (> 0.5 years) on 1,047 farms in 2022, Tyrol Mountain has the largest number of animals among the registered sheep breeds in Austria. Tyrol Mountain is a non-seasonal breed; in 2022, 10,351 lambings from 8,147 ewes were recorded (ÖBSZ, 2023). Official breeding values for Tyrol Mountain sheep in Austria were published for the first time in 2017. At the same time, genetic evaluations have been introduced for other sheep and goat breeds. For the mountain sheep breeds, genetic evaluation currently includes the traits age at first lambing, lambing interval, lambs born and lambs born alive (both maternal and paternal) and the fitness index based on these traits. However, longevity, one of the most important functional traits, is missing from the current breeding objectives. While survival analysis has long been the "state of the art", especially in dairy breeding, many newly established routine genetic evaluations are based on linear models. Therefore, a genetic evaluation for a longevity related trait was developed for Tyrol Mountain, which can be implemented in routine breeding value estimation based on linear models. For this purpose, 5 cumulative periods from first lambing onwards were defined. The traits are the number of lambings in the periods 1, 2, 3, 5 and 8 years after the first lambing. The average number of lambings is 1.8 in the first year and 4.5 within 8 years, the maximum number of lambings after 8 years is 15. Apart from the random animal genetic effect, the evaluation model includes the fixed effects of age at first lambing, year-month, herd, and the random effect of herd-year. The last uncompleted period of living animals is considered by extrapolating their expected performance. All following periods are then set to missing for these animals. Estimated heritabilities for the 5 periods range from 0.03 (period 1) to 0.14 (period 8). Genetic correlations between traits are consistently high, ranging from 0.81 to 0.99. Period 3, i.e. the number of lambings within 3 years after the first lambing, is considered the target trait. The first official breeding values for longevity will be published in June 2024, along with new breeding values for conformation and a total merit index.

Keywords: sheep, longevity, genetic parameters, breeding values.
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Introduction

A considerable variety of sheep breeds exists, which are adapted to different climatic conditions and/or are particularly suitable for different purposes. Accordingly, the Austrian Sheep and Goat Association (ÖBSZ, Österreichischer Bundesverband für Schafe und Ziegen) categorises the different sheep breeds into four groups, namely mountain sheep, land sheep, dairy sheep and meat sheep (ÖBSZ, 2013). Mountain sheep breeds account for the highest proportion of animals (>50% of herd book ewes). These breeds are mainly kept in alpine regions, are non-seasonal, and are used mostly for lamb production, landscape management, and conservation grazing. Within this group, the Tyrol Mountain breed has the highest proportion of animals among the registered sheep breeds in Austria, with 15,166 registered ewes (> 0.5 years) on 1,047 farms in 2022. In the same year, 10,351 lambs were recorded from 8,147 ewes (ÖBSZ, 2023).

In 2017, official breeding values for Tyrol Mountain sheep in Austria were published for the first time. In parallel, genetic evaluations have been introduced for other sheep and goat breeds (Fuerst-Waltl and Fuerst, 2021; 2022). For the mountain sheep breeds, genetic evaluation currently includes the traits age at first lambing, lambing interval, lambs born and lambs born alive (both maternal and paternal) and the fitness index based on these traits. Breeding values are calculated on two main dates, the beginning of January and the end of June. In addition, breeding values and reliabilities are calculated on a weekly basis, but are only uploaded if the change in reliability exceeds 5% points or if a new result is available from individual performance recording (e.g. lambing). All estimated breeding values (EBV) are standardised to relative breeding values with a mean of 100 (rolling base, rams aged 5-15 years) and a genetic standard deviation of 12 points, with higher values being desirable. Longevity, one of the most important functional traits, is however missing from the current breeding objective. While survival analysis has long been the “state of the art”, especially in dairy breeding, for several reasons many newly established routine genetic evaluations are based on linear models and survival yes/no in certain periods (e.g. van Pelt *et al*., 2017; Heise and Simianer, 2019, Zuchtwert AUSTRIA, 2023). In livestock without dairy focus, such as beef or sheep with focus on lamb production, the number of births in a certain period may be of higher interest. Venot *et al*. (2013) proposed the number of calvings at a target age, called “productive efficiency”, as a suitable trait for beef cattle. Following the latter concept, a genetic evaluation based on linear models was developed for Tyrol Mountain.

Material and methods

Five cumulative periods were defined from the first lambing onwards, i.e. 1, 2, 3, 5 and 8 years. The longevity related trait was defined as the number of lambings within each period. Following the concept of Brotherstone *et al*. (1997), the last uncompleted period of animals that are alive is considered by extrapolating their expected performance. All other periods are subsequently set to missing for these animals. Genetic parameters were calculated using the VCE6 software package (Groeneveld *et al*., 2008) and bivariate linear animal models. In addition to the random animal genetic effect, the model included the fixed effects of age at first lambing, year-month and herd, and the random effect of herd-year. Depending on the period, between 12,935 and 22,383 ewes were considered (Table 1); the pedigree data set comprised 41,135 animals. Following intensive discussion with the breeding associations and contrary to dairy breeds, no correction for voluntary culling was made.

Results and discussion

Table 1 shows the number of animals, as well as the mean, standard deviation, minimum and maximum of the defined trait in each period. For period 1, the mean number of lambings is 1.75 ± 0.46, for period 8 it is 4.48 ± 2.90. In the higher periods, the number of lambings is somewhat lower than in other non-seasonal sheep breeds (data not shown). However, this may also be due to conscious management decisions by farmers, as seasonal lambing is potentially advantageous in the case of alpine grazing, which is particularly common in the federal province of Tyrol.

Table 1. Number of animals and mean, standard deviation, minimum and maximum for the number of lambings in the defined periods (1, 2, 3, 5 and 8 years).

Period (yrs)	N	Average number of lambings	Standard deviation	Minimum	Maximum
1	22,383	1.75	0.46	1	3
2	21,332	2.67	1.01	1	5
3	19,890	3.36	1.53	1	7
5	17,454	4.20	2.38	1	10
8	12,935	4.48	2.90	1	15

Table 2. Heritabilities (on diagonal) and genetic correlations (above diagonal) and their standard errors in parenthesis for the number of lambings in the defined periods (1, 2, 3, 5 and 8 years).

Period	1	2	3	5	8
1	0.034 (0.01)	0.97 (0.04)	0.92 (0.06)	0.86 (0.06)	0.81 (0.02)
2		0.063 (0.01)	0.99 (0.01)	0.97 (0.02)	0.96 (0.03)
3			0.089 (0.01)	0.99 (0.01)	0.99 (0.01)
5				0.128 (0.01)	0.99 (0.01)
8					0.140 (0.01)

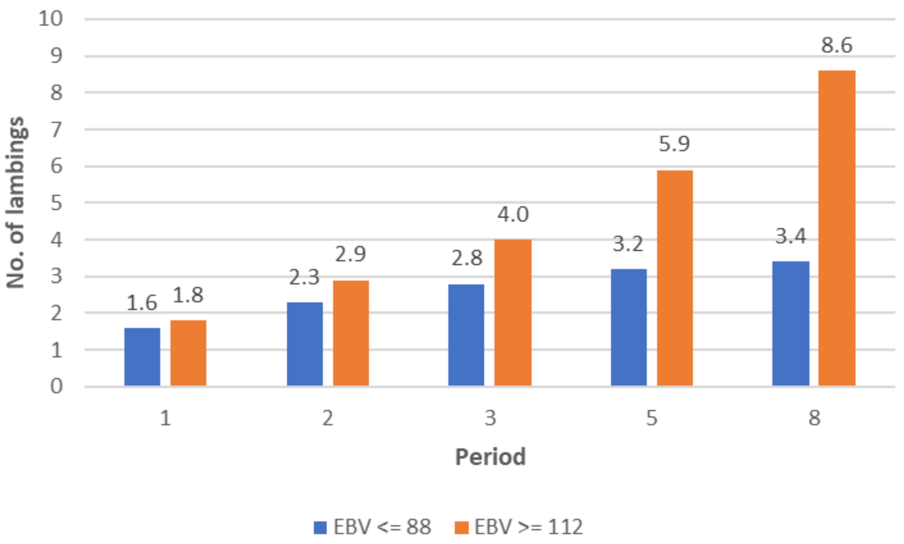

Figure 1. Mean number of lambings of daughters of rams with period 3 breeding values (EBV) of <= 88 and >=112.

Table 2 shows the heritabilities and genetic correlations as well as their standard errors for the five traits, number of lambings in a defined period. All bivariate runs finished with optimal status. Both the heritabilities ($h^2 = 0.034$ to 0.14) and the genetic correlations ($r_a = 0.81$ to 0.99) lie within a reasonable range and are consistent. Heritabilities increase markedly with increasing period. Results for other mountain sheep breeds such as Jura or Brown Mountain are however very similar ($h^2 = 0.10$ and 0.13 in period 8, respectively; data not shown). For the land sheep breed Merinoland, also being non-seasonal, but bred with a focus on meat production (Fuerst-Waltl and Fuerst, 2021) lower heritabilities were observed in all periods (e.g. $h^2 = 0.06$ in period 8), though. The genetic correlations range from 0.81 between period 1 and 5 and 0.99 between e.g. period 4 and 5. The periods at younger ages will thus provide suitable predictors for the number of lambings later in life.

Based on the genetic parameters, genetic evaluation test runs were performed by MiX99 (MiX99 Development Team, 2022), which is also used in the routine. In accordance to other traits, natural EBV were standardised to a mean of 100 and 12 points per genetic standard deviation. Figure 1 illustrates the mean number of lambings for daughters of rams with EBV for period 3 <=88 and >=112. The difference between these two ewe groups is 0.2 lambings in period 1, in period 8 it is as high as 5.2 lambings.

The first official EBV will be published in June 2024. Breeding values for all periods are estimated multivariately, but only those for period 3 are published for animals with a minimum reliability of $r^2 = 0.20$.

The introduction of the new EBV for longevity will be accompanied by the introduction of a genetic evaluation for conformation traits based on linear scoring and a total merit index (TMI). Within the TMI, longevity will have a relative weight of 30%, which is half the weight of all functional traits. The inclusion of conformation traits as auxiliary traits for longevity is not yet feasible as the estimated genetic correlations still have too high standard errors and are therefore unreliable. Linear scoring has only recently been introduced, so ewes with phenotypes for conformation have not had the chance to survive higher periods. Therefore, genetic correlations between conformation and longevity will be re-estimated over the next few years and appropriate traits will be selected based on the results.

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Breeding dairy goats for organic farming – sustainable and animal-friendly

P. Herold[1], A. Lange[2], M.-R. Wolber[3], G.M. Seyfang[1] and H. Hamann[1]

[1]Landesamt für Geoinformation und Landentwicklung Baden-Württemberg, Stuttgarter Str. 161, 70806 Kornwestheim, Germany
[2]Bildungs- und Wissenszentrum Boxberg, Seehöfer Straße 50, 97944 Boxberg, Germany
[3]University of Hohenheim, Institute of Animal Science (460), 70593 Stuttgart, Germany
Corresponding Author: pera.herold@lgl.bwl.de

Abstract

The proportion of organic farms in dairy goat farming in Bavaria and Baden-Württemberg is over 80%. As part of the GoOrganic project (2016-2022), the development of a breeding value estimation for lifetime performance as well as the establishment of targeted mating taking into account the natural mating as well as the establishment of a network of those involved in goat breeding, including advisory and further training structures, were addressed. Possibilities of breeding for health and robustness are being pursued in the current HealthyGoats project (2021-2024).

Keywords: dairy goats; breeding; lifetime effectiveness; longevity; health.
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Introduction

In Bavaria and Baden-Württemberg, over 80% of professional dairy goat farming takes place on organic farms (Manek *et al*., 2017). Professional dairy goat farming with populations of over 80 to 500 milked goats has increased significantly in recent years. Still, dairy goat farming is a small niche within agricultural production in Germany. Unfortunately, there are no exact figures available, only estimates but for example, there are approximately 50 farms with around 5.000 dairy goats in Baden-Württemberg (Kern, 2019). Goat breeding structures in Germany are significantly less developed than in other livestock species. Artificial insemination of goats plays a minor role here; there is only one EU-approved insemination station for small ruminants in Germany. Reproduction usually takes place seasonally and through natural mating. A few goat breeders import goat semen or live goat bucks from France or the Netherlands. The economic viability of dairy goat farms is primarily determined by milk production and, in particular, the milk ingredients. An increase in milk yield on organic farms is linked to good forage utilization, especially pasture or green fodder, as well as tolerance or resistance to parasites. This places new demands on the breeding goals of dairy goat breeding and corresponds to a high degree with the conceptual breeding goals of an organic breeding program. This was the initial situation for the GoOrganic project - development of a sustainable breeding program “Goats for organic farming”. The project was carried out from 2016 to 2022 by the University of Hohenheim together with various actors from science and practice in Bavaria, Baden-Württemberg and Thuringia. The goals of GoOrganic were to develop a breeding value estimation for lifetime performance as well as to establish targeted mating taking into account the (prevailing) natural mating as well as to build a network of those involved in goat breeding, including advisory and further training structures.

Breeding value estimation for lifetime performance

GoOrganic was able to build on the breeding value estimation for milk production (Herold *et al*., 2018) and conformation traits (Lange *et al*., 2018) of the State Office for Geoinformation and Rural Development (Landesamt für Geoinformation und Landentwicklung Baden-Württemberg, LGL) for the two domestic breeds German Fawn and German White in Bavaria and Baden-Württemberg. Before the new breeding value could be developed, the influence of continuous milking on the lifetime performance of the goats had to be examined. Continuous milking is increasingly being practiced on agricultural dairy goat farms meaning that they have been milking part of their herd over several years, without any lambing in between. There are mainly three reasons for this:

1. The high workload during seasonal lambing;
2. The aim of producing milk all year round if possible;
3. The poor sales opportunities for goat kids that are not needed for breeding.

Wolber *et al*. (2018) and Wolber *et al*. (2019) proofed an influence of continuous milking on lifetime performance. Based on this, Wolber *et al*. (2021) estimated genetic parameters for various traits that describe an animal's lifetime performance. These are length of productive life (LPL), lifetime efficiency (LEF) and milk yield efficiency (EDM). From these traits, LEF as lifetime milk production (kg) per days of life appears to be particularly suitable for depicting an animal's lifetime performance. This means LEF combines the ability to achieve high levels of performance with the ability of a long and healthy life. According to Wolber *et al*. (2021) the heritability of LEF is 0.29 ± 0.03 and it is highly correlated with LPL ($r_G = 0.65 \pm 0.06$). Additionally, a system for linear description of dairy goats was developed in 2013 and since 2018, breeding values for conformation traits are estimated (Lange *et al*., 2018). The information on conformation traits could also be included in a breeding value estimation for lifetime performance. A breeding value estimation for LPL based on a section model is currently being developed (Herold and Chagunda, 2023). The next step is to develop a breeding value estimation for LEF.

Breeding for health and robustness

As a performance test for health traits, a monitoring system for goats (GMON goat) was established in the goat herd manager (ZDV) of the performance testing organisations (LKV) in Bavaria and Baden-Württemberg. It is based on a central health key for dairy goats. The GMON goat is based solely on the observations of goat farmers and not on diagnoses by veterinarians. This is because there are only a few goat farms and the veterinarians usually only look after one or very few goat farms and therefore would not come up with the required number of diagnoses for data validation. Besides health data the farmers can also monitor measures such as vaccinations, deworming or hoof trimming. Even if the GMON goat is now well accepted, it must be taken into account that the number of farms that use ZDV is small. By the end of 2023, 716 observations from 11 farms had been entered in Baden-Württemberg and 6,471 observations from 49 farms in Bavaria. The GMON goat is important information for farmers when it comes to herd management or individual animal selection. The database is currently not sufficient to estimate breeding values. Additionally, Wolber *et al*. (2021) estimated genetic parameters for the indirect health traits fat:protein ratio (FPR) and urea content

(UC) which are recorded during the milk performance test. Heritabilities were 0.32 ± 0.03 and 0.47 ± 0.04.

In order to further advance breeding for health and robustness in goats, the HealthyGoats project started in 2021 - expanding breeding for health and robustness in dairy goats (www.gesundeziegen.de; Bernau *et al*., 2023). The HealthyGoats project deals with new health and robustness traits in dairy goats as well as advice on animal health and breeding. The detailed recording of possible new traits is carried out on ten farms. In addition, the GMON goat was expanded to another database, serv.it OVICAP, in order to expand data collection to other goat breeders and to carry out health trait monitoring throughout Germany. One project goal is to recommend new traits for breeding for health in goats and thus for performance testing.

Advice and further training

In addition to the development and establishment of new traits as well as the new performance testing systems, goat breeders and goat keepers should be actively involved in breeding. The method *Stable School* appears to be particularly suitable here. The project team of GoOrganic implemented the *Stable School* method as a breeding working group concept: A steady group of goat farmers meets regularly, moderated by a representative of the breeding association. A farm from the group is the host and determines the topic (an important / current challenge on the farm). The meetings always follow a strict schedule; at the end of the meeting, the farm has a portfolio of recommendations from the participants on how to overcome the operational challenge. The method was transferred to the HealthyGoats project, where the methodology was further developed in order to be able to offer online working groups. In the HealthyGoats project, the goat breeding associations of Bavaria, Baden-Württemberg and Thuringia also want to address the continuation of the method. In addition, the HealthyGoats project is working to establish the method of *breeding location decision* developed within the framework of GoOrganic in breeding advice and to train and coach interested consultants (Wolber *et al*., 2023).

Conclusion

Dairy goat farming in Bavaria and Baden-Württemberg is a small but growing niche. Predominantly organic goat farming is perceived positively by consumers. The work of the breeding value estimation team and the various goat breeding projects support goat farmers in Bavaria as well as in Baden-Württemberg and Thuringia. In Bavaria and Baden-Württemberg, goat farmers also benefit from performance testing organizations services in the areas of milk performance testing and LKV goat herd manager. This ensures that the goat farmers are well positioned for the future in the area of breeding and that progress in breeding in the sense of sustainable and animal-friendly breeding is possible.

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Standard operating procedures for efficient management of small ruminant farms

*G. Arsenos[1], S. Vouraki[1,2], V. Papanikolopoulou[1], A. Argyriadou[1], G. Batikas[1],
A.M. Iatrou[1], V. Fotiadou[1], P.D. Carvalho[1] and S. Priskas[1]*

[1]Laboratory of Animal Husbandry, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University, 54124 Thessaloniki, Greece
[2]Department of Agriculture, School of Agriculture, University of Ioannina, 47100 Arta, Greece
Corresponding Author: arsenosg@vet.auth.gr

Abstract

Success in dairy sheep and goat farming depends largely on efficient management of available resources and expertise of farmers. Both, combined with the low level of implementing innovation at farm level dictate animal productivity and farm profitability. The objective was to develop farmer-friendly standard operating procedures (SOPs) for training employees as well as recording protocols to cater the needs of efficient farm management. Initially, a comprehensive review of existing guidelines and relative research studies regarding farm management practices was performed. In particular, milk production recording and udder morphology assessment guidelines of the International Committee of Animal Recording (ICAR) and protocols for Animal Welfare Indicators (AWIN) were examined.

Refereed publications about reproduction technologies, newborn management, nutritional management, milking procedures, milking parlor critical points, and biosecurity measures were studied. Decision support tools for farm management and economic performance assessment were also explored. Based on the above, detailed SOPs, tailored for dairy farms, were developed. Each SOP was divided in subsections detailing objectives, step-by-step procedures, and required equipment/technologies. Specifically, SOPS for reproduction management focused on artificial insemination and natural mating, pregnancy diagnosis, and lambing/kidding management.

SOPs for newborn lambs/kids focused on colostrum quality and storage, artificial rearing, and prevention of diarrhea incidents. SOPs for nutritional management included guidelines for meeting energy and protein requirements of each animal category according to their physiological and production stage, and sample collection of feeds for chemical analysis. Milking parlor and milking procedure guidelines focused on maintenance, cleaning, and education of milkers on milking practices. SOPs for animal health and welfare included vaccination protocols, and practical welfare indicators (body condition score, water availability, fleece cleanliness panting, stocking density, hoof overgrowth, body and skin lesions, lameness, fecal soiling, ocular discharge, mastitis).

SOPs for milk production and udder health detailed procedures for milk yield recording with volumetric milk meters, milk sample collection for chemical analysis as well as udder morphometric measurements. Biosecurity SOPs, focused on internal and external measures for disease prevention and control. Lastly, the applicability and usefulness of existing decision support tools for efficient farm management and economic performance of small ruminant farms was assessed. Next steps include the integration of these protocols in an online interactive platform. The notion is that those customized SOPs will help farmers become more efficient in managing dairy sheep and goat farms while ensuring animal health and welfare.

Keywords: small ruminants, training, standard operating procedures, recording protocols, production, profitability.
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Introduction

Dairy sheep and goats are primarily reared in Mediterranean countries, having a substantial socio-economic impact. Their milk is mainly processed into cheese products, many of which are Protected Designation of Origin. Moreover, they provide ecosystem services and employment opportunities, especially in mountainous and less-favored areas (Arsenos *et al*., 2021).

However, many sheep and goat farmers do not follow established guidelines and management protocols. Moreover, they have not adopted new technologies, and innovations that could help modernize farm practices (Paraskevopoulou *et al*., 2020). All the above result in low levels of animal productivity, poor animal health and welfare, high production costs, and low farm income. To overcome these challenges education on farm management practices detailing step-by-step procedures is imperative.

The objective here was to develop farmer-friendly standard operating procedures (SOPs) for training employees and recording protocols to cater the needs of efficient farm management.

Reproduction management SOPs

Ten weeks prior to the mating period, males should be assessed to detect issues that could adversely affect reproductive ability. Evaluation includes assessment of body condition score (BCS; 3-4), testicular size (>30 cm and >25 cm in rams and bucks, respectively), genitalia, jaw, teeth, and limbs. Females should be also assessed based on age (first mating at 7-8 months), BCS (2.5-3.5), health, and genetic improvement criteria.

Ideally, artificial insemination (AI) should be performed. AI enhances genetic improvement by increasing selection intensity and genetic evaluation precision. To increase cervical AI success rates the following guidelines (Priskas *et al*., 2022) are suggested:

1. Selection of the best females based on age (first to third lactation period), BCS (2.5-3.5), health, productivity (milk yield and quality), udder morphology, and somatic cell count.

2. Estrus synchronization using intravaginal fluogestone acetate sponges, or CIDR devices and insemination 50-55 hours following their removal.

3. Proper animal handling after AI.

Any animals not artificially inseminated are naturally mated. Hormonal interventions are suggested to increase reproduction and management efficiency. These include estrus synchronization or melatonin implants to accelerate the onset of reproductive activity (1 to 10 and 1 to 25 male to female ratio, respectively). Random mating practices should be avoided, and pedigree records should be kept for purposes of genetic improvement.

Newborn management SOPs

Colostrum is imperative for newborn survival. To guarantee an efficient amount of high-quality colostrum for lambs/kids the following are suggested:

1. Individual housing of ewes/goats with their newborns for 2-4 days following parturition,
2. Assessment of colostrum quality using a digital Brix refractometer,
3. Pasteurization to reduce microbial load,
4. Storage (refrigerator or freezer) to accommodate any future needs,
5. Thawing and warming prior to administration.

Artificial rearing is an important management practice to increase farm profitability. The following guidelines are suggested for efficient implementation:

1. Smooth transition, observation, and assistance of lambs/kids,
2. Use of high-quality milk replacer that should be prepared and administered according to manufacturer's instructions,
3. Provision of a warm and dry environment to avoid hypothermia,
4. Provision of feedstuffs at the age of one week,
5. Weaning at the age of 35-40 days and at a body weight of 15 kg.

Nutritional management SOPs

During the mating period and first month of gestation the nutritional management of the respective lactation stage is followed; flushing is advised to achieve a BCS of 2.5-3.5. During the last month of gestation energy and protein demands increase substantially but feed intake decreases. Therefore, it is suggested to gradually decrease forages and increase concentrates; BCS at this stage should be 3-3.5. During the early stages of lactation, animals are typically fed with 1.5-2 kg of lucerne hay and/or silage, 0.15-0.2 kg of straw and 1-1.5 kg of concentrates offered at least twice daily to avoid ruminal acidosis. In general, feeding of males is designated to cover mainly maintenance requirements. Two months prior to mating period the amount of concentrate should be increased to 1 kg/animal/day to meet energy demands and improve semen quality. Finally, from weaning to five months old, lambs/kids are fed with concentrates and straw ad libitum and 500-600 g of lucerne hay. From the age of five months and until the first mating period, 500-700 g of concentrate feed, 500-600 g of lucerne hay, and straw ad libitum are suggested.

Milking procedure SOPs and milking parlor critical points

Milking procedure includes the following steps:

1. Use of gloves by milkers and frequent disinfection or replacement.
2. Use of discrete measures to indicate animals with mastitis that should be milked separately.
3. Pre-stripping and observation of milk for any signs of mastitis.
4. Attachment of milking units.

5. Cluster removal after vacuum cessation to prevent teat injuries.
6. Post-milking disinfection (post-dipping).

Milking parlor should be cleaned both externally and internally after every milking. External cleaning is performed using high-pressure water. Critical points for efficient internal cleaning include:

1. Water temperature at 70-80^{o} C.
2. Use of alkaline detergents every time.
3. Use of acid detergents once per week if the water is not hard, otherwise 2-3 times per week.
4. Cleaning duration for at least 30 min and 90 min when using only alkaline or both alkaline and acid detergents, respectively.

Vacuum level in the manometer should be checked daily. Moreover, vacuum level, pulsation rate and pulsation ratio in the milking units should be monitored twice per year by authorized technicians using designated equipment. Vacuum level differences between clusters and the vacuum pump should not exceed 2 kPa; such differences have been associated with increased risk for subclinical mastitis and teat-end hyperkeratosis (Vouraki *et al*., 2018). Finally, clusters should be replaced at 2,500-5,000 milkings per milking unit.

Biosecurity, animal health and welfare

External biosecurity aims at reducing the risk of disease introduction to a farm. External biosecurity measures include:

1. Disinfection of vehicles' wheels when entering farm premises.
2. Use of gloves, clean clothing and footwear by all visitors and employees.
3. Low animal purchasing frequency and quarantine for at least three weeks.
4. Handling of dead animals with gloves, and storage in a freezer until disposal, and
5. Vermin control.

Internal biosecurity aims at reducing the spread of a disease within a farm. It is suggested that animals of different age groups are housed separately; younger animals should be visited first since they are more susceptible to infections. Sick animals should be separated from the healthy ones and remain in a hospital pen. Diagnoses, treatments, and deaths should be recorded. Moreover, for efficient cleaning and disinfection the following steps should be followed:

1. Dry cleaning and removal of organic material.
2. Soaking of surfaces with detergents to loosen all remaining organic material.
3. High pressure cleaning with water.
4. Drying to avoid disinfectant dilution.
5. Disinfection and drying.

Vaccinations are suggested for enterotoxemia (ewes/does one month prior to parturition and lambs/kids at the age of three weeks), contagious agalactia (ewes/does two months prior to parturition and lambs/kids at the age of two months), enzootic abortion (one month prior to first mating) and paratuberculosis (in goat farms where animals have been diagnosed with the disease and at the age of 2-3 weeks to 6 months). Finally, welfare indicators to be routinely assessed include BCS, water availability, fleece cleanliness, panting, stocking density, hoof overgrowth, body and skin lesions, lameness, fecal soiling, ocular discharge, and mastitis. Scoring is performed according to AWIN guidelines (Dwyer *et al*., 2015).

Milk production and udder morphology recording protocols

Milk yield recording is performed using volumetric milk meters. Recording starts after weaning and is performed monthly (five monthly records per milking period are suggested) following the guidelines of International Committee of Animal Recording (ICAR, 2018). Inddividual milk samples should be collected from the milk meters monthly (at least for three months in early lactation) to assess milk quality (fat, protein, lactose, solids-non-fat content). Udder morphology assessment includes morphometric measurements of udder depth, udder attachment, degree of separation of udder halves, and teat placement. Scoring is performed using the nine-point linear scale proposed by Casu *et al*. (2006) and ICAR (2018).

Farm economic performance recording and assessment

Decision support tools can be used to record and evaluate farm economic performance. Specifically, a web-based application has been developed within the ProudFarm project to familiarize farmers with financial management of small ruminant farms and help towards decision making. This app illustrates the expected daily net income against feeding costs. Input data include daily milk production and animal feed intake. Moreover, web applications, such as Happy Goats (https://happygoats.eu/), are available to assess annual farm economic performance in relation to farm management practices. Towards this end, records should be kept and used as input parameters for the following categories:

1. Flock size.
2. Production (milk and meat),
3. Nutritional management and grazing.
4. Income, farm prices, and costs.

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Conformation recording as auxiliary trait for functionality and fitness in dairy goats

P. Herold[1], A. Lange[2], M. Simčič[3], B. Fuerst-Waltl[4], J. Stoeckl[5], R. Taferner[5], V. Clément[6], A. Bailly[7], M. Sanchez[8], J. Caballero-Villalobos[8], C. Iglesias Pastrana[9], E. Wallin[10], I.A. Boman[10], M. Valencia Posadas[11], J. Conington[12] and T. Neuenschwander[13]

[1]Landesamt für Geoinformation und Landentwicklung Baden-Württemberg, Stuttgarter Str. 161, 70806 Stuttgart, Germany
[2]Bildungs- und Wissenszentrum Boxberg, Seehöfer Straße 50, 97944 Boxberg, Germany
[3]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[4]Universität für Bodenkultur Wien, Gregor-Mendel-Straße 33, 1180 Wien, Austria
[5]Österreichischer Bundesverband für Schafe und Ziegen (ÖBSZ), Dresdner Straße 89/B1/18, 1200 Wien, Austria
[6]Idele, Campus INRAE AUZEVILLE - 24, Chemin de Borde Rouge, 31320 Auzeville-Tolosane, France
[7]Capgenes, 2135 Route de Chauvigny, 86550 Mignaloux-Beauvoir, France
[8]Department of Animal Production, Faculty of Veterinary Sciences, University of Córdoba, 14071 Córdoba, Spain
[9]Department of Genetics, Faculty of Veterinary Sciences, University of Córdoba, 14071 Córdoba, Spain
[10]The Norwegian Association of Sheep and Goat Breeders (NSG), PO Box 104, 1431 Ås, Norway
[11]División de Ciencias de la Vida, Universidad de Guanajuato, Carretera Irapuato-Silao km 9,ExHda. El Copal, Irapuato, Gto. CP 36824, Mexico
[12]SRUC, W Mains Rd., Edinburgh EH9 3JG, UK
[13]Holstein Switzerland, Rte de Grangeneuve 37, 1725 Posieux, Switzerland
Corresponding Author: pera.herold@lgl.bwl.de

Abstract

In 2016, the International Committee for Animal Recording (ICAR) published the first international guidelines for the linear scoring of dairy goats. A working group with the goal to review and revise these guidelines was established in 2022. First, a literature and internet search were carried out on conformation recording systems for dairy goats that existed worldwide. Ten organizations were identified that carried out a linear scoring of dairy goats (Austria, Canada, France, Germany, Mexico, Norway, Slovenia, Spain, United Kingdom and USA). All organizations were contacted and asked whether they would like to participate in a working group to revise the ICAR guidelines. Eight of them take part in the working group. The working group's proposed changes are to be implemented in 2024.

The ICAR guidelines include 21 traits in three categories as follows: udder (9), legs (5) and body frame (7). The focus of all eight participating organizations was on udder traits. Leg traits are recorded in four countries according to the ICAR guidelines. The

ICAR body frame traits are recorded by four countries; they are often measured and not described linearly.

A breeding value estimation for conformation traits was developed in five of the eight participating countries. In addition, relationships with other traits, in particular *length of productive life* (LPL), were examined. The traits recommended by ICAR will therefore provide even better information in the future as auxiliary traits for breeding healthy and long-living dairy goats.

Keywords: dairy goats; conformation; linear type traits
Presented at: Session 7, Breeding for agroecological transition in sheep and goats

Introduction

One goal in dairy goat breeding is to breed healthy and long-living animals to achieve the desired performance. Conformation traits play a major role for production, longevity and profitability (Massender *et al*., 2022; Castañeda-Bustos *et al*., 2017; McLaren *et al*., 2016). While much farm animal's performance can be measured objectively, such as milk production and daily gain, conducting an objective conformation assessment is more complex. ICAR published international guidelines for conformation recording in dairy goats in 2016 to help to ensure the equivalence of conformation recording collected worldwide and to provide practitioners with description procedures that are backed up by scientific knowledge. At that time, four countries were involved in developing the guidelines. Since then, conformation recording has been introduced in several other countries. Practical experience in carrying out the performance test according to the published guidelines as well as scientific studies on the genetic basis of the traits made it necessary to revise the guidelines. Therefore, a working group of experts to review and revise the ICAR guidelines was established in 2022. The working group's proposed changes are to be implemented in 2024. The following describes the distribution of the conformation recording in dairy goats worldwide as well as experience with using the traits from the ICAR guidelines from 2016.

Material and methods

A literature and internet search were carried out in 2022 on conformation recording systems for dairy goats that existed worldwide. All identified organizations were contacted and asked whether they would like to participate in a working group to revise the ICAR guidelines. In a series of online meetings, participants presented and discussed the conformation recording system in their countries. All the information collected was supplemented with references from the literature and shared among the participants. The results of the working group were agreed with the ICAR conformation working group at the ICAR meeting in Bled in May 2024 and are to be incorporated into the guidelines.

Results

Ten organizations were identified that carried out a linear description of dairy goats (Austria, Canada, France, Germany, Mexico, Norway, Slovenia, Spain, United Kingdom and USA). All organizations were contacted and asked whether they would like to participate in a working group to revise the ICAR guidelines. Eight of them responded positively. The stakeholders are representatives of state institutions, universities and breeding organizations. In two countries, only male animals are reported, in two countries both males and females are included and in all other countries female animals only are reported, providing the information relevant to the sires of those female progeny. Describing the goats includes the responsibility of goat breeders, employees of the breeding associations and state employees.

The ICAR guidelines currently include 21 traits in three categories of udder (9), legs (5) and body frame (7). The focus of the majority of participating organizations is on udder traits. Eight of the ICAR traits are recorded in four countries, seven in two countries and two countries record only two of the ICAR udder traits. Some countries record additional traits such as *udder profile/form*, *rear udder side view* or deficiencies like *teat thickness* or *supernumerary teats*. Leg traits are recorded in four countries according to the ICAR guidelines. Further traits are *front legs set front view*, *front legs set side view* and *feet angle opening*. *Splayed toes* are recorded as a deficiency in one country. The ICAR body frame traits are recorded by four countries; they are often measured and not described linearly. Additionally, *body length* and *chest circumference* are measured in two countries, other traits are *chest depth*, *width of pelvis*, *withers height* and *sacrum height* (all measured). Two countries describe *dairyness*, which considers assessments of for example length, cleanness and flatness of bone, length and leanness of neck, definition and sharpness of withers, ribs angularity, degree of fleshing, femininity and

Table 1. Heritabilities (± SE, if available) of exemplary ICAR goat conformation traits.

Trait	Heritability	Study
Stature	0.20 ± 0.02	Castañeda-Bustos et al., 2017
	0.76 ± 0.18	Lange et al., 2018
	0.52	Luo et al., 1997
	0.18	Muñoz-Mejías et al., 2023
	0.72 ± 0.07	Valencia-Posadas et al., 2022
	0.52	Wiggans and Hubbard, 2001
Loin strength	0.09-0.12	Fuerst-Waltl and Fuerst, 2022
	0.0 ± 0.02	Lange et al., 2018
Rear legs set side view	0.20 ± 0.02	Castañeda-Bustos et al., 2017
	0.04-0.09	Fuerst-Waltl and Fuerst, 2022
	0.15 ± 0.10	Lange et al., 2018
	0.21	Luo et al., 1997
	0.03	Muñoz-Mejías et al., 2023
	0.19 ± 0.09	Valencia-Posadas et al., 2022
	0.21	Wiggans and Hubbard, 2001
Rear legs set rear view	0.0-0.08	Fuerst-Waltl and Fuerst, 2022
	0.0 ± 0.10	Lange et al., 2018
	0.25 ± 0.05	McLaren et al., 2016
	0.23	Muñoz-Mejías et al., 2023
Fore udder attachment	0.13-0.29	Biffani et al., 2020
	0.25 ± 0.02	Castañeda-Bustos et al., 2017
	0.30	Clément et al., 2002
	0.09-0.26	Fuerst-Waltl and Fuerst, 2022
	0.11 ± 0.10	Lange et al., 2018
	0.25	Luo et al., 1997
	0.26-0.33	Manfredi et al., 2001
	0.26 ± 0.02	Massender et al., 2022
	0.15 ± 0.04	McLaren et al., 2016
	0.23	Muñoz-Mejías et al., 2023
	0.30 ± 0.01	Rupp et al., 2011 (Alpine)
	0.25 ± 0.02	Rupp et al., 2011 (Saanen)
	0.13 ± 0.08	Valencia-Posadas et al., 2022
	0.25	Wiggans and Hubbard, 2001

refinement, and fineness and texture of skin (extremes are coarseness and sharpness). A *loose shoulder* is recorded as a defect in two countries, describing the lateral deviation of the shoulder joint due to relaxation of the muscles between the chest and front legs. A breeding value estimation for conformation traits was developed in five of the eight participating countries. Table 1 shows example genetic parameters for a few selected ICAR traits. The genetic and phenotypic relationships amongst conformation traits and milk yield in mixed breed UK dairy goats scored during their first lactation in the UK were reported by McLaren *et al*. (2016). The genetic correlations estimated between the conformation traits and milk yield, across the first lactation, demonstrate the changes that occur during this time period. The majority of the correlations estimated between milk yield and both the udder and teat traits were negative and antagonistic. These results are consistent with those of Clément *et al*. (2002), who found null or negative correlations in French goat breeds.

In addition, relationships with other traits, in particular length of productive life (LPL), were examined. Genetic correlations were estimated between the final score and functional productive life at 72 months of age (FPL72) (0.52 ± 0.11), fore udder attachment and FPL72 (0.37 ± 0.09) and udder depth with FPL72 (0.36 ± 0.10). This suggests that selection for these type traits can improve FPL72 in dairy goats (Castañeda-Bustos *et al*., 2017). Findings from these genetic analyses were incorporated into the revision of the ICAR traits.

Finally, the working group agreed to skip the traits rear udder height, locomotion, chest and rump width. The following traits are to be included: teat orientation rear and side view, feet angle opening and chest circumference. Teat orientation is the direction of the teats in relation to the udder viewed from rear/side. Feet angle opening is the angle between the hind legs, when the goat is walking, and chest circumference is the circumference measured behind the shoulder blades. The angularity trait is renamed rib structure. Chest circumference replaces chest width. At rear udder height and rump width, the general opinion was that these traits are not relevant for the majority of the population. For locomotion, all group members agreed that recording the trait was not

Table 2. Summary of the working group's resolutions.

Trait group	Keep	Skip	New
Udder traits	Fore udder attachment Central ligament Rear udder width Udder depth Teat placement rear view Teat length Teat form	Rear udder height	Teat orientation rear view Teat orientation side view
Leg traits	Rear legs set rear view Rear legs set side view Front pasterns side view Hind pasterns side view	Locomotion	Feet angle opening
Body frame traits	Stature Body depth Rump angle Loin strength Rib structure (before: Angularity)	Chest width Rump width	Chest circumference

Table 3. Examples of defects in dairy goats

Defect	Definition
Loose shoulder	Weakening of the muscles between the chest and the front limbs, which can cause problems when standing up
Jaw: undershot and overshot	Underdevelopment of the lower or upper jaw
Supernumerary teats	More than two teats (which sometimes produce milk)
Double teats	Teat splits in two teats
Unbalanced udder	Udder halves are expressed to varying degrees
Udder warts	Non-milk-producing protrusions on the udder
Weeping teats/udder	Milk can filter through pores in the skin to the udder surface
Splayed claws	Enlarged gap between the claws

practical. Table 2 provides an overview of the existing, new and deleted traits for the guidelines for linear scoring in goats

A list of defects has also been drawn up to supplement the guidelines. Table 3 shows the defects and their definition.

Discussion

Milk produced from dairy goats is an important source of nutrition for many populations worldwide, and is considered to be a niche market for others. As the formalisation of breeding programs to enhance productivity has become more accessible world-wide, it is important to consider functional fitness traits as being integral to these breeding programmes. From four countries in 2016, there are now (in 2024) ten countries, which have introduced linear scoring in dairy goat breeding. The importance of the ICAR guidelines increases with the extension of linear scoring in different countries. It is therefore necessary to regularly adapt the guidelines to new developments, which has now been done by the sub-working group. At the same time, dissemination to all relevant organizations and harmonization among describers within and across populations will be essential to ensure adequate data quality and comparability. Studies of the genetic basis show that all of the traits proposed by ICAR are heritable, mostly in the low to moderate range, depending on the populations studied. Therefore, the traits can be improved by selection. Findings of McLaren *et al*. (2016) and Clément *et al*. (2002) on negative or null correlations between conformation traits and milk yield show the importance of scoring conformation scores in dairy goats as breeding programmes would benefit from including these traits in order to ensure that selection for increased productivity is not accompanied by the deterioration of functional fitness. In addition, morphological traits are genetically favourably correlated with somatic cell count (Rupp *et al*., 2011). Thus, taking morphology into account in the selection objective is also a way of acting on udder health. Through the improvement of conformation traits based on the ICAR guidelines, the productive life of goats can be extended, the economic value of the animals can be improved and the income of breeders can be increased. To ensure this, further efforts should be made to investigate genetic relationships with LPL and other health-related traits in the target populations..

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Assessment of Mediterranean buffalo lactation curves shape using lactation models

L. Trapanese[1], N. Pasquino[1], A. Salzano[2], and M. Hostens[3,4]

[1]Department of Electrical Engineering and Information Technologies,
University of Naples Federico II, Italy
[2]Department of Veterinary Medicine and Animal Production,
University of Naples Federico II, Naples, Italy
[3]Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience
Engineering, Ghent University, 9000 Ghent, Belgium
[4]Department of Animal Science, College of Agriculture and Life Sciences,
Cornell University, United States
Corresponding Author: lucia.trapanese2@unina.it

Abstract

In Italy, the number of buffalo has increased by the 173% from 1996 until now with a total of around 425000 buffaloes. In contrast to dairy cows, adoption of new technologies such as automatic systems is limited in buffalo. Milk yields are recorded by hand, making it time-consuming and error prone. As a result, few data are available in buffalo lactations. The analysis of lactation curve shape has been shown to be useful for technicians and farmers to understand the evolution of milk yields and support management decisions. Lactation curve models have not yet been explored in buffaloes opposed to dairy cows where lactation curve model have been adopted by the entire industry (e.g. Wood, Wilkmink or Milkbot). The aim of our work was to explore lactation curve models applied to Mediterranean Italian buffaloes by performing a comparative assessment of Wood and Milkbot equations. The analysis was performed on a large dataset containing the milk yield, calving date, lactation number and days in milk from 333 376 animals on 295 herds over a 4-year period from 2013 until 2016. Performance of the final models was evaluated using the coefficient of determination (R^2). Wood's model performed slightly better than Milkbot model with $R^2 = 0.75 \pm 0.24$ and 0.66 ± 0.23, respectively illustrating the ability of both models to fit buffalo daily milk production. These results encourage adopting a more analytical approaches to buffalo to obtain in-depth phenotypes on their milk productive capacity. Although, Milkbot performed slightly worse than Wood, it directly provides information on the loss of productivity capacity which can be converted into a measure of persistency. In conclusion, the final aim was promoting the use of mathematical models in the buffalo.

Keywords: Buffalo, Lactation curves, Wood, Milkbot, Mozzarella cheese.
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Holstein cows are generally recognised as the most important dairy species due to their high milk production. Even if dairy cattle represented the majority of the milk market, other species, such as sheep, goats or buffaloes, are still important producers of milk

Introduction

and dairy products. In particular, the buffalo sector has been constantly growing in the last years, especially in the Mediterranean countries. In Asia and South America buffalo farming is considered an important sector from an economic and social point of view. The buffalo sector in Italy changed from an extensive to intensive livestock system where small herds with few animals were replaced by bigger herds with more than 100 cows. Indeed, nowadays buffalo sector counted a total of around 425 000 buffaloes especially located in the South of Italy (BDN, Italy). The main output of buffalo farm is the “Mozzarella di bufala Campana PDO” and thanks to its organoleptic properties and taste, it is appreciated globally (Levante *et al*., 2023). To manage and cover the market request of buffalo milk a more data-driven approach to the industry is needed.

Lactation models (LC) are one of the pivotal tools to understand and forecast milk production and helps breeder make better management decisions. Due the key role of this tool, different lactation curves model has been developed over the last 50 years.

Since 1923, with the advent of the model theorized from Brody *et al*. (1923), various mathematical models have been introduced to characterize the shape of dairy lactation curves (LC) and provide insights into milk yield production. Among these models, the Wood equation stands out as one of the most utilized benchmarks for model evaluation (Radjabalizadeh *et al*., 2022). This equation, formulated by Wood in 1967, is based on an incomplete gamma function and comprises three key parameters: scale (a), ramp (b), and declining slope (c) (Wood, 1967).

A more recent addition to the repertoire of lactation curve models is the MilkBot model (Ehrlich, 2013). Like the Wood equation, the MilkBot model is an exponential equation, but it incorporates an additional parameter, enhancing its ability to capture the slope of lactation. Furthermore, this model introduced characteristics such as the time of maximal creation of productive capacity (offset, c) and the loss of productive capacity (decay, d). These parameters offered a more comprehensive understanding of the lactation process and can be easily translated into a measure of persistency.

All of those LC models have been poorly explored in buffaloes and only few papers were available in the literature (Khan *et al*., 2023; Metry *et al*., 1994; Şeahin *et al*., 2015). The purpose of this work is providing evidence on how the Wood and Milkbot equations describe the buffalo LC, giving information to benchmark animal performance and ultimately improve milk production in buffalo.

Material and methods

The analysis was performed on a large dataset containing milk yield, calving date, lactation number and days in milk from 333376 animals on 295 buffalo herds from 2013 until 2016 with lactation numbers ranging from 1 to 3. Animals with at least five observations per lactation were chosen to ensure a coherent fitting with at least the number of observations equal to the number of the regression parameters of the model. All data pre-processing was done through R software (version 4.3.2).

The first mathematical model fitted to the data was the Wood equation (Wood, 1967) with the following formula:

$$Y(t) = at^{b}e^{-ct} \tag{1}$$

Where *Y* is the milk production, *t* the days in milk, the magnitude *a*, *b* the time to peak and *c* is the decay.

The second model employed was the MilkBot (Ehrlich, 2013). The full MilkBot equation is shown as:

$$\mathrm{Y}\,(t) = a\left(1 - \frac{e^{\frac{c-t}{b}}}{2}\right)e^{-dt} \tag{2}$$

Where *Y* is the milk production, *t* the days in milk, *a* the magnitude, *b* the time to peak*c* the offset and *d* the decay.

In this case, the fitting happened through the API version of the model (1.3), which was available online (Jim Ehrlich, API Milkbot). Wood and Milkbot equations required the employment of priors for the parameters a, b,c and d. Prior values were used as initial guesses to search the optimal solution. Initially, Wood and Milkbot models were fitted using priors based on a literature search (Khan *et al*., 2023; Şeahin *et al*., 2015). After the first fitting step, mean and standard deviation (sd) of regression parameters from the results were used to fit all lactations for a second time, At the end, the performance of the models was evaluated through the coefficient of determination (R^2).

$$R^2 = \frac{SS_{res}}{SS_{tot}} \tag{3}$$

where SS_{res} is the sum of the squared residuals and SS_{tot} is the total sum of squares. The *curve_fit* function from the *scipy* package was used to fit lactation data using Python v3.10.

Results and discussion

The presented research provides a preliminary approach to a mathematical model of the buffalo LC shape. The results suggest an overall better performance of the Wood equation compared to Milkbot.The results of the fitting of the Wood and Milkbot equations are shown in detail and discussed in this section. Results are shown for lactation number 1, 2 and 3.

Wood analysis

Table 1 reports the mean of parameters a,b,c ± *s.d*. and the R^2 ± *s.d*.

The mean values of *a, b, c* are coherent with Khan *et al*. (2023). We achieved high R^2 values, especially for lactations 2 and 3. Our results suggest that the Wood model achieved a good approximation of real milk yield despite the low sampling rates of time series. On the other hand, the standard deviation for each parameter and R^2 suggest that data are strongly variable about the mean, probably due to the variability in the number of milk points available for each lactation.

This is one of the first analysis applying the Milkbot model to buffalo LC. No literature was available to compare the obtained results. However, results seem coherent based

Table 1. Fitting metrics of the Wood model.

Parity	$\bar{a} \pm \sigma_a$	$\bar{b} \pm \sigma_b$	$\bar{c} \pm \sigma_c$	$\overline{R^2} \pm \sigma_{R^2}$
1	6.1±4.2	0.30±0.30	0.005±0.003	0.72±0.26
2	7.6± 5.1	0.29±0.30	0.006±0.004	0.78±0.22
3	7.9± 5.2	0.30±0.30	0.007±0.004	0.79±0.21

Table 2 reported the result for Milkbot equation.

Parity	$\bar{a} \pm \sigma_a$	$\bar{b} \pm \sigma_b$	$\bar{c} \pm \sigma_c$	$\bar{d} \pm \sigma_d$	$\overline{R^2} \pm \sigma_{R^2}$
1	13.5±2.4	30.67±0.06	-0.4992±0.001	0.0015±0.0001	0.58±0.26
2	15.9± 3.2	22.74±0.02	-0.7751±0.001	0.0026±0.0003	0.69±0.22
3	17.1± 3.6	25.07±0.75	0.0039±0.002	0.0029±0.0003	0.69±0.20

Milkbot analysis

on dairy cow parameters and their interpretation (Chen *et al*., 2022). Milkbot performed worse than Wood in terms of R^2 for each lactation.

The R^2 values suggest that Milkbot and Wood equations seem to be a promising technique for evaluating the LC of buffalo considering the very few milk points available during lactation that negatively affect the results. However, since the models are strongly influenced by the choice of the initial priors, more efforts to find suitable values of *a, b, c,* and *d* can improve the model performance. Finally, like in Holstein domain, the first lactations achieved worse result compared to the lactations 2+.

Conclusion

This work reported and compared the performance of Wood and Milkbot equations to describe the behaviour of buffalo milk yield. The results suggested that Wood performed better than Milkbot in terms of R^2 in buffalo cows. Moreover, Wood equation achieved better result than Milkbot employing few milk points available. Results are promising, but more efforts are needed to establish more accurate priors for buffalo cows.

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Early detection of multifetal pregnancies in Alpine Goats using pregnancy-associated glycoprotein (PAG) concentrations in milk

Y.T. Chen[1], M.K. Yang[1], Y.H. Yeh[1], Y.H. Chen[1], J.W. Shiau[1], J.F. Huang[2] and P.A. Tu[1]

[1]Northern Region Branch, Livestock Research Institute, Ministry of Agriculture, No.207-5
Pitoumian, Xihu Township, Miaoli 368003, Taiwan
[2]Taiwan Livestock Research Institute, No.112,
Farm Road, Hsinhua,Tainan 71246, Taiwan
Corresponding Author: tpa@mail.tlri.gov.tw

Abstract

The nutritional metabolic demands and foetal development needs of pregnant goats carrying multiples (≥3 foetuses) are significantly different from those of single and twin pregnancies, including requirements for energy, protein, and minerals. Early prediction of the number of foetuses can allow for timely adjustment of management practices for goats pregnant with multiples. In this study, milk samples from 348 pregnant Alpine goats were collected and analysed for PAG concentrations during early (10–43 days), mid (49–78 days), and late (85–94 days) pregnancy. To evaluate the number of foetuses in Alpine goats during early pregnancy and to assist dairy farmers in corresponding reproductive management, receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity, specificity, and area under the curve (AUC) for different PAG concentration thresholds at various days of pregnancy. In this research, the fatal number of 1, 2, and 3 or more kids accounted for 21%, 71%, and 8% of total pregnant goats, respectively. The results showed that at 49 days of pregnancy, a milk PAG threshold of 1.208 for determining multiple pregnancies had a sensitivity of 100%, a specificity of 80.6%, and an AUC of 0.903; at 57 days of pregnancy, a milk PAG threshold of 2.643 resulted in a sensitivity and specificity of 100% with an AUC of 1. Moreover, the Youden index at 57 days was higher than at 49 days (1 vs. 0.806). We then used Canonical Discriminant Analysis (CDA) to evaluate the significant differences between the groups of multiple and single or twin pregnancies based on different milk PAG concentration thresholds and days of pregnancy. Starting from 49 days of pregnancy, the Wilk's Lambda value was 0.786, indicating a significant difference in milk PAG concentrations between goats with multiples and those with single or twin foetuses ($P<0.01$); at 57 days of pregnancy, the Wilk's Lambda value was 0.104, denoting a highly significant difference ($P<0.001$). It is concluded that 49 to 57 days of pregnancy are effective timepoints to use milk PAG concentrations to differentiate the number of foetuses in pregnant Alpine goats.

Keywords: Alpine goats, pregnancy-associated glycoprotein, foetal number. Presented at the ICAR Anual Conference 2024 in Bled at the Session 7: Breeding for agroecological transition in sheep and goats.

Introduction

Nutritional metabolism and foetal development requirements of goats carrying multiple foetuses (≥ 3 foetuses) are significantly different from those of single and twin pregnancies, including energy, protein, and mineral requirements, and the energy demand might gradually increase with gestational days (El-Sayed *et al*., 2022; Mongini and Van Saun, 2023). Insufficient energy intake may cause increased incidence of ketosis and toxaemia in ewes (Ji *et al*., 2023), so early determination of pregnancy numbers in ewes and providing appropriate care might reduce the incidence of disease (Dinç *et al*., 1994).

Pregnancy-associated glycoproteins (PAG) are members of the inactive aspartic proteinase family and secreted by binucleated cells in the ruminants placental nutritive layer (Singh *et al*., 2019a). Plasma caprine pregnancy-associated glycoprotein (caPAG) concentration started to increase at 22 days of gestation, reached the peak at 45 days of gestation, and rapidly declined after parturition, reaching baseline at 14 days postpartum (Singh *et al*., 2019a). Additionally, plasma PAG concentration in ewes carrying twins was significantly higher than those carrying singles at 28 days of gestation (Singh *et al*., 2019a), with concentrations in twins being 1.4-1.8 times higher than those in singles in the mid and late gestation (Singh *et al*., 2019a). As above, it is evidence that plasma PAG concentration is influenced by gestation and number of foetuses.

The objective of the experiment is to collect daily milk samples from Alpine goats after artificial insemination to measure PAG concentration in milk, determine the threshold for differentiating the number of foetuses by milk PAG concentration and confirm the optimal time point as a basis for foetus's number prediction.

Material and methods

Management of experimental animals and collection of milk sample

The data in this study were derived from 348 Alpine multiparous goats (3-5 pregnancies). Pregnancy detection was performed by ultrasound (ALOKA Prosound 2, Japan) between days 45 and 50 post-breeding. Pregnancy was confirmed based on the visualization of embryos, foetal membranes, and foetuses via ultrasound. Goats confirmed pregnant would advance to the next experiment, and the number of foetuses was recorded. The proportion of singles, twins and triplets are 21%, 71%, and 8%, respectively. Milk samples were collected from 10 days post-breeding and weekly until day 94 of pregnancy. Collected 1 mL of milk into sterile centrifuge tubes before milking and stored at 4°C before being sent to the milk testing laboratory of the Northern Branch of the Livestock Research Institute, Ministry of Agriculture. Samples were kept at room temperature before testing.

Analysis of PAG concentration in milk samples

The measurement of PAG concentration in milk samples was performed by commercially available antigen capture enzyme-linked immunosorbent assays (ELISA) (IDEXX Laboratories, Inc., Westbrook, Maine, USA). There are 2 wells for positive control (PC) and 2 wells for negative control (NC) in each 96-well plate. Incubated the 96-well plate in a 37°C oven with agitation for 2 hours, and measured the optical density (OD) of samples in 96-well plate at 450 nm and 630 nm wavelengths by a SpectraMax® Absorbance Reader CMax Plus (USA), and calculated the average values of PC and NC as PC mean and NC mean. The validity of the results was confirmed by the difference between PC mean and NC mean being more than 0.5 and NC mean being below 0.2. The PAG concentration in milk was calculated by subtraction of the NC mean with the OD value of the samples as PAG (sample-negative) value. A difference more than 0.25 (including 0.25) was considered as positive, indicating pregnancy.

Statistical analyses

For statistical analysis of milk PAG concentration in Alpine goats at different pregnancy days, receiver operating characteristic (ROC) analysis were used to establish a predictive model for multiple pregnancies. The area under the curve (AUC) was used to determine the optimal prediction time point for the highest accuracy in predicting multiple pregnancies in Alpine goats. The optimal cutoff point was determined based on the maximum value of the Youden index = sensitivity + specificity - 1. Subsequently, canonical discriminant analysis (CDA) coefficients were evaluated based on linear combinations of various variables to confirm the accuracy between different sampling time points (10, 33, 43, 49, 57, 65, 71, 78, 85, and 94 days of pregnancy). Wilk's Lambda value was to assess overall significance of multiple pregnancies on PAG concentration in Alpine goat milk at different pregnancy days. Wilk's Lambda value was ranging from 0 to 1. When Wilk's Lambda value is closer to 0, it can be considered that multifetal pregnancies have a significant influence on Alpine goat milk PAG concentration at different pregnancy days.

Results

Evaluation of the ROC curve on milk PAG concentration thresholds at different pregnancy days in Alpine goats

To confirm the number of pregnancies in Alpine goats in early gestation, this experiment collected milk samples from 348 pregnant Alpine goats during early pregnancy (10-43 days), mid-pregnancy (49-78 days), and late pregnancy (85-94 days). This experiment calculated the milk PAG concentration thresholds at different pregnancy days by ROC curve to verify the accuracy of multifetal pregnancies determination. Results showed that while the milk PAG concentration threshold is 1.208, the sensitivity is 100%, specificity is 80.6% and an AUC is 0.903 at 49 days of pregnancy. At 57 days of pregnancy, the milk PAG threshold is increase to 2.643, the sensitivity and specificity are both 1, and AUC is 1. Furthermore, using the Youden index as the criterion for determining the optimal cutoff timepoints. Results showed that Youden index values are of 0.29, 0.67, 0.806, and 0.806 at 10, 33, 43, and 49 days of pregnancy, respectively. The Youden index values were all 1 at 57, 65, 71, 78, 85, and 94 days of pregnancy. This indicated that the most effective timepoints to confirm multifetal pregnancies by milk PAG is between 49 and 57 days of pregnancy.

Table 1. Result of receiver operating characteristic curve (ROC curve) analysis [sensitivity (Se), specificity (Sp) and area under curve (AUC)] of milk pregnancy-associated glycoprotein (PAG) ELISA assessment for determination of triple foetuses based on different threshold values of circulating PAG concentration (S-N) at different days of gestation in Alpine goats.

Days of pregnancy	PAG threshold values	Se (%)	Sp (%)	AUC and P value	95% Confidence interval	
					Lower bound	Upper bound
10	0.024	100.0	29.0	0.452 (P = 0.779)	0.113	0.790
33	0.300	100.0	67.7	0.720 (P = 0.006)	0.563	0.878
43	0.446	100.0	80.6	0.828 (P < 0.001)	0.696	0.960
49	1.208	100.0	80.6	0.903 (P < 0.001)	0.785	1.000
57	2.643	100.0	100.0	1.000 (P < 0.001)	1.000	1.000
65	3.326	100.0	100.0	1.000 (P < 0.001)	1.000	1.000
71	3.421	100.0	100.0	1.000 (P < 0.001)	1.000	1.000
78	3.756	100.0	100.0	1.000 (P < 0.001)	1.000	1.000
85	2.126	100.0	100.0	1.000 (P < 0.001)	1.000	1.000
94	3.146	100.0	100.0	1.000 (P < 0.001)	1.000	1.000

Evaluation of CDA on milk PAG thresholds at different pregnancy days in Alpine goats to differentiate the number of foetuses

To assess the milk PAG concentration of multiple pregnancies (twins or triplets) versus single or twin pregnancies in Alpine goats at different gestational days, CDA was conducted. Results showed that on 49 days of pregnancy, the Wilk's Lambda value was 0.786, indicating a significant difference in milk PAG concentration between multiple pregnancies and single or twin pregnancies (*P* < 0.01). On 57 days of pregnancy, the Wilk's Lambda value was 0.104, indicating an extremely significant difference in milk PAG concentration between multiple pregnancies and single or twin pregnancies (*P* < 0.001). It demonstrated that from 49 to 57 days of pregnancy, milk PAG concentration can effectively differentiate between multiple pregnancies and single or twin pregnancies.

Table 2. Canonical discriminant analysis (CDA) results for discrimination of single or twin foetuses from triple foetuses with milk pregnancy-associated glycoprotein (PAG) enzyme-linked immunosorbent assay (ELISA) assessment at different days of pregnancy in Alpine goats

Canonical discriminant function coefficients		Days of pregnancy									
		10	33	43	49	57	65	71	78	85	94
Wilk's Lamba value		0.996	0.993	0.996	0.786^{**}	0.104^{***}	0.179^{***}	0.255^{***}	0.317^{***}	0.278^{***}	0.068^{***}
Unstandardized coefficients		5.648	4.110	2.276	2.369	4.213	2.689	2.198	1.826	3.469	4.802
Group centroids	Single or dual foetuses	0.020	-0.025	-0.020	-0.157	-0.885	-0.647	-0.515	-0.443	-0.486	-1.115
	Triple foetuses	-0.207	0.253	0.208	1.627	9.150	6.684	5.324	4.581	5.022	11.526
Eigenvalue		0.004	0.007	0.004	0.272	8.608	4.594	2.915	2.158	2.593	13.659
% of variance		100	100	100	100	100	100	100	100	100	100
Canonical correlations		0.066	0.081	0.067	0.463	0.947	0.906	0.863	0.827	0.850	0.965

[1] ** indicates a significant difference (P < 0.01).
[2] *** indicates a highly significant difference (P < 0.001).

Discussion

Goats carrying multiples requires precision feeding management to face the metabolic demands. If goats carrying multiples didn't intake sufficient energy to meet metabolic demands, lipid mobilization is an adaption, resulting in the increase of blood non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentration (2.1 and 3.7 times, respectively) than those carrying single pregnancies before delivery (Moallem *et al*., 2012). Excessive related metabolites could be transferred into ketones in the liver, increasing the incidence of pregnancy toxaemia in ewes (Ji *et al*., 2023). PAG has been studied as an indicator for early pregnancy diagnosis and even predicting the number of foetuses in ruminants (Hussein *et al*., 2017). Therefore, detecting the changes in milk PAG concentration during pregnancy might be a way to differentiate the number of foetuses, improve the nutritional management of ewes carrying multiples and reduce the incidence of disease.

In the experiments showed that at 10, 33, and 43 days of pregnancy, the sensitivity was 100%, but the specificity was only 29.0%, 67.7%, and 80.6%, respectively, with AUC of 0.452, 0.720, and 0.828, indicating that the accuracy of detecting the number of foetuses at 10, 33, and 43 days of pregnancy was low. At 49 days of pregnancy, with a milk PAG concentration threshold of 1.208, the sensitivity was 100%, specificity was 80.65%, and AUC was 0.903; while at 57 days of pregnancy, the milk PAG concentration threshold was 2.643, with both sensitivity and specificity at 100% and AUC at 1, suggesting that at 57 days of pregnancy, goats carrying multiples could be identified accurately. Yang *et al*. (2022) showed that milk PAG concentration in goats carrying multiples started increasing at 49 days of pregnancy compared to single or twin pregnancies. In the present study, the determination of milk PAG concentration at 49 days of pregnancy has showed a certain degree of accuracy (80.65%), in agreement with the results in Yang *et al*. (2022). Although the threshold in the present study was higher than Singh *et al*. (2019a)'s value of 0.830, it might attribute to the threshold in Singh *et al*. (2009a) based on PAG in blood and the differentiation between single and twin pregnancies. Furthermore, the target breed in Singh *et al*. (2019a) was Indian Barbari goats, whereas the present study was focused on Al-Bayda goats, contributing to differences in PAG concentrations (De Carolis *et al*., 2020). Additionally, we measured milk PAG concentration, with the threshold of 1.208 to differentiate between singles and twins or multiples. Some studies showed that PAG in blood was about 1.9-2.0 times higher than in milk (Singh *et al*., 2019b), indicating that blood PAG threshold of 0.830 divided by 2 was the milk threshold of about 0.415. The value (0.415) was close to the value of singles and twins in Yang *et al*. (2022), presuming that the number of foetuses might be a main factor of PAG concentration threshold. Moreover, it was noteworthy that the timepoints which Singh *et al*. (2019a) distinguished the number of foetuses was over 45 days of pregnancy. This result was consistent with Yang *et al*. (2022), pointing out PAG concentration in mid pregnancy was related to number of foetuses. Therefore, it could serve as evidence to determine the timepoints of the number of foetuses (49 to 57 days of pregnancy).

In the present study, Canonical Discriminant Analysis (CDA) was used to measure milk PAG concentration at different pregnancy days to distinguish between singles, twins, and multiples. Results showed that at 49 days of pregnancy, Wilk's Lambda value was 0.786, indicating a significant difference ($P < 0.01$) in milk PAG concentration between goats with single or twin pregnancies and with multiple pregnancies. At 57 days of pregnancy, Wilk's Lambda value decreased to 0.104, indicating an extremely significant difference ($P < 0.001$) in milk PAG concentration between goats with singles or twins and with multiples. This suggested that milk PAG concentration could start to differentiate the number of foetuses between 49 and 57 days of pregnancy. The analysis by Youden index to determine the optimal detection timepoints was also between 49 and 57 days of pregnancy. Also, other studies indicated that at 45 days of pregnancy, it was a significant difference in serum PAG concentration in singles, twins and triplets, with concentrations of 58.23 ± 2.64, 48.55 ± 2.86, and 34.77 ± 1.53 ng/ml, respectively.

These results suggested that serum PAG concentration can be used to differentiate the number of foetuses at 45 days of pregnancy (Hussein *et al*., 2017). Similarly, the difference of PAG concentration between the number of foetuses, especially in multiples, was also mentioned in Yang *et al*. (2022). However, Hussein *et al*. (2017) measured goats serum PAG concentration, while Yang *et al*. (2022) used milk samples from pregnant goats. Although the target substances in these two studies were different, there was a strong positive correlation ($R^2 = 0.64$) between blood and milk PAG concentrations (Singh *et al*., 2019a). Additionally, both blood and milk PAG concentrations showed high accuracy in determining pregnancy after 32 days of pregnancy, with accuracies 92% and 89%, respectively. Based on the studies, the timepoints for blood and milk PAG concentrations to determine the number of foetuses were quite close, indicating that milk PAG concentration can be used to determine the number of foetuses between 49 and 57 days of pregnancy.

Conclusion

According to the above, at 49 days of pregnancy, the milk PAG concentration could be used to detect for multiple pregnancies in goats. Additionally, at 57 days of pregnancy, milk PAG concentration could differentiate between single and multiple pregnancies in goats. Therefore, 49 to 57 days of pregnancy is the most ideal time for detecting milk PAG concentration and determining the number of foetuses in Alpine goats. These findings will enable farmers to confirm the number of foetuses in early pregnancy, apply for precise feeding and management and reduce the incidence of metabolic disorders in goats.

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The estimation of variance components for litter size in two Slovenian sheep breeds

G. Šen, M. Bizjak and M. Simčič

University of Ljubljana, Biotechnical Faculty, Department of Animal Science,
Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
Corresponding Author: Gregor.Sen@bf.uni-lj.si

Abstract

The aim of this study was to estimate variance components for ewe litter size in two autochthonous Slovenian sheep breeds, the Jezersko-Solčava sheep (JS) and the Improved Jezersko-Solčava sheep (JSR). Both breeds are fertile all year round and are mainly bred for lamb production. Litter size records were collected from the farms according to the breeding programs for 17,071 ewes with 79,387 lambings (40,172 - JS, 39,215 - JSR) in the period from 2007 to 2023. A pedigree file with 24,425 animals was created from the Central database for small ruminants in Slovenia. The fixed part of the model was analysed with the SAS statistical package using the MIXED procedure and included the breed effect (JS, JSR), ewe parity (from 2 to 10) and the year-season interaction (1, 2, 3, …, 68), while the lambing interval was included as a linear covariate. The variance components were estimated using the REML method implemented in the VCE-6 program. The random part of the model consisted of the additive genetic effect, the permanent environmental effect and the flock effect. JSR ewes had a significantly higher litter size (1.39 ± 0.01 lambs per litter) compared to JS ewes (1.26 ± 0.01 lambs per litter). Litter size was significantly the lowest in the second parity (1.22 ± 0.01 lambs per litter) and increased until the sixth parity (1.36 ± 0.01 lambs per litter). Thereafter, it gradually decreased until the tenth parity (1.33 ± 0.01 lambs per litter). Litter size increased with increasing lambing interval and was also affected by year-season interaction. The estimated heritability for litter size was 0.06. The effect of permanent environment explained 0.02 variability, while the flock effect explained 0.11 variability in litter size. As expected, the variance components including estimated heritability for litter size were relatively low. Nevertheless, it is expected that they could contribute to more effective selection in the future, and for this reason the estimated variance components will be used in predicting breeding values for ewe litter size from 2024 onwards.

Keywords: parity, lambing period, heritability, permanent environmental effect, flock effect.
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Introduction

Fertility traits are economically very important factors which affects the profitability of lamb production. Apart from lambing interval, the most important fertility trait is ewe's litter size. A higher litter size could increase the profit of farmer through a higher quantity of lamb meat produced per ewe. In Slovenia, the most widespread sheep breeds are two autochthonous breeds - the Jezersko-Solčava sheep (JS) and the Improved Jezersko-Solčava sheep (JSR). Both are bred mainly for lamb production and are fertile all year round. They are distinguished by several phenotypic traits as well as in some production traits such as litter size. Nevertheless, JSR sheep is the result of improving of JS sheep with the Romanov sheep in order to achieve better fertility and this goal was achieved (Cividini *et al*., 2024a; Cividini *et al*., 2024b). Still, no study about variance components for litter size in sheep breeding has been made in Slovenia.

The main objective of the study was to determine the main factors affected the litter size and to estimate variance components for litter size in two Slovenian sheep breeds, the Jezersko-Solčava sheep, and the Improved Jezersko-Solčava sheep.

Material and methods

Records were provided by the breeding programs of JS and JSR (Cividini *et al*., 2024a; Cividini *et al*., 2024b) for sheep collected from the year 2007 to the year 2023. Data about ewe's breed, flock, lambing date, parity, and litter size were acquired from the Central Database for Small Ruminants in Slovenia. Only ewes with lambing interval between 150 and 550 days were included in the analysis. Ewes with more than 10 parities were excluded. Due to a low number of quadruplets, they were counted to triplets. After the records control, 17,071 ewes with 79,387 lambings (40,172 JS, 39,215 JSR) were included in the analysis. A pedigree file with 24,425 animals was also created from the Central Database for Small Ruminants. The fixed part of the model was analysed with the SAS statistical package using the MIXED procedure (SAS Institute Inc., 2014). The model included breed (JS, JSR), parity (2, 3, 4, 5, 6, 7, 8, 9, 10), and the year-season interaction (1, 2, 3, …, 68), while the lambing interval was included as a linear covariate. The variance components were estimated using the REML method implemented in the VCE-6 program (Groeneveld *et al*., 2010). The random part of the model consisted of the additive genetic effect, the permanent environmental effect and the flock effect.

Results and discussion

Table 1 shows p-values for fixed effects included in the model for litter size. All effects (breed, parity, year-season interaction and lambing interval) significantly affected litter size ($P<0.001$)

The litter size of JS ewes and JSR ewes is presented in Table 2. JSR ewes had significantly higher litter size (1.39 ± 0.13 lambs per litter) than JS ewes (1.26 ± 0.12 lambs per litter). This was expected because JSR sheep is the result of improving JS sheep with the Romanov sheep in order to achieve better fertility.

Ewe's litter size by parity is shown in Figure 1. Litter size was significantly the lowest in ewes at the second parity (1.22 ± 0.01 lambs per litter) and increased until the sixth parity (1.36 ± 0.01 lambs per litter). Thereafter, it gradually decreased until the tenth parity (1.33 ± 0.01 lambs per litter).

Estimated variance components for litter size are presented in Table 3. Estimated heritability was 6%, while permanent environmental effect explained 2% of the variability. The flock effect explained 11% of variability in litter size, while 81% of phenotypic variance remained in the residual. Habtegiorgis *et al*. (2023) estimated genetic parameters for some growth and fertility traits in Dawuro sheep in Ethiopia. The estimate of direct heritability for litter size was 0.10, which is higher in comparison with the present study (0.06). The estimate for permanent environmental effect was 0.31, which is quite high compared to the present study (0.02). In the study of Hamman *et al*. (2004), permanent environment also explained a higher proportion of phenotypic variance (0.05) while estimated heritability (0.04) was slightly lower than in our study. However, Schmidova *et al*. (2014) estimated variance components for litter size in

Table 1. p-values of included effects in the statistical model for ewe's litter size.

	Effects			
	Breed	**Parity**	**Year-season interaction**	**Lambing interval**
Litter size	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. Litter size (LSM ± SE) by the sheep breed.

	Breed (LSM ± SE)	
	Jezersko-Solčava sheep	**Improved Jezersko-Solčava sheep**
Litter size	1.26 ± 0.12	1.39 ± 0.13

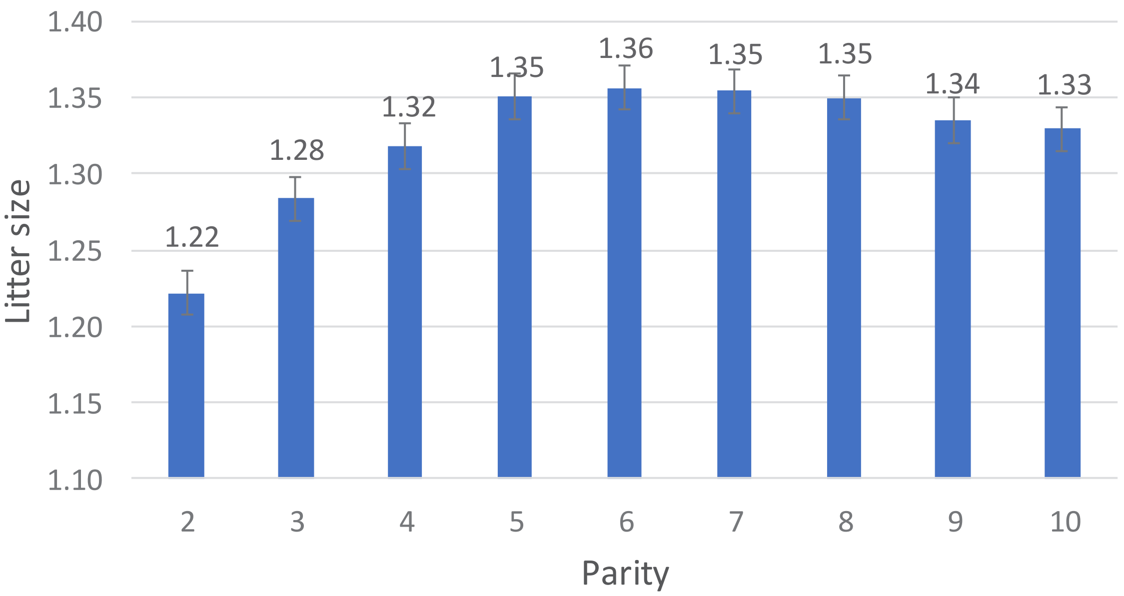

Figure 1. Ewe's litter size by parity.

Table 3. Estimated variance components ratios for litter size of ewes in two Slovenian sheep breeds.

	Variance components (ratios)			
	Additive genetic effect	**Permanent environment**	**Flock**	**Residual**
Litter size	0.06	0.02	0.11	0.81

seven sheep breeds and found that the heritability estimate in Šumava sheep and Romney sheep was 0.06 while the estimate for permanent environmental effect was 0.02 which is the same as in our study.

Conclusions

The variance components including estimated heritability for litter size were relatively low what was expected. Still, it is expected that they could contribute to more effective selection in the future, and for this reason, the estimated variance components will be used in predicting breeding values for ewe litter size from 2024 onwards.

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J. Conington[1], N. Lambe[1], F. Tortereau[2], F. McGovern[3], E. Navajas[4], I. De Barbieri[4], G. Ciappesoni[4], J. Jakobsen[5], E. Smith[6], J. Yates[6], M McCleod[1], Q. Le Graverand[2], K. McDermott[7], G. Steinheim[8], B. Aspeholen Aby[8], I. Dønnem[8], N. McHugh[3], L. Farrell[3], C. Marie-Etancelin[2], P. Johnson[10], D Brown[9] and S. Rowe[10]

[1]SRUC W Mains Rd, Edinburgh EH9 3JG, Scotland UK
[2]GenPhySE, Université de Toulouse, INRAE, Castanet Tolosan, France
[3]Teagasc, Animal and Grassland Research and Innovation Centre,
Athenry, H65 R718, Co. Galway, Ireland
[4]Instituto Nacional de Investigación Agropecuaria, INIA Las Brujas, 90100,
Rincón del Colorado, Canelones, Uruguay
[5]Norwegian Association of Sheep and Goat Breeders, Box 104, 1431, Ås, Norway
[6]The British Texel Sheep Society, Stoneleigh Park, Warwickshire, CV8 2LG;
[7]SheepIreland Highfield House, Shinagh, Bandon, Co. Cork, P72 X050; Ireland
[8]Norwegian University of Life Sciences, Universitetstunet 3, 1433 Ås, Norway;
[9]AGBU, UNE, Armidale, Australia,
[10]AgResearch, Invermay Agricultural Centre, Private Bag 50034,
Mosgiel 9053 New Zealand
Corresponding author joanne.conington@sruc.ac.uk

Abstract

Reduction of greenhouse gas (GHG) emissions, and particularly enteric methane (EM) emissions, from ruminant livestock is a global problem faced by all producers. In 2015 there were 1.18 billion sheep in the world emitting 158 Mt of carbon dioxide (CO_2) equivalents of EM, or 6% of the total EM emissions (FAO 2022). Animal breeding is a highly cost-effective strategy to achieve reductions in EM and has already been included in national plans and Nationally Determined Contributions (NDCS) to achieve mitigation targets in several countries (https://unfccc.int/NDCREG).

An international project (‘Grass To Gas’, 2019-2023) combined expertise and generated new knowledge towards the reduction of methane (CH_4), a potent GHG, from sheep. Proxy measurements were investigated including individual animal feed intake and N-alkane measures indoors and at pasture, rumen microbiome-generated data, nuclear magnetic resonance (NMR) spectroscopy with plasma and rumen samples, and rumen volume by Computer-Tomography (CT), amongst others, in native, improved and unimproved sheep breeds and with different feed and forage qualities. Direct measurements of CH_4, and CO_2 from ewes and lambs were compared using portable accumulation chambers (PACs), sheep and Greenfeed to enable animal ranking and first breeding values for GHG emissions for sheep. Heritabilities (with s.e.) for PAC raw CH_4 (g/day) were reported as being between 0.26(0.03) and 0.34(0.09) from 4 countries, with methane intensity CH_4 / (CH_4+CO_2) moles/day having lower values [0.21(0.03) to 0.29(0.06)]. Estimates for residual feed intake (RFI) were higher at between 0.37(0.08) to 0.45(0.08). As expected, genetic correlations between RFI and feed intake were moderate to strong (0.41(0.14) to 0.79(0.09), and low or close to zero with body weight or growth. Comparing low vs high RFI progeny of Romane sires showed that after 4 generations, the dry matter intake difference between RFI lines reached 123 g/day of concentrate and 80 g/day of forage in favour of the efficient line. A larger difference of 20% in feed intake was reported when comparing Australian Merino lambs contrasting in RFI.

The links between RFI, feed intake and CH_4 emissions are more complex and require further investigation. However, high CH_4 was associated with higher levels of feed intake with preliminary genetic correlations reported as being between 0.33(0.17) and 0.43(0.19) and those between CH_4 and metabolic body weight being 0.58(0.15) to 0.68(0.11). The use of routine CT scans enabled retrospective computation of rumen volume which has been shown to be moderately heritable. The genetic correlation between rumen volume and CH_4 is yet to be determined although larger reticulo-rumen volumes, as measured by CT scanning, were associated with increased methane emissions but not with RFI at the phenotypic level.

The rumen microbiota is a complex ecosystem, which include bacteria, archaea, protozoa, fungi and viruses, that provides ruminants with the ability of digesting fibres in plant cell walls into nutrients for the animals. The composition of the rumen microbiota did not enhance prediction accuracies over and above more conventional zootechnical predictors of feed efficiency for French sheep. In contrast in New Zealand, promising results were reported based on rumen microbial composition using high-throughput, restriction enzyme-reduced representation sequencing. The EBV accuracies for methane yield and RFI were comparable with those achieved only using the animals' genomic information.

Additional results emanating from the project demonstrated that incorporating methane emissions into national sheep breeding programmes is a cost-effective mitigation measure with significant abatement potential. Importantly, working internationally to share protocols, information, knowledge and experience benefits all countries, avoiding duplication and fostering shared responsibility to be part of the solution to a global problem. A new international project, 'Sustain Sheep' (2024-2027) is underway to advance and extend our understanding of some of the key issues highlighted in this paper.

Keywords: enteric methane, greenhouse gas, genetic parameters, genetic improvement, sheep.
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Introduction

Reduction of greenhouse gas (GHG) emissions, and particularly enteric methane (EM) emissions, from ruminant livestock is a global problem faced by all producers. Although large ruminants make up the majority of the enteric methane emissions from livestock, in 2015 there were 1.18 billion sheep in the world emitting 158 Mt of carbon dioxide (CO_2) equivalents of EM, or 6% of the total EM emissions (FAO 2022). Animal breeding is a highly cost-effective strategy to achieve reductions in EM and has already been included in national plans and Nationally Determined Contributions (NDCS) to achieve mitigation targets in several countries (https://unfccc.int/NDCREG). Animal breeding is a long-term strategy to make cumulative reductions in methane and GHG emissions, which are permanent and highly cost-effective, for meat (Lambe, 2022), wool (Navajas *et al*., 2022a) and potentially for dairy sheep.

An international project ('Grass To Gas', 2019-2023) combined expertise and generated new knowledge towards the reduction of methane (CH_4), a potent GHG, from sheep. The countries involved in this project all have established national sheep breeding programmes and prior to the onset of the project, were independently measuring some or all of the following: individual animal feed intake, RFI, GHG emissions (CO_2 and CH_4), N-alkane measures at pasture, rumen microbiome-generated data, NMR spectra and rumen volume by Computer-Tomography (CT), amongst others, in native, improved and unimproved sheep breeds and with different feed and forage qualities. The overall aim of the project was to bring together the collective expertise to validate existing protocols, benchmark, and enhance the measurement and analyses of these new phenotypes for their development and use in sheep breeding programmes. The project also aimed to determine the use of selective animal breeding as a tool for GHG abatement, considered in relation to other measures available with modelling applicable to one country (UK). Specifically, the objectives were,

1. To validate predictors of feed intake (FI), residual feed intake (RFI) and CH_4|emissions.
2. To compare indoor and outdoor FI and RFI.
3. To investigate the opportunity to use genetics and genomics to reduce CH_4 emissions by determining the genetic basis of new phenotypes including rumen microbiome.
4. To quantity the economic and environmental benefits of more feed-efficient and lower GHG-emitting sheep, and ensure that the relevance of the project from farm to international impact scale.

Material and methods

Feed intake, Feed efficiency

Residual feed intake (RFI) is a measure of feed efficiency defined by Koch *et al*. (1963) as the difference between actual and predicted dry matter feed intake, based on average daily gain and metabolic body weight. In France, the methodology used to collect feed intake and feed efficiency data in 951 male Romane lambs is documented in Tortereau *et al*., 2020, using an 8 week testing period per batch of between 92-149 lambs at around 70d of age and weighed at the start and end of the trail period. In Uruguayan Merino sheep, phenotypic records of 1138 animals with feed intake, and 1120 with RFI were recorded using Intergado® automated feeders as described by Amarilho-Silveira *et al*., 2022 and genetic parameters analysed according to the methodology by Marques *et al* 2024. In New Zealand maternal sheep, individual measures of feed intake, feeding behaviour (length and duration of eating events) CH_4 and CO_2 were generated on 986 growing maternal ewe lambs sourced from three pedigree recorded flocks, the methodology for which was reported by Johnson *et al*., 2022. Norway compared 40 Old Norwegian with Norwegian White dry adult ewes fed different silage qualities as described by Aby *et al*., 2023a. In Ireland, 242 individual feed intake measurements taken at grass from 242 Texel-cross lambs at grass was measured using the N-alkane technique as described by McGovern *et al*., 2021 and in the UK, 250 indoor- and outdoor- reared Texel-cross lambs were used to compare sire ranking of full-sib offspring reared in the 2 systems as described by Conington *et al*., 2022. More detail on the predictions of feed intake and methane emissions in sheep using different proxies has been provided in Le Graverand *et al*., 2024.

Methane measurements

Direct methane measurements were undertaken using different technologies. Portable accumulation chambers (PACs) were used in Norway, New Zealand and Ireland, using the protocols first developed in Australia (Goopy *et al*., 2011, applied in New Zealand by Jonker *et al*., 2018, and validated by O’ Connor *et al*., 2021. A PAC is an airtight chamber in which an animal is kept for less than one hour. Emissions of CO_2 and methane are estimated based on the difference in the concentration of gases when the animal is entering and leaving the PAC. Temperature and pressure are also measured, and with the body weight of the animal, are used to calculate the emission of these two gases in grams per day. In Uruguay, PACs to measure GHG emissions were used to measure 930 Merino as described by Marques *et al*., 2022; RFI and CH_4 have been measured at post-weaning in Corriedale, Dohne Merino, Merelin and Texel lambs since 2018. Animals evaluated in Uruguay belong to the Selection Nucleus (Australian Merino), Information Nucleus (Corriedale, Dohne Merino, Texel) and commercial stud-flocks (all breeds) and are strongly connected with populations in the genetic evaluation (performance recorded) (Navajas *et al*., 2022)In France, the C-lock® GreenFeed individual small animal measurement system was used to quantify daily CH_4 and CO_2 emissions from individual animals as described by Tortereau *et al*., 2023.

CT-derived rumen volume and other proxy traits

In the UK, and New Zealand, Computer Tomography (CT) is used routinely as part of national meat sheep breeding programmes to quantify, with almost perfect precision, the body composition of elite rams. Using additional data generated as part of that process, the rumen size was determined to estimate the correlations between reticulo-rumen size and CH_4 emissions, as documented by Lambe *et al*., 2022 and Hitchman *et al*., 2023. CT-measured rumen volumes were measured within the Grass to Gas project in the UK (from unselected Texel-crossbred lambs) and France (from Romane lambs divergently selected for RFI) and related to feed intake and efficiency measures. In Norway, rumen tissue volume was undertaken on samples collected post-mortem as described by Åby *et al*., 2023b.

Different traits were considered as proxy measurements for feed intake, feed efficiency or methane emissions depending on the study. Most of the proxy traits were recorded during feed intake trials, such as bodyweight or body composition. In Scotland, New Zealand and Norway, the number of feeding events per day were collected and tested as a possible proxy. In France and New Zealand, blood and rumen fluid samples were collected during (New Zealand) or at the end of the intake trial (France). DNA was extracted from blood samples, and genotyping was performed using the Illumina 50K SNP chip. Ruminal microbiota was assessed through meta-barcoding or meta-genomics in France (Le Graverand *et al*., 2023) and New Zealand (Hess *et al*., 2023), respectively. For meta-barcoding to determine the rumen microbiota composition, to predict feed intake, the details are provided in Le Graverand *et al*., 2023a. In New Zealand, metagenome profiles were generated using restriction enzyme-reduced representation sequencing (RE-RRS) (Hess *et al*., 2020).

Impact of breeding for reduced methane emissions

The use of animal breeding as a tool for GHG reduction in small ruminants has not received serious attention by some Governments as a tool for the estimation of the abatement potential, despite the unequivocal evidence of the contribution of animal breeding to improve animal efficiency. The contribution to the reduction in GHG emissions in 5 Irish sheep breeds reared in 4 flocks was summarised by McHugh *et al*., 2022 and the difference in high- vs low-genetic merit Irish flocks was reported by Farrell *et al*., (2022). Bioeconomic modelling to compare profitability of high- vs low-emitting sheep flocks was used in the study.

In the UK, animal breeding for efficiency and directly breeding to reduce CH_4 emissions were included in new marginal abatement cost curves (MACC) for sheep which was estimated in terms of the cost-effectiveness and abatement potential.

Results

McGovern *et al*., (2024) provides full details of summary statistics and the models used to analyse the phenotypic results from each participating country. Table 1 summarises the heritability estimates for traits used in the estimation of feed efficiency and GHG emissions. Full details of the genetic models to estimate these parameters can be seen in the respective publications.

The genetic correlations of RFI with other traits are summarised in Table 2. They confirm a strong association of RFI with feed intake, and low, or close to zero correlation with body weight and growth during the feed efficiency tests.

The comparison of progeny of high feed efficiency (low RFI) and low efficiency (high RFI) Romane sires, after one generation of selection showed that improving RFI represents 3% less concentrate per day at the same level of performance (Tortereau *et al*., 2020). After 4 generations of selection, these differences between the 2 RFI lines reach 123 g of concentrate and 80 g of forage in favour of the efficient line (Marie-Etancelin *et al*., 2023). A larger difference of 20% in feed intake was reported by De Barbieri *et al*. (2020), when comparing Australian Merino lambs of contrasting RFI. In Norway, Åby *et al*. (2023a) found differences in methane emissions between two Norwegian breeds which were strongly linked to feed intake. The breed differences were still present after adjusting for feed intake, which suggest that other factors such as rumen size and anatomy may influence methane emissions. In France, comparisons of RFI lines in term of GHG showed that the most efficient animals (with lower feed intake) produce significantly more CH_4 than the least efficient animals, whether in males fed mixed

Table 1. Heritability estimates (standard error) for residual feed intake (RFI), traits used for the estimation of RFI and individual methane (CH_4) and carbon dioxide (CO2) emissions.

Study	Johnson et al. (2022)	Hickey et al. (2022)[1]	Tortereau et al. (2020)	Marques et al. (2022)	Jakobsen et al. (2022)
Breed	NZ maternal	Several	Romane	Australian Merino	Norwegian White
RFI	0.42 (0.09)	--	0.45 (0.08)	0.37 (0.08)	--
Feed intake	0.35 (0.10)	--	0.28 (0.08)	0.41 (0.08)	--
Average daily gain	0.42 (0.10)	--	0.22 (0.07)	--	0.19(0.01)[5] 0.20(0.01)[6]
Metabolic body weight	0.44 (0.11)	--	--	--	--
Body weight	--	--	0.21 (0.07)[2]	0.41 (0.01)[3]	--
Backfat thickness by US	0.57 (0.09)	--	0.39 (0.08)	0.32 (0.04)	--
Muscle depth by US	--		0.41 (0.08)	0.39 (0.04)[4]	--
CH4 (g/day)	0.32 (0.08)	0.26 (0.03)	--	0.34 (0.09)	0.17(0.04)
CO2 (g/day)	0.32 (0.08)	--	--	--	--
CH4/(CH4 + CO2)	0.29 (0.06)	0.21 (0.03)	--	--	--

[1] Gas measurement by Respiration Chambers, the other studies used PACs.
[2] Includes body weight at the end of feed efficiency test;
[3] body weight at shearing;
[4] muscle area.
[5] Direct & maternal h^2 summed for 42d weight
[6] Direct & maternal h^2 summed for 140d weight.
[7] CH_4 g/hr

Table 2. Genetic correlation (S.E.) between RFI and performance traits recorded in feed efficiency tests.

Study/trait	**Johnson et al. (2022)**	**Tortereau et al. (2020)**	**Marques et al (2022)**
Feed intake	0.41 (0.14)	0.78 (0.08)	0.79 (0.09)
Metabolic body weight	-0.23 (0.17)	--	--
Body weight	--	-0.03 (0.19)[1]	-0.22 (0.14)[2]
Average daily gain	-0.09 (0.17)	-0.03 (0.19)	--
Ultrasonic Backfat thickness	-0.14 (0.15)	0.00 (0.16)	-0.17 (0.16)
Ultrasonic Muscle depth	--	-0.30 (0.16)	-0.15 (0.20)[3]

[1] Body weight at the end of feed efficiency test;
[2] Body weight at shearing;
[3] Muscle area.

Table 3. Genetic correlations (standard errors) of methane emissions (CH4), carbon dioxide (CO2), total greenhouse gas (CH$_4$+C0$_2$) and methane intensity CH$_4$/(CH$_4$ + CO$_2$) with RFI and traits recorded in feed efficiency test

	CH$_4$	**CO$_2$**	**CH4 + CO2**	**CH4/(CH4 + CO2)**
		Johnson et al. (2022)		
RFI	-0.28 (0.16)	0.05 (0.17)	0.04 (0.17)	-0.41 (0.15)
Feed intake	0.33 (0.17)	0.59 (0.14)	0.60 (0.14)	-0.24 (0.09)
Metabolic body weight	0.68 (0.11)	0.62 (0.13)	0.73 (0.08)	-0.06 (0.08)
Average daily gain	0.34 (0.10)	0.06 (0.19)	0.07 (0.19)	0.10 (0.16)
Backfat thickness by US	-0.04 (0.15)	-0.33 (0.15)	-0.32 (0.15)	0.06 (0.08)
		Marques et al., (2022)		
RFI	0.43 (0.19)	--	--	--
Feed intake	0.79 (0.09)	--	--	--
Body weight	0.58 (0.15)	--	--	--
ADG	--			
Ultrasonic backfat	0.37 (0.17)	--	--	--
Ultrasonic Muscle	0.36 (0.15)			

diet or in females on pasture (Tortereau *et al*., 2023). Table 3 confirms that methane production is closely associated with feed intake. Higher feed efficiency measured by RFI (i.e. low RFI) is associated with lower feed intake, which lead to the hypothesis that increased efficiency also means less methane production, although these results are not conclusive from our studies.

Proxy traits

Microbiome

Predictions of host feed efficiency from 16S (assessed as the average correlation between actual RFI and its prediction) ranged from 0.11 under a concentrate diet to 0.35 under a mixed diet, while predictions from fixed effects varied from 0.31 to 0.55, respectively (Le Graverand *et al*., 2023a). When young rams fed concentrate only, SNPs were the best predictors of RFI (accuracy of 0.44), but blood NMR spectra were also promising with an accuracy of 0.33 (Le Graverand *et al*., 2023b). In New Zealand, accuracy of methane and feed intake prediction was significantly improved by including a microbial relationship matrix in the linear mixed model alongside the genomic relationship matrix (Hess *et al*., 2023). Bilton *et al*., 2022 further showed that the genetic correlation between direct measures from PAC chambers and methane predicted from rumen microbial profiles was 0.76 (s.e. 0.12) and 0.64 (s.e. 0.11) for feed intake.

Computer Tomography

Rumen volume measurements, taken from CT scans in the UK and France, were significantly correlated with body weight, growth and feed intake, and in the UK also with age and muscle depth. However, no significant link between RFI and rumen volume was identified. Selecting lambs on RFI under a concentrate diet in the growing period did not seem to impact rumen volume later in life. These results suggest that rumen volume cannot be proposed as a proxy of feed efficiency. However, it might be tested in further analyses as a proxy of feed intake.

Predictions from other phenotypes

In general across all available data sets from the project, when investigating various proxy measurements (Le Graverand *et al*., 2024), validation prediction accuracies were higher for feed intake than for feed efficiency criteria (residual feed intake and feed conversion ratio). The best predictions for feed intake were obtained when body weight and the average number of feeding events per day were included in the models (R^2=0.78). Methane emissions were predicted with the highest accuracy when feed intake was considered among the proxies, alongside body weight, average daily live-weight gain and ultrasound measured body composition (R^2=0.34). Prediction accuracies for methane emissions obtained with metagenome were higher than with the sheep genome, although this accuracy remains quite low (r=0.32).

Economics and abatement potential

The results from the Irish study comparing high-vs low- genetic merit flocks for differences in profitability and predicted GHG emissions, indicated that the flock of high genetic merit was more profitable with a higher net profit of €18/ewe than the flock of low genetic merit (Farrell *et al*., 2022). Although the GHG emissions, assessed by life-cycle analysis (LCA) showed an increase of 2.9% in total emission (expressed as CO2eq), the emissions intensity (kg CO2eq/ kg carcass weight sold) was 6.9% lower for the flock with high genetic merit. In the UK,

the results of the marginal abatement cost curve (MACC) in terms of cost effectiveness (CE) and abatement potential (AP) are presented in Table 4. The maximum AP estimated for UK sheep was of 2.7Mt, which is equivalent to 27% reduction in emissions. Sheep breeding strategies explain almost 30% of this reduction.

Another example is the integration of sheep genetics for reducing GHG emissions in the context of a Regenerative Livestock Farming programme in Uruguay. Research and development for this initiative have been supported by national and international funding. As the industry captured the demands of high-value markets for high intrinsic quality wool produced in regenerative livestock farming systems, nowadays there are 19 farms included in this initiative (Blumetto *et al*., 2023), and the approach is expanding

Table 4. Cost effectiveness (CE) and abatement potential (AP) of mitigation measures applied to UK sheep systems, with interactions.

Name	Applied to	CE (£/tCO2e)	AP (ktCO2e)	AP as %
Bio N fixation in grasslands	Managed grass	-1034	250	2.5%
Optimising pH for grass growth	Managed grass	-31	278	2.8%
Breeding for improved productivity	All sheep	-10	504	5.0%
Breeding for lower CH4	All sheep	20	252	2.5%
Better health planning for sheep	All sheep	38	391	3.9%
3NOP	Non-grazing sheep	119	99	1.0%
3NOP	All sheep	158	925	9.2%
Total			2699	27%

to other industries. Modelling work carried out based on Life Cycle Analysis indicates a potential abatement of emission intensity associated with wool products, ranging from 6 to 20%, assuming the flock has a CH4 emission equivalent to the top 25% genetically superior (animals with lower emission) (Blumetto *et al*., 2023).

In 2023, GEPDs for RFI and CH4 emission were made available for an Australian Merino breed in Uruguay as research breeding values, being a first step before incorporating routinely these traits into the breeding program. These estimates were based on 1.200 lambs phenotyped for RFI and CH4 and 3000 genotyped animals.

In New Zealand, the cost of mitigating a tonne of CO_2 equivalent through breeding for reduced methane in the national scheme was estimated at less than NZ$2. Empirical evidence showed that a 0.5-1% per year reduction is possible for a moderate economic weighting on carbon demonstrating that breeding has major potential as a national mitigation strategy (Rowe *et al*., 2021). The authors also demonstrated use of methane emissions in a selection index for a 750 ewe research flock where methane emissions were being reduced by greater than 1% per year whilst maintaining genetic gain in the production index.

Discussion

Some viable proxy traits for predicting feed efficiency and methane emissions are emerging. Work continues to progress on rumen microbial profiling to predict methane emissions (Rowe *et al.* 2019; Hess *et al.* 2020), which has the potential to provide an accurate and relatively quick method to determine genetic merit for use in breeding programmes. Likewise, CT-measured rumen volumes are now being measured in greater numbers within research flocks and breeding programmes in various countries to relate to methane emissions at the genetic level. If this proxy trait proves to accurately predict methane at the genetic level, then a mechanism is already in place in some countries (e.g. UK, NZ, Ireland) to incorporate these alongside other CT traits in breeding programmes. However, direct measurement of methane emissions, using PAC technology, is emerging as the most promising route to large-scale measurement of emissions from sheep across different production systems. PACs are being used within research flocks (e.g. in Australia, NZ, Uruguay, Ireland, Norway), often with strong genetic links to national breeding programmes. In some cases (e.g. NZ, Ireland, Norway, UK), PACs are being taken to breeders' farms to measure methane from industry flocks participating in progeny testing or national breeding programmes. The GrassToGas team has produced standardised protocols for both PAC and feed efficiency measurements, which could help in collation of methane records from different countries via international collaborations. Measurement of more animals can help accelerate the contribution of sheep breeding to methane mitigation.

The next steps are to develop environmental selection indices, including methane emissions, for breeding programmes, once sufficient data are available. Mitigation targets can be expressed as absolute methane emissions or "methane intensity" (methane per kg product). Improving productivity and reducing production inefficiencies favourably impacts methane intensity, but may increase absolute emissions. However, direct genetic selection for low emitting animals reduces total methane emissions. One main project conclusion is that selection for lower methane emissions should be considered in the framework of a multi-trait selection index, allowing optimised genetic improvement across several important traits, even when some genetic associations are unfavourable.

One of the most successful outcomes for the project is truly independent validation of methods and protocols to monitor complex phenotypes associated with environmental impact. The consortium will continue working together under the newly-funded Green-Era-Hub 'Sustain Sheep' project, and hopefully into the future via the Global Methane Hub.

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Developing livestock breeding strategies for enteric methane mitigation in developing countries – the case of Latin America

E.A. Navajas[1], J.M. Alvarez[2], S. Munilla[3,4], G. Ciappesoni[1], V.E. Vega-Murillo[5], R.J.C. Cantet[4], R. Calderón-Chagoya[6], M. Montaño-Bermúdez[6] and A. Berndt[7]

[1]Instituto Nacional de Investigación Agropecuaria, INIA Las Brujas,
Ruta 48 km 10, 90200 Canelones, Uruguay
[2]Instituto Nacional de Tecnología Agropecuaria. Universidad Nacional de Río Negro - EEA Valle Inferior-UIISA Ruta nac. 3 km 971, camino 4, Idevi, Viedma 8500, Argentina
[3]Universidad de Buenos Aires, Facultad de Agronomía, 1417 Buenos Aires, Argentina
[4]Instituto de Investigaciones en Producción Animal,
INPA CONICET, Buenos Aires, Argentina
[5]Facultad de Medicina Veterinaria y Zootecnia,
Universidad Veracruzana, Veracruz, Ver, México
[6]Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal,
INIFAP, Querétaro, México
[7] Embrapa Southeast Livestock, São Carlos, SP, Brazil
Corresponding Author: enavajas@inia.org.uy

Abstract

Meat, dairy and wool industries are particularly important in Latin American (LA) countries given their significant contributions to national economies, generation of employment and rural development. Livestock industries are also relevant for ensuring food security in the region, and for the rest of the world attending the predicted increase in food demand of a growing global population. The LA region is responsible for 25% and 11% of the world beef and dairy production, respectively, with Argentina, Brazil, Mexico and Uruguay being among the major beef and dairy producing and exporting nations. Reducing enteric methane emissions (EME) is one of the challenges that the livestock industry faces in Latin America. Cattle and sheep are major sources of greenhouse gas (GHG) emissions in LA, particularly EME which represents from 11% (Mexico) to 42% (Uruguay) of the total GHG emissions in the considered countries. Animal breeding provides the opportunity to harmonise production growth and EME mitigations targets in the framework of the Paris Agreement and the Global Methane Pledge. The most important Bos taurus (Angus, Hereford, Holstein, Simmental, Charolais), Bos indicus (Nelore, Brahman and Guzerá) and composites (Brangus, Braford, Montana, Simbrah) breeds have genetic evaluation in place in Argentina, Brazil, Mexico and Uruguay, some of them established 30 years ago. Similarly, wool, dual purpose and meat sheep breeds are genetically evaluated in Argentina and Uruguay. Genetic evaluations systems include relevant traits related to production (reproduction, growth, carcass weight, milk production, fleece weight), and product quality (meat intramuscular fat, milk protein content, fibre diameter). Feed efficiency, assessed by residual feed intake (RFI), has been recently incorporated into beef cattle genetic evaluations in Argentina (Angus, Brangus, Braford), Brazil (Nellore, Guzera and Brahman) and Uruguay (Hereford). Genomic information has been integrated in many breeding programmes of cattle and sheep. This is particularly relevant for difficult to measure traits, such as RFI and EME, although expanding the reference population sizes for improving prediction accuracies remains a difficult task. EME phenotypes for breeding purposes are still scarce in the region. In 2021, Uruguay started recording

EME in Hereford, using GreenFeed units, during the RFI tests, and a similar approach will be implemented in Argentina in several breeds. An intensive phenotyping platform was developed in Uruguay for sheep, in which RFI and EME are measured. Based on data recorded with Portable Accumulation Chambers, the first genomic breeding values for EME have been published in Merino. The genetic evaluation system in these LA countries have been implemented based on associations between breed societies and academic institutions, including national research institutes and universities. These relevant and long-lasting collaborations between public and private sectors in Argentina, Brazil, Mexico, and Uruguay provide the basis for a coordinated regional programme for animal breeding strategies with the aim of mitigating EME and improving livestock performance. The critical step is implementing the phenotyping platform for EME for the main breeds. This implies improving EME recording in association with RFI and expanding it to grazing conditions. The first approach delivers key information to disentangle the links among feed intake, animal performance and EME. Data recorded in grazing animals allows investigating EME in the most relevant livestock production environment in the region, and potentially developing proxy measures for larger phenotyping carried out by breeders. Although feed intake, which is a main driver of EME, is a very difficult-to-measure trait, the information in the grazing system would provide valuable data for quantifying EME intensities and estimating EME factors. An integrated and collaborative approach among the mentioned countries would be able to provide breeding tools and information for breeders to contribute to current and future challenges, considering environmental, social, and economic sustainability.

Keywords: genomic selection, sustainability, greenhouse gas, residual feed intake, cattle, sheep.
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Introduction

Latin America (LA) is one of the main providers of meat and fibres of the world, comprising 30% and 6% of cattle and sheep stock in the world. The region has the largest reserve of land with agricultural potential determined by 16% of the world's agricultural land and 33% of its unused agricultural area. LA countries play a key role for food security not only in the region, but also for the rest of the world (OECD *et al*., 2023). Reinforcing the path towards a more sustainable livestock production is relevant for the region to face current and future challenges, given that food consumption is expected to increase by 1.4% per year in the next decade due to the population growth. Estimates indicate that for the next decade a global growth of beef production of 8%, with LA accounting for 33% of this growth and reinforcing its position as world primer exporter of agricultural products such as beef (OECD-FAO, 2022).

Most of the greenhouse gas (GHG) emissions in LA countries are from the agricultural sector accounting for 40% of total emissions (CO2 eq)(Cárdenas and Orozco, 2022). In particular, enteric methane emissions (EME) from ruminants explain between 6 and 43%, proportion explained by combined effect of the relevance of the livestock sector and relative magnitude of other economic sectors, such as the energy sector (Tedeschi *et al*., 2022). These figures highlight the significant impact of reducing EME on total GHG emissions and, at the same time the importance of implementing EME mitigations strategies in the livestock industry.

Addressing EME strategies in LA must take into account the economic, social and environmental relevance of livestock production, and drastic alternatives, such as reducing national flocks and herds to decrease total EME would have significant unfavourable implications in all dimensions defining sustainability. LA is integrated by developing countries where livestock production is economically very relevant and a key activity for rural development and source of employment globally in the livestock industry. Additionally, it is important to mention that livestock farming is also related to other characteristics associated with the environmental dimension of sustainability, as an important proportion of cattle and sheep are raised in grazing conditions, with an important proportion of natural grasslands (OECD-FAO, 2022). Carbon sequestration by soil and biodiversity conservation (i.e. Duarte-Guardia *et al*., 2024) are examples of aspects related to GHG mitigation and environmental sustainability in the livestock production systems.

Animal selection offers the opportunity to harmonise production growth and EME mitigations targets in the framework of the Paris Agreement and the Global Methane Pledge, subscribed by LA countries. This work focuses on four main beef and sheep producers and exporters countries in LA: Argentina, Brazil, Mexico and Uruguay. The genetic evaluations systems in place in these countries provide the basis for the assessment of the impact of animal breeding on EME and the integration of EME phenotyping. It will help investigate alternatives to accelerate genetic selection for lower EME while reconciling all sustainability dimensions. In this article we describe the contribution of these countries to the EME in the region and relevance as livestock producers, their current breeding programmes and how the integration of EME phenotyping can contribute to reduce EME and achieve mitigation targets.

Livestock production and GHG mitigation targets

Cattle and sheep stock in Argentina, Brasil, Mexico and Uruguay represent more than 75% and 65% of the total in LA for each species, respectively. Livestock production explains a proportion of the national gross domestic product (GDP) that varies between 28 to 43% (Arango *et al*., 2020), being among the major beef producing and exporting nations.. In all cases, the agricultural sector (agriculture, forestry and other land use sector in the national GHG inventories, AFOLU) is responsible for significant proportion of total GHG emissions, and EME represents a high proportion of the AFOLU GHG emissions (Ruden *et al*., 2023).

Global national mitigations targets have been defined in all countries, in accordance with the Paris Agreement, in terms of emission intensity and total emissions. For 2025, total reductions in GHG intensities of 25% and 50% have been proposed by Mexico and Uruguay, respectively. For 2030 specific mitigation targets in absolute terms for each GHG have been defined in Uruguay, as well as a specific goal of lowering by 35% the EME intensity in the cattle sector. Argentina committed to a reduction of total GHG emissions of 27,7%, without a reduction in livestock stocks, this implies lowering EME intensities by increasing efficiency. México committed to an unconditional reduction

Table 1. Livestock production and enteric methane emissions by country.

	Argentina	Brazil	Mexico	Uruguay
Economic characterization [1]				
Cattle numbers (million heads)	53.9	214	33.5	11.3
Area (million ha)	110	168	197	13.3
Contribution to national GDP (%)	3	6.8	1.6	6
Contribution to agricultural GDP (%)	38	30	43	28
GHG emissions profile [2,3]				
AFOLU contribution to total GHG (%)	45.0	48.5	19.0	75.4
Contribution of EME to AFOLU (%)	33.3	45.6	78.3	57.5

[1] Adapted from Arango et al. (2020);
[2] Adapted from Ruden et al. (2023);
[3] Adapted from MAyDS (2023)

of 25% of its GHG and Short-Lived Climate Pollutant (SLCP) emissions by 2030. This commitment implies a 22% reduction of GHGs and a 51% reduction of Black Carbon.

Genetic evaluations in cattle and sheep

Cattle and sheep national genetics evaluations have been in place South America since late-80´s (Mueller *et al*., 2016; Navajas and Baldi, 2016; Ravagnolo *et al*., 2023) and established in Mexico in the early 2000s (Ríos Utrera *et al*., 2021).The genetic improvement programs in both species have been implemented through collaborative development among academic institutions and breed societies. The main cattle and sheep breeds are generically evaluated, and as described in Table 2. The most important Bos taurus, Bos indicus and synthetic Taurus x Indicus breeds are considered in the genetic evaluations systems, as well as the most important wool, dual purpose and meat sheep breeds.

Main production traits in beef cattle and sheep genetic evaluation

A comprehensive set of traits directly related to reproductive performance, growth and carcass and meat quality are considered overall in beef cattle in the different countries, as reported by Navajas and Baldi (2016). More detailed description of specific traits can be found at FAGB (2023) for Argentina, Pampaplus (2023) and Embrapa-Geneplus (2024) for Brazil, Ríos Utrera *et al*. (2021) for Mexico, and Ravagnolo *et al*. (2023) for Uruguay. Across countries and beef cattle breeds, there is a prevalence of growth traits such as birth weight, weaning weight, milk production and final weight (recorded at 15/18 months of age).

A second group of traits considered in the genetic evaluation systems are indicator traits of carcass and meat quality composition, which are measured by ultrasound: eye muscle areas, subcutaneous fat depth and intramuscular fat. Regarding reproductive performance, scrotal circumference is the most common trait seen in the genetic evaluations, as well as mature cow weight and calving ease, although other specific reproductive traits are present in the genetic evaluations (gestation length, age at first

Table 2. Genetic evaluations: main beef cattle and sheep breeds by country..

Cattle and sheep breeds	Argentina	Brazil	Mexico	Uruguay
Cattle				
Angus	X	X	X	X
Hereford	X	X		X
Limousin	X		X	X
Brahman	X	X	X	
Nellore		X		
Simmental			X	
Simbrah			X	
Charolais			X	
Brangus	X	X	X	
Braford	X		X	X
Sheep				
Australian Merino	X			X
Dohne Merino	X			X
Corriedale	X			X
Texel	X			X

calving, etc). A similar situation is observed at sheep genetic evaluations in which wool production and quality are very relevant, in addition to growth and reproductive traits (Álvarez *et al*., 2014; Ravagnolo *et al*.,2023).

Inclusion of feed efficiency

Feed efficiency is a new trait included in the genetic evaluation of beef cattle. Residual feed intake (RFI), defined as the difference between actual and predicted feed intake (Koch *et al*., 1963), implies reducing feed intake without compromising animal performance. Consequently, improving feed efficiency by selecting for RFI is an appealing new breeding objective because it leads to improved net income by reducing feed costs without affecting economic income. At a production system or national level, improving RFI could also be interpreted as a contribution to optimise the use of limiting resources, such as land in pasture-based production systems (Navajas *et al*., 2022).

Feed efficiency tests to assess RFI are performed in the four countries. In Argentina, INTA has five RFI phenotyping stations placed in the most relevant livestock production areas and other private facilities are recording RFI (Pordomingo, 2022). A public-private partnership between INTA, the University of Buenos Aires, Breeders Associations and private organisations is responsible for the evaluation of bulls of many breeds, such as Angus, Brangus, Braford and Hereford. Because RFI recording recently started the number of evaluated animals is still limited, but rapidly increasing and the EBVs are being published by the breeder societies. Argentina also started to register RFI in Merino and Dohne Merino at INTA Chubut Experimental Station and another facility is planned in southern Patagonia, which will also include Corriedale.

Several initiatives are in place in Brazil for measuring RFI in Bos taurus and Bos indicus and composites. For example, Embrapa Beef Cattle Unit has had a continuous private-public partnership with the company Geneplus Consulting Ltd, giving rise to the Embrapa Geneplus Program that involves 10 beef cattle breeds. In Nellore, since 2021, EPDs for RFI have been released for all breeders. Vast research has been carried out in the Canchim breed by Embrapa Southeast Livestock, where RFI measurements take place in this breed. a. Furthermore, in the Embrapa Southern Livestock Unit, the beef cattle breeding and research team has collected RFI data in Angus, Brangus, Hereford, Bradford and Charolais breeds, an initiative implemented in partnership with the breeders associations.

Similarly, initial tests for RFI have been conducted in Mexico since 2019 in collaboration with the Mexican Simmental and Simbrah Cattle Association, using the GrowSafe system. In Uruguay, RFI is in the Hereford genetic evaluation based on the information recorded in the post-weaning feed efficiency tests of Hereford bulls and steers, carried out at the e Central de Prueba Kiyú of the Hereford Breeders Society of Uruguay (Pravia *et al*., 2022). More recently, RFI at finishing (feedlot) is also evaluated in steers immediately after the post-weaning test, although this trait is not included in the genetic evaluation (Navajas *et al*., 2022). In the case of sheep, Australian Merino has the first estimations of genetic merit for RFI, although RFI information is being collected at INIA Experimental Station La Magnolia where lambs of the main wool, dual purpose and meat sheep breeds of Uruguay have been measured post-weaning since 2018: Australian Merino, Corriedale, Dohne Merino, Merilin and Texel lambs. Animals evaluated here belong to Selection Nucleus (Australian Merino), Information Nucleus (Corriedale, Dohne Merino, Texel) and commercial stud-flocks (all breeds) and are strongly connected with populations in the genetic evaluation (performance recorded) (Navajas *et al*., 2022).

Implementation of genomic selection

In the last decade, several of the cattle breeding programs have implemented genomic selection. This represents an important step for accelerating genetic progress by enhancing prediction accuracies at an earlier age. Additionally, it facilitates the inclusion of hard-to-measure traits, such as feed efficiency and EME, which can be improved more effectively.

Brangus and Braford breeds in Argentina have recently implemented genomic evaluations in collaboration with the Animal Breeding Group of the University of Buenos Aires, based on the methodology by Cantet *et al*. (2022). Argentinian Angus also implemented the genomic evaluation a few years ago, which was carried out by INTA (Instituto Nacional de Tecnología Agropecuaria) (Curutchet *et al*., 2023). In the case of the Hereford breed in Argentina, as well as in Uruguay, genomic evaluation was available in the context of the Pan-American Hereford Evaluation that included genotypes of all countries in the reference population (Navajas and Baldi, 2016).

Embrapa has implemented genomic evaluations in several breeds in partnership with different organisations. For example, in association with ABCZ (Brazilian Zebu Breeders Association), Embrapa Geneplus Program is responsible for the biggest Nellore genomic evaluation in the world (13 million animals, 306k genotypes). In the south, Embrapa Southern Livestock in collaboration with three animal genetic improvement programs (Pampaplus, Promebo and Brangus+) has contributed to the implementation of genomic selection in Angus, Hereford, Braford and Brangus.

In Mexico, genomic data has been included in genetic evaluation in Simmental and Simbrah breeds. In 2016 the first genomic evaluation was made, with the support of genetic improvement researchers from the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), establishing a strategic alliance to develop the procedure for estimating direct genomic values in the Mexican Simmental and Simbrah cattle population. There is a reference population of 1,250 Simmental and Simbrah animals, selected on the basis of their marginal genetic contribution in the population and with EPDs for all traits included in the Genetic Evaluation, in particular for growth traits, frame score, scrotal circumference and stayability. On the other hand, activities have been carried out to integrate reference populations for feed efficiency and carcass characteristics measured by ultrasound. Once the appropriate number of animals with phenotypic information is available, genotypic characterisation will proceed with a high density microarray and the development of equations to predict genomic values for RFI, marbling, and ribeye area.

Genomic data has been included in the genetic evaluations in Angus and Hereford breeds in Uruguay that lead to the publication of genomic breeding values since 2016 and 2021, respectively (Ravagnolo *et al*., 2023). In the case of RFI, an initial binational reference population comprising 731 Uruguayan and 1168 Canadian Hereford bulls and steers was the basis for estimating genomic expected progeny differences (GEPD), which have been published since 2017. The assessment of prediction ability using two validation strategies concluded that it is possible to predict accurate and unbiased RFI GEPDs for non phenotyped selection candidates based on genomic prediction (Pravia *et al*., 2023).

Incorporation of methane emissions in genetic evaluations

Several studies confirm that EME is a heritable trait (cattle, range 0.10-0.45, Dressler *et al*. 2024); sheep, range 0.17-0.34, Jakobsen *et al*., 2022, Marques *et al*., 2022), confirming that selective breeding can support the reduction of EME. To make this possible, phenotyping EME is required in a large number of animals to be able to obtain accurate estimations of genetic correlations between EME and production traits. Having in mind that EME is a difficult-to-measure trait, genomic selection will have an important impact and a large reference population needs to be built. Recording EME is the first main challenge for implementing genetic selection for lowering EME. Although different methods to measure are available, all have advantages and limitations in terms of accuracy, ease of use and setting in which they can be used (Tedeschi *et al*., 2022). In LA, several of these methods are being used with a variety of purposes.

Respiration chambers are the gold standard method, and four units are available at INTA in Balcarce and Leales Experimental Stations (Argentina) and four at Embrapa Dairy Cattle (Brazil). Expertise regarding the use of SF6 is available in the region and the method has been used extensively for the evaluation of other mitigation interventions such as forage quality, finishing systems (grazing vs feedlot) and different pasture-based systems (Loza *et al*., 2024). However, for measuring EME in association with feed efficiency tests and expanding it for grazing conditions and massive data recording, GreenFeed and Sniffers are the preferred options for cattle and Portable Accumulation Chambers (PAC) for sheep.

Methane emission and feed efficiency

The first EME measurements using GreenFeed units in South America were at Embrapa Southeast Livestock. In 2014 and 2015, animals from different lineages of the Canchim breed were monitored for EME, both on pasture and in confinement, as well as in relation to feed efficiency in confinement (Méo-Filho *et al*., 2020). In Uruguay, EMEs started being recorded during the post-weaning RFI tests in Hereford using GreenFeed units, as well as during the finishing RFI evaluations. Similarly, new GreenFeed units have been installed in feed efficiency facilities at INTA for the evaluation of Angus, Hereford, Braford and Brangus located in several INTA experimental stations in different regions in the country (Anguil, Rafaela, Mercedes, Valle Inferior, Cesáreo Naredo).

The EMEs in Mexico have been measured using Sniffers, which operate based on the methodology developed by Garnsworthy *et al*. (2012), involving continuous analysis of gas concentrations using an infrared methane analyzer. This system utilises the Guardian NG - Infrared Gas Monitor to retrieve concentration data of gases second by second in the ambient environment. Before implementation, the equipment underwent calibration using methane gas at known concentrations, and were then installed in modified feeders to establish a closed environment, thereby mitigating external influences on measurements.

Incorporating EME measures to the feed efficiency test in both cattle and sheep provide very valuable information, particularly considering that animals recorded are part or linked to the populations in the genetic evaluations. In addition to the possibility of estimating genetic parameters for EME, the combination of feed intake and animal performance data allows to disentangle the associations between them and EME. This is very relevant for understanding the impact of genetic selection on the three EME metrics:

1. absolute EME measured in the animal,
2. EME intensity express emissions relative to livestock production (g/kg meat, milk or wool produced), and
3. EME yield, which is the ratio between absolute emissions and the feed intake expressed as dry matter intake (g/kgDMI) (Beauchemin *et al*., 2022).

Additionally, quantifying the role and impact of RFI as indirect selection criteria for reduced EME would be possible in different breeds.

Measuring methane emission in grazing conditions

EME in grazing conditions is a particularly important and challenging step that needs to be considered, given the relevance of pasture-based livestock farming in the region. In this context the use of GreenFeed units provides a feasible alternative for EME recording. Focusing these measurements on animals also linked to the genetic evaluations will be beneficial. Given the limited experience and the technical demands of continuous use of the equipment a first step would be to implement EME recording in experimental stations, before designing the expansion to commercial settings such as breeder and stud farms.

In Uruguay, two Hereford herds (INIA Glencoe and INIA Las Brujas) and the Central de Prueba Kiyú where feed efficiency and EME are measured in bulls and steers constitute the Hereford information nucleus. Sires selected by genetic merit for feed efficiency are used in both herds, which also provide steers for the feed efficiency test at finishing that are evaluated for carcass and meat quality traits. Growth and reproductive performance in grazing conditions are recorded in the herds. Therefore, the inclusion of EME recording in the herds will complement the information measured at the feed efficiency tests.

Other challenges

Beyond the expected significant impact of genetic selection on EME mitigation, it is a slow process. This emphasises the need of implementing not only phenotyping platforms for EME recording associated with genetic evaluations to accelerate genetic progress but also addressing other challenges to promote the adoption of genetic tools by breeders and encourage finance support by policy makers and funding bodies. Examples of these challenges include quantification of impact of genetic selection at farm and regional levels, developing linkages with GHG inventories to be able to report mitigation impact by animal breeding, reinforcing knowledge transfer programs and promoting breeders' engagement in this process and implementing direct or indirect economic incentives for farmers to select for this trait.

Genetic evaluations systems of the four countries considered here are carried out by or with the support of their national research institutes and universities, in strong partnership with the private sector. Additionally, national research institutes have direct links with national government agencies and regional forums and initiatives. This provides a valuable opportunity for the development of effective communication strategies with key stakeholders of the private and public sectors.

Conclusions

The LA region has genetic evaluations systems in place in the main livestock producers and exporters countries, with the capabilities to incorporate EME platforms that would enable accelerating genetic progress to reduce EME and achieve the GHG mitigation targets. An integrated and collaborative approach among Argentina, Brazil, Mexico and Uruguay will facilitate providing breeders and farmers with genetic tools for current and future challenges, considering environmental, social, and economic sustainability. In direct connection with policy makers and regional forums, effective communication strategies could be implemented to position animal breeding as a major contributor to improve livestock productivity and reduce EME in the region.

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Milk cell transcriptome opens a new dimension in the mammary gland biology research

M. Zorc and P. Dovc

University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
Corresponding Author: peter.dovc@bf.uni-lj.si

Introduction

The mammary gland is a highly regenerative organ that undergoes most of its development after birth (Inman *et al*. 2015). The cyclical phases of growth, differentiation, lactation and involution of the mammary gland are regulated by hormones and growth factors (Neville *et al*. 2002). A consequence of the complex function of the mammary gland and intense secretion of milk, which differs significantly among different species, is also the presence of somatic cells in milk. The main fractions of somatic cells in milk are epithelial cells, lymphocytes, polymorphonuclear neutrophils (PMN), and macrophages. The majority of exfoliated epithelial cells present in milk are viable and exhibit characteristics of fully differentiated alveolar cells (Boutinaud and Jammes 2002). The somatic cell count (SCC) in milk, widely used as a marker for udder health, only provides the cumulative number of somatic cells in milk, whereas the differential somatic cell count (DSCC) allows differentiation between two groups of cells: PMN and lymphocytes versus macrophages (Wall *et al*. 2018) and represents therefore a significant step forward in understanding the dynamics of the somatic cell population in the mammary gland during lactation and at infection. In cattle and sheep, the epithelial cell fraction represents only a relatively small part of somatic cells in milk, whereas, in porcine and goat milk, as well as in human milk, epithelial cells are the predominant cell type in milk (Boutinaud and Jammes 2002). In different organs, adult stem cells are present with their primary role of maintaining tissue homeostasis (Biteau *et al*. 2011). However, stem cells in the adult mammary gland serve both, development and homeostasis. Mammary stem cells (MaSCs) can self-renew and differentiate into different cell types during the mammary gland's developing cycles (Visvader and Stingl 2014). In 2006, mouse MaSCs were identified and isolated for the first time (Shackleton *et al*. 2006). Since then, plenty of strategies have been used to identify and characterize MaSC and to delineate the mammary epithelial hierarchy (Inman *et al.* 2015).

Considerable efforts have been made to find a noninvasive way to obtain biological material for molecular analyses of mammary gland cells. The comparison of five different sources of RNA (biopsies of the mammary gland tissue, laser microdissected mammary epithelial cells, milk somatic cells, milk fat globules and antibody-captured milk mammary epithelial cells) for analysis of the bovine mammary gland transcriptome, showed that isolation of a total RNA directly from somatic cells released into milk during lactation, is an effective alternative to mammary gland tissue biopsies and laser microdissection of mammary tissue (Canovas *et al*. 2014).

The organ-specific gene expression studies in the mammary gland were performed using expression microarrays a decade ago (Maningat *et al*. 2009) and allowed a comparative approach between species but were limited with the selection of genes on the chip. The next important step represented bulk RNA sequencing from mammary gland isolates (Medrano 2010). Sequencing of bulk RNA isolated from milk cells in three different lactation stages in Holstein cows revealed expression of more than 19,000 genes as a cumulative number of genes expressed in different cell types present in cow's milk. Regardless of the lactation stage, approximately 9,000 genes showed ubiquitous expression, however, genes encoding lactoproteins and enzymes in the lactose synthesis pathway showed higher expression in early lactation and the majority of genes in the fat metabolism pathway had high expression in transition and peak lactation (Wickramasinghe *et al*. 2012). Several recent studies examined the distinct gene expression profiles of different mammary epithelial cell lineages at the single-cell level in human and mice (Cristea and Polyak 2018). In mouse, the analysis revealed 11 luminal and four basal clusters (Bach *et al*. 2017). The main advantage of single-cell RNA sequencing over the bulk mammary RNA sequencing is that single-cell RNA sequencing provides a reliable information about gene expression differences among different cell types and allows reliable assignment of transcripts to different cell types. The single-cell RNA sequencing opens a new horizon for documentation of cell type specific expression profiles in the mammary gland and even the possibility to determine different cell types based on cell type specific transcriptomic profile (Nguyen *et al*. 2018). In four studies a complete murine (Han *et al*. 2018; Schaum *et al*. 2018) and bovine (Becker *et al*. 2021; Zorc *et al*. 2024) mammary gland cell population was sequenced at the single-cell level, revealing a number of distinct cell types which exceeds the initially expected number. This approach also allows the identification of cellular sources for several milk components, which did not have defined origin before (Dallas *et al*. 2015).

The amount of data in a typical single-cell sequencing experiment is much larger than in bulk RNA sequencing experiments. The increased amount of data represents a computational challenge and an opportunity to apply advanced approaches such as machine learning. Machine learning concepts are applied in computational pipelines for scRNA-seq data analyses (Hwang *et al*. 2018). From a mathematical point of view, identification of cell-populations in scRNA-Seq data is unsupervised clustering, a problem widely studied in the field of machine learning (Andrews and Hemberg 2018). Dimension reduction is needed before clustering because scRNA-Seq data is high-dimensional (~104 dimensions for mammalian samples) and suffers from the curse of dimensionality (Wagner *et al*. 2016). Methods used for dimension reduction are either Principal Component Analysis (PCA), t-distributed Stochastic Neighbour Embedding (tSNE) or diffusion maps (DM).

Here we report the application of scRNA-seq to elucidate the cell type repertoire in bovine milk based on the transcriptomic differences among different cell clusters. Milk contains mammary epithelial cells and immune system cells (lymphocyte, macrophage, neutrophils), which reflect the activity of the mammary gland and illustrate the response of the mammary gland to environmental challenges.

Material and methods

Milk samples were collected from two healthy Holstein cows (less than 50.000 SSC) in mid lactation. The cells were pelleted and washed in cold PBS. Single-cell library was generated using 10X Genomics technology and Chromium Single Cell 3' Reagent Kit. Library samples were diluted to a concentration of 10 nM and loaded onto NovaSeq 6000 (Illumina) instrument. Sample demultiplexing, barcode processing, read alignment to the bovine reference genome (ARS-UCD1.2.108), quantification and initial quality control of the paired-end sequencing data were performed for each sample using Cell Ranger software (version 7.1.0, 10X Genomics). Genes expressed in less than three cells were removed from the gene expression matrix.

We applied "anchor-based" Seurat's workflow (Stuart *et al.* 2018) to integrate two datasets. After filtering, we log-normalized the raw counts with LogNormalize and identified highly variable genes with FindVariableFeatures for each batch at default settings. We then ran FindIntegrationAnchors with dims = 1:30. The resulting anchors were used for IntegrateData with the 30 dimensions. The most variable genes based on their expression in the entire population were determined using the FindVariableGenes function with default parameters (selection.method = ”vst”, nfeatures = 2000). Clusters were identified using the FindClusters function with a resolution of 0.8 and then visualized using the RunTSN and RunUMAP functions (reduction = “pca”). For fully automated cell-type identification we used ScType with ScType’s marker database (https://www.nature.com/articles/s41467-022-28803-w).

Results

A total of ~361M reads were obtained with 36,315 mean reads per cell for the first cow and ~257M reads with 17,459 mean reads per cell for the second. The efficiency of read mapping was between 94.1 and 96.6%. In total, 15,630 and 16,497 genes were identified. We performed an anchor-based integration analysis to explore all cells in both samples simultaneously. After UMAP reduction, a clear cell clustering highlighting 22 distinct cell populations was obtained (Figure 1).

The identified clusters can be grouped into two larger categories, including immune and epithelial cells. We identified classical and intermediate monocytes, naïve, effector and memory CD8+ T cells, naïve and effector CD4+ T cells, ISG expression immune cells, neutrophils, macrophages, mast cells, platelets, neutrophils, naïve B cells, progenitor cells, dendritic cells, luminal, ductal and alveolar cells. Identification of milk producing cells was based on expression of casein (CSN1S1, CSN1S2, CSN2, CSN3) and whey protein (PAEP, LALBA) genes. The cell cluster with significantly higher levels of caseins and whey proteins was annotated as alveolar cell cluster. The expression of casein (CSN1S1, CSN1S2, CSN2, CSN3), whey protein (PAEP, LALBA), MUC15 and BTNA1 genes allows identification of cell-type specific profiles indicating differences among bovine somatic milk cell clusters.

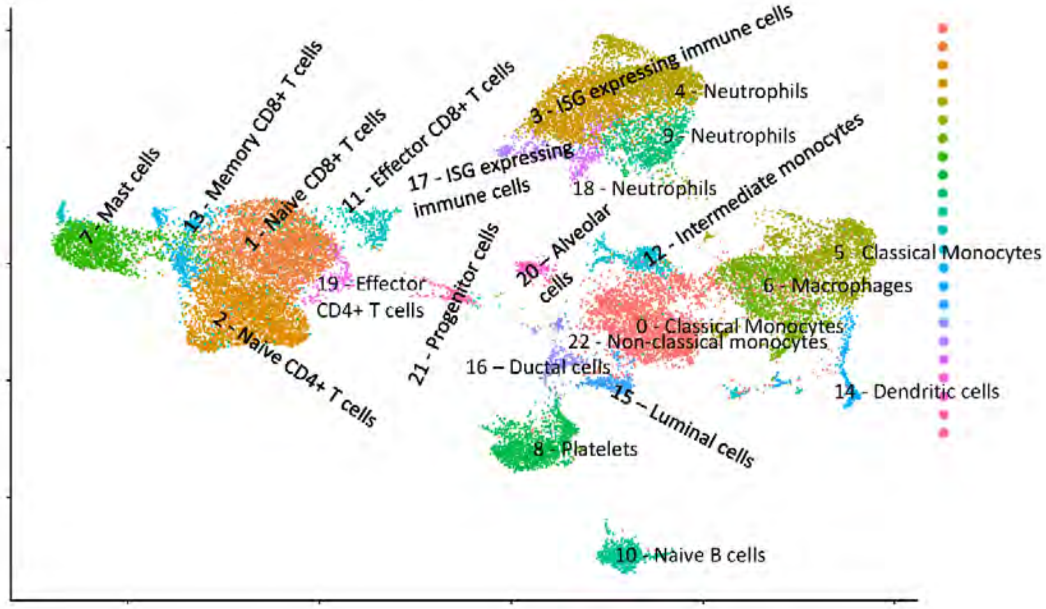

Figure 1. Cell type clusters in bovine milk somatic cells. Cluster 20 (alveolar cells) represents a typical milk producing cluster.

Discussion

Traditionally, somatic cells in the milk are expected to belong to myo/epithelial mammary gland cells, different types of immune cells (lymphocytes, neutrophils, macrophages) and stromal cells (Wickramasinghe *et al*. 2011). However, since precise markers for sub differentiation of cell types in the mammary gland are not present in all mammalian species (agricultural species are there not very well covered), the number of different cell types in the somatic cell fraction was normally underestimated. The analysis of bulk RNA transcripts from milk somatic cells revealed a very wide range of expressed genes and consequently indicated a wider range of cell-types in the milk somatic cell fraction. Single-cell sequencing of human and mouse mammary somatic cells revealed a much wider range of cell types, which are present in the milk (Nguyen *et al.* 2018; Schaum *et al.* 2018). Our single-cell RNA sequencing analysis of bovine milk has unveiled a cellular landscape of bovine milk somatic cells, highlighting a rich diversity of cell types pivotal for lactation, immune response and tissue homeostasis. Similar to the findings of Becker *et al*. (2021) (Becker *et al.* 2021), our study confirms the mammary gland's complexity, revealing a broad array of immune and epithelial cells.

A recent analysis of single-cell transcriptomes in mice has revealed important differences in gene expression between different cell types, which can significantly vary during the development of the mammary gland as well as in the course of lactation (Giraddi *et al*. 2018). The identification of a considerably higher number of cell-types in the milk somatic cell fraction compared to the traditional expectation opens a new horizon for more complex interpretation of the biological processes in the mammary gland.

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Unlocking genetic potential: The national genotyping program for Ireland's cattle herd

M. Waters, K. O'Connell, K. Downing, K. McDermott, C. Vigors, T. Browne, E. Fitzgerald, D. Matthews and R. O'Kelly

ICBF, Link Rd, Ballincollig, Cork, P31 D452, Ireland.
Corresponding Author: mwaters@icbf.com

Abstract

The National Genotyping Programme (NGP), a collaborative initiative between ICBF, the Department of Agriculture, Food and the Marine (DAFM), industry stakeholders and participating farmers, is the first step in achieving a fully genotyped national bovine herd in Ireland. The NGP operates under a co-funded model, sharing the cost across Irish Government, Industry Stakeholders and direct farmer contributions from the more than 10,000 participating herds. Phase one of NGP saw over 780,000 animals genotyped in 2023, including each participant's mature breeding herd. This was achieved, at no cost to the farmer, using Irish Government funding. Phase two, which began in January 2024, sees participating herds submit genotype samples at birth for all calves born in the herd, through the DNA Calf Registration Process, for the remainder of the programme (2024 to 2027 incl.). The cost of genotyping each calf in the programme is shared equally, 3 ways, between the farmer, DAFM and Industry, with each contributing approximately €6/calf. Since January 2024, over 660,000 registrations have been processed, with an average lab turnaround of 4.5 days, calves fully registered by an average of 12 days old, and with over 98,000 samples processed in a single week during the spring peak.

Keywords: National Genotyping Programme, Genotyping, Genomics, Calf Registration, Parentage.
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Introduction

Agriculture is the Republic of Ireland's single largest source of emissions, representing 34.3% of total national GHG emissions. 62.5% of agricultural emissions in Ireland are due to enteric fermentation. Manure management constitutes a further 11% of the agricultural emissions profile, meaning that combined, livestock are responsible for approx. 74% of emissions in the sector. The Irish government has committed to achieving climate neutrality across all sectors no later than 2050 with a 51% reduction in GHG emissions targeted by 2030 (Government of Ireland, 2023).

Genetic gain, particularly in this context of lowering Ireland's agricultural Green House Gas (GHG) emissions, is a key strategic goal of both industry and government (Department of Agriculture, Food and the Marine, 2020). Animal breeding, improved animal health and reduced age at slaughter all directly and indirectly reduce GHG emissions, while proving cost negative, which equals a significant "win win" in terms of GHG mitigation measures (Teagasc, 2023).

Genotyping the entire national bovine herd will underpin and accelerate the rate of genetic gain by leveraging genomic information and technologies against the existing integrated national database in ICBF to increase the accuracy of Ireland's national breeding indexes (EBI, Beef-Eurostar, DBI) and provide further tools to improve the national breeding programme. The advancement in the scale and accuracy of genomic selection, alongside numerous auxiliary benefits, such as enhanced traceability of beef and dairy products, labour saving, reduced administration, prevention of cattle theft etc, provided by NGP, offers Irish agriculture a profound opportunity which is estimated to provide up to a 4:1 return on investment (Abacus bio, 2022).

Laying the foundations for full National Genotyping

As of the mid 2023, ICBF, Ireland's integrated national cattle breeding database, had collected over 3 million genotypes. Large scale genotyping had already been achieved in the Irish suckler herd through government schemes such as the Beef Data and Genomics Programme (BDGP), and the Suckler Carbon Efficiency Programme (SCEP).

Up to 400,000 genotypes have been submitted each year as part of these programs since 2015 leading to approximately 60% of the national suckler herd being genotyped by 2023.

Lacking equivalent external investment, the dairy herd has lagged behind in terms of genotyping, with only approximately 6.5% of the national herd genotyped by same juncture.

DNA calf registration pilots

Proof of concept trials were carried out from spring 2018 to spring 2023 on the process of DNA calf registration, a system designed to integrate the genotyping and parentage verification process into the national calf registration system. The system consists of a genotype sample being submitted for each calf being registered, prior to the full registration of the calf on the national bovine traceability database, identifying and correcting errors in the recorded parentage, sex and breed of these calves in the process.

This system has the two major advantages of (i) reducing the administrative burden of subsequently correcting parentage errors, and (ii) allowing the calf to receive a full genomic evaluation at the earliest possible opportunity.

Over this period the scale of the pilots increased from an initial 18 herds to almost 600, with the number of calves genotyped in each pilot increasing accordingly from just over 1,500 calves in 2018, to almost 50,000 in 2023.

The process was also, refined and improved over the course of these trials, with infrastructure developed which would be fundamental to the future scalability of the system.

Data integration was improved between ICBF and both the Department of Agriculture, Food and the Marine (DAFM), and the farm software providers authorized to submit calf registrations. The turnaround time to process samples was reduced, and a new double tissue national calf tag was developed by tag suppliers capable of taking both a BVD sample and a DNA sample from a single set of national calf tags.

The National Genotyping Programme

Following on from the success of the DNA Calf Registration pilots, The National Genotyping Programme (NGP) was launched in June of 2023. A collaborative initiative, between ICBF, DAFM, beef and dairy industry stakeholders and participating farmers, the NGP is the first major step in achieving a fully genotyped national bovine herd in Ireland. The NGP operates under a co-funded model, sharing the cost across Irish Government, Dairy Industry Ireland (D.I.I.), Meat Industry Ireland (M.I.I.) and direct farmer contributions. The NGP consists of two main phases.

Phase one, 2023

Phase one of the NGP began in June of 2023 with a drive to recruit herds which lead to over 10,600 herds signing up to participate, including ~3,800 dairy herds and ~6,800 beef herds, accounting for almost 700,000 cows in total. The first phase also saw over 780,000 animals genotyped in 2023, including each participant's mature cow herd, breeding heifer replacements and expected calf sires which had not already been genotyped. This was achieved, at no cost to the farmer, using Irish Government funding. This This influx of genotypes brought the total number of genotypes in the ICBF database over 4 million and led to the largest single publication of new genomic evaluations to date, with some 620,000 females receiving genomic evaluations for the first time in January evaluation run of 2024.

Phase two, 2024 to 2027

The second phase, which began in January 2024, has seen participating herds submit genotype samples at birth for all calves born in the herd through the DNA calf registration process, which will continue for the remainder of the programme (2024 to 2027 incl.). The cost of genotyping each calf in the programme is shared equally, 3 ways, between the farmer, DAFM and industry, with each contributing approximately €6/calf. Continued process improvements, along with meticulous planning and coordination among the various stakeholders, has allowed the process to admirably handle the over 660,000registrations since January 2024, which equates to a third of all the calves registered in Ireland in the same period. This was achieved while processing samples in an average lab turnaround of 4.5 days, fully registering calves by an average of 12 days old, and with over 98,000 samples processed in a single week during the spring peak.

Correcting parentage, sex and breed errors

Since this phase of the programme began in January 2024, across all herd types, parentage errors have been identified in an average of 16.49% of cases. Errors in the recorded sex were identified and corrected in 1.69% of cases. In the 9.65% of calves submitted for registration without sire details, a sire was identified by the genotype and added in 81.26% of cases.

Genotyping and evaluation infrastructure

This level of large scale genotyping is achieved on the ICBF custom International Dairy and Beef SNP Chip (V5), a ThermoFisher Applied Biosystems™ Axiom™ Genotyping array, combined with the ThermoFisher Axiom Propel Express 384HT Workflow. In order to accommodate the volume of genotypes included in weekly genomic evaluations, significant changes were made to the imputation pipeline by limiting imputations to only new genotype samples or those belonging to animals that had parentage changes since the last imputation, and by adding an additional 4 new

High Performance Computing Nodes, each with 64 cores and an additional 3TB of RAM, which drastically reduced the run time of the imputation process and allowed for more evaluations to be run concurrently.

Table 1. Number of participating herds in DNA calf registration pilots and the corresponding number of calves genotyped at birth.

Pilot year	DNA Calf Registration Pilots Number of participating herds	 Number of calves genotyped at birth
2018	18	1,537
2019	35	1,652
2020	269	21,141
2021	401	33,404
2022	560	40,201
2023	594	43,676

Conclusion

Large scale genotyping and the DNA calf registration process offer many technical and logistical challenges. The operational aspects of the programme are multifaceted, involving the collection, processing, and analysis of huge amounts of genetic data and the seamless integration and flow of data across multiple databases and stakeholders. Practical innovations were also required at farm level such as the development of calf identification tags capable of taking multiple different tissue samples, while still conforming to all the existing statutory requirements around identification and traceability. With such a complex and interconnected project, absolute commitment and cooperation is required from all stakeholders involved including; farmers, government, industry, genotyping labs, tag suppliers, farm software providers, Animal Health Ireland and farm advisory bodies such as Teagasc, to ensure success at such scale and within such time sensitive timelines.

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Opportunities and obstacles of the use of genomic data in sheep breeding; large versus small populations

M. Špehar[1], J.M. Astruc[2], J. Ramljak[3] and A. Kasap[3]

[1]Croatian Agency for Agriculture and Food, Svetošimunska cesta 25, 1
0000 Zagreb, Croatia
[2]IDELE, Castanet-Tolosan, France
[3]University of Zagreb, Faculty of Agriculture, Department of Animal Science and
Technology, Svetošimunska 25, 10000 Zagreb, Croatia
Corresponding Author: marija.spehar@hapih.hr

Abstract

The objective of this paper is to draw the benefit and obstacles of using genomic data in sheep breeding through two contrasting situations: Croatia and France. Istrian (IS) and Pag (PS) sheep are Croatian native breeds undergoing selection on dairy traits (milk, fat, and protein) based on traditional pedigree BLUP. Genetic improvement of dairy performance via selection is important for their long term productive and economic viability, while maintaining the existing genetic variability is vital for their resilience in unpredictable future environment. Different SNP arrays have been used in many novel breeding programmes for the purpose of selection and conservation, and inclusion of genomic information in existing genetic evaluation of IS and PS seems to be promising and beneficial from both, the selection and conservation perspectives. The intention of the stakeholders involved in genetic evaluation of these breeds is to implement the basic principles of genomic optimum contribution selection (OCS) in existing breeding program in order to provide selection progress on targeted trait/s with minimal loss of genetic variability (ultimate goal of the OPTI-SHEEP project, CSF, IP: 2019-04-3559). So far, almost the whole breeding population of IS and PS (altogether ~4,000 animals) was genotyped using the Illumina OvineSNP50K BeadChip®. Transition from pedigree based BLUP to single-step GBLUP and development of pipelines for routine genomic OCS are still in progress. However, prior to major financial investments in genotyping and picking up the best strategy for long-term OCS, many practical and scientific questions need to be answered. Since the benefits of genomic selection heavily depend on population specific genetic parameters such as linkage disequilibrium, genetic connectedness between flocks, heritability of the traits, effective population size, etc., their estimation highlighted the potential benefits of genomic selection and some weaknesses that need to be worked on to maximize the benefits from this promising strategy. All these efforts will definitely impact long-term sustainability of Croatian sheep breeds which will benefit the breeders, consumers and corresponding (dairy) industries. By showing how to optimize selection with maintenance of genetic diversity, the results could also be beneficial to other sheep and livestock breeding programs. In France, all dairy sheep breeds have switched to genomic selection, with large reference population of rams for artificial insemination (Corse breed, n=0.4 K; Red – Faced Manech, n=3.5 K, Black – Faced Manech, n=0.7 K; Basco – Béarnaise, n= 1.2 K Lacaune breed, n= 7.4 K).

Each year, more than 8,000 new animals are genotyped with more and more affordable medium density SNP arrays (including all candidate rams and also some females in flocks with no AI nor control mating). In addition to single-step genomic evaluation, SNP markers are used for prediction of monogenic traits such as resistance to scrapie, and for parentage verification and discovery. The extra genetic gain achieved through the genomic program has already permitted to include novel traits, such as resistance to parasites, and will hopefully enable the inclusion of novel efficiency and resilience traits in the near future. As the French sheep populations are large, it is not feasible to genotype the whole female population, unlike the Croatian situation. In this respect, and through the comparison between those populations (large vs small populations, ancient vs recent breeding programs), we highlight similarities and differences in terms of opportunities and obstacles in the use of genomic data.

We also assess how an initiative to establish an EU Reference Centre (EURC) for performance testing and genetic evaluation in small ruminants could offer opportunities to better valorise genomic data, including across country evaluation, in more sheep breeds.

Keywords: genomic selection, genotyping, sheep, genetic variability,
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Introduction

In traditional breeding methods animals are selected as parents for the next generations using breeding values estimated based on phenotypic information and genetic relationships (pedigree-based BLUP). In the last decade, genomic selection (GS) has been implemented in animal breeding by 'upgrading' traditional breeding methods with genomic information, i.e. genotypes of thousands of single-nucleotide polymorphisms (SNPs) covering the whole genome. Dairy breeding programs were the first to adopt GS showing many benefits (Schaeffer, 2006). In small dairy ruminants, implementation of GS is quite challenging compared to dairy cattle due to incomplete data, lack of large and excellent reference population, higher genetic diversity between and within sheep breeds, the cost of maintaining reference populations, constraints of fresh semen for artificial insemination (AI), and the higher costs of genotyping compared to the value of the animal (Van der Werf *et al.*, 2014). Despite these obstacles, genomic evaluation methods can increase the accuracy of estimation compared to traditional pedigree-based BLUP as reported in numerous dairy-oriented sheep breeding programs (Legarra *et al.*, 2014, Baloche *et al.*, 2014). A good strategy in designing reference populations in combination with efficient genotyping strategies gives possibility for well-designed breeding programs to apply cost effective GS. The benefits of GS heavily depend on population specific genetic parameters such as linkage disequilibrium (LD), genetic connectedness between flocks, effective population size, etc., and their estimation brought to light potential benefits of GS. Genomics can also be used as an important tool for assessment of genetic diversity of local sheep breeds providing accurate estimates of relationship between animals than pedigree records and avoiding inbreeding (Eynard *et al.*, 2015). This is particularly important in cases when accurate pedigree records are unavailable or incomplete which is often the case in sheep populations.

Sheep milk production is highly important in Mediterranean and Middle Eastern countries where most of the milk is processed into cheese of high quality. Consequently, milk recording is economically important to the dairy industry. According to the ICAR online survey, the number of European dairy ewes in official milk recording reached around 900,000 ewes in 2021 (Astruc *et al.*, 2023). More than half of these populations was recorded in France. On the other hand, countries with smaller dairy sheep populations like Croatia account less than 1% of milk recorded ewes. Both countries have used SNP arrays for the purpose of selection and conservation of dairy sheep breeds in their breeding programmes. The objective of this paper was to draw the benefits and obstacles of using genomic data in contrasting countries, i.e. large vs. small dairy sheep populations: France vs. Croatia. By comparing French (Lacaune, Red – Faced Manech, Black – Faced Manech, Basco – Béarnaise, Corsican) and Croatian (Istrian, Pag) dairy sheep breeds, similarities and differences in the feasibility of GS in dairy sheep breeds are highlighted.

Material and methods

Investments in breeding programs are often related to possibilities and improvements in:

- Collecting phenotypes.
- Prediction of breeding values.
- Dissemination of realised selection progress.

The main peculiarities of dairy sheep breeding programs in France and Croatia are disclosed in the next paragraph.

French dairy sheep population

Breeding programs are implemented for five dairy sheep breeds that have traditionally been farmed in three mountainous areas in southern France: The Lacaune breed (LAC) reared in Southern Massif Central (200,000 ewes in selection and 300 new AI rams each year), Red – Faced Manech (MTR), Black – Faced Manech (MTN), and Basco – Béarnaise (BB) reared in the Pyrenean mountains (altogether 120,000 ewes in selection and 250 new AI rams each year), and the Corsican breed (COR) reared in Corsica with (18,000 ewes in selection and 20 new AI rams each year). Breeding programs are designed on a pyramidal structure with the production flocks at the base and open nucleus flocks at the top. The latter is the basis for selection based on genetic and nowadays genomic evaluation having official milk and pedigree recording. The key point in breeding programs is usage of AI based on fresh semen (seasonal reproduction) mainly from the nucleus population (Larroque *et al.*, 2014; Astruc *et al.*, 2022). Before genomic era, conventional breeding schemes were based on progeny-tested AI rams. The AI rate in the flocks under selection ranges from 45% (COR) to 85% (LAC) and the number of yearly progeny-tested rams varied from 20 rams in COR breed to 300 in LAC breed (Astruc *et al.*, 2022). Genomic evaluation has been implemented in 2015 for the LAC breed followed by Pyrenean breeds (2017) and COR breed (2020). The reference populations (Table 1) are constituted of AI progeny-tested rams across the breed (from 0.4 K in COR to 7.4 K in LAC) with a depth getting back to the early 2000's. Each year, more than 8,000 new animals are being genotyped, including all candidate rams and some females in flocks without AI and control matings are performed with medium density SNP arrays.

The single step GBLUP method using the package BLUPf90 (Misztal *et al.* 2002) has been used for genomic evaluation. Genotypes are also used for predicting major

Table 1. Reference population sizes across breed in France.

Breed*	Genotyped rams	Genotyped AI rams with daughters	Rams genotyped yearly (2023)
LAC	36 K	7.4 K (since 1996)	~3,500
MTR	7.9 K	3.5 K (since 1998)	~700
MTN	1.4 K	0.7 K (since 1996)	~100
BB	2.8 K	1.2 K (since 1999)	~250
COR	2.8 K	0.4 K (since 2003)	~350

* LAC – Lacaune; MTR – Red – Faced Manech; MTN – Black – Faced Manech; BB – Basco – Béarnaise; COR – Corsican

genes (such as scrapie, SOCS2, horn) and for parentage verification and discovery of unknown parents. The extra genetic gain obtained with the genomic program has already permitted to include novel traits more easily, such as resistance to parasites, and will hopefully allow other novel traits (efficiency and resilience) to be included in the near future.

Creating an initial reference population is a large investment and therefore assistance from funding through research and development projects is required. In France four projects have been carried out to build the reference population and the engineering of the main breeds of small dairy ruminants.

Croatian dairy sheep population

Dairy sheep breeding in Croatia is concentrated in the Mediterranean part of the country (islands and hinterland). Two dairy breeds are included in the breeding programs: Istrian sheep (IST) reared in the Istrian peninsula (1,026 ewes and 35 rams in 12 flocks) and Pag sheep (PAG) reared in the island of Pag (4,086 ewes and 78 rams in 34 flocks). Milk recording started two decades ago, while BLUP genetic evaluation has been carried out for about a decade (CAA, 2017) based on test-day repeatability animal model (Špehar *et al.*, 2020). Unlike French breeding programs, there is no AI, which hampers establishment of genetic connectedness between flocks and consequently leads to bias in the ranking of animals' genetic merits (BVs) between the animals belonging to different flocks. The breeders regularly exchange rams between the flocks, but not systematically to exploit all the benefits of the across-flock BLUP. Furthermore, the population size is relatively small in both breeds, which leads to substantial level of inbreeding, so specially designed mating plans are required to minimize the inbreeding rate in both populations. Since the inbreeding is inevitable in the small population under selection, the Optimum Contribution Selection (OCS) approach appears to be the optimal selection strategy in this population to achieve genetic gain and mitigate the loss of genetic variability.

The future breeding strategies for these breeds are improvement of dairy performance through selection, which is necessarily for their long term productive and economic viability and maintenance of the existing genetic variability which is crucial for resilience in an unpredictable future environment. Implementation of the basic principles of genomic OCS in existing breeding program is a method of choice which enables selection progress on targeted trait/s with minimal loss of genetic variability.

So far, almost the whole breeding population of IST and PAG breeds (altogether ~4,000 animals) was genotyped (Table 2) with the Illumina OvineSNP50K BeadChip® for the purpose of selection and conservation. Since the Croatian breed populations are small, it is feasible to genotype the whole female population unlike the French situation where genotyping is dominantly based on males.

For the sake of higher accuracy, the existing genetic evaluation is planned to be upgraded to the single-step genomic BLUP (Legarra *et al.*, 2009). Development of pipelines for routine genomic evaluation based on BLUPF90, a family of programs is

Table 2. Number of genotyped animals by breed and sex in Croatia.

Breed*	**Genotyped rams**	**Genotyped ewes**	**All**
Istrian sheep	86	1,293	1,379
PAG sheep	140	2,543	2,683

in ongoing phase. Knowledge of the genetic architecture of the population is important to predict the potential of GS. Therefore, prior to transition to GS, especially due to additional financial investments, it is wise to examine some important population genetic parameters that reflect genetic diversity within and/or between these populations such as effective population size, inbreeding rate, genetic connectedness between flocks, linkage disequilibrium, etc. It is important not only to have some clues about the current state of the population under consideration, but also to set up the most promising strategy for OCS.

The main investment in the GS is the cost of genotyping, and so far several funding sources have been used for genotyping as follows: Scientific Project OPTI-SHEEP (CSF, IP-2019-04-3559) of Croatian Science Foundation, Rural Development Programme of the Republic of Croatia: Sub-Measure 10.2 – support for conservation and sustainable use and development of genetic resources in agriculture, and financial support by Breeding Association of Sheep and Goats.

Results and discussion

The French situation

In France, the annual genetic gain for economic index was estimated to be between 0.12 (BB) and 0.35 (LAC) genetic standard deviation. This observation is consistent when:

- Observing the evolution of the indices over several year.
- Calculating the gain realised each year to generate a new cohort through actual selection intensity, accuracy and generation interval.

As the selection indices are calculated to have the same variability of the milk yield, the genetic standard deviation is the one of milk yield in each breed. Based on the comparison between the genetic gain observed recently in genomic selection and the genetic gain observed several years ago before genomic selection, in the French sheep breeding schemes, Figure 1 showed that the extra annual genetic gains obtained in genomic selection vs traditional selection ranged from 16% (BB) to 57% (LAC). GBLUP resulted in greater accuracies of estimated breeding values (EBV) compared to pedigree-based BLUP although for some traits and population, the increase in accuracy was small. The gain in generation interval is limited. The key factor is the selection intensity realised for the choice of the males (which directly depends on the number of genotyped candidates).

In addition to single-step GBLUP, genotyping is used for prediction major genes and for parentage verification and discovery. Major genes were associated with various reproductive, disease or production traits of interest to breeders. The following major genes were identified and included in breeding programs: PRP (scrapie resistance), SOCS2 (susceptibility to inflammation of the mammary gland), and Horn (management of horn in MTR). As the costs of genotyping decreases, it is expected that new major genes like those for lethal mutations and cryptorchidism will be available in the near future. SNPs have proved to be useful to infer pedigree information and could be used both to detect misidentification of parents and to assign true parents among candidates. Parentage verification followed by parentage discovery has been applied on males chosen for genomic selection (before genomic pre-selection). For example, the proportion of false wrong sires in LAC and Pyrenean breeds was 4.6 and 4.4 and sire discovery was successful in 92% for LAC and in 87% for Pyrenean breeds. Parentage verification is of particular importance for sire discovery in flocks that does not use AI (including organic farms where synchronisation by hormones is forbidden). The extra genetic gain obtained with GS will be used for inclusion of new resilience (functional

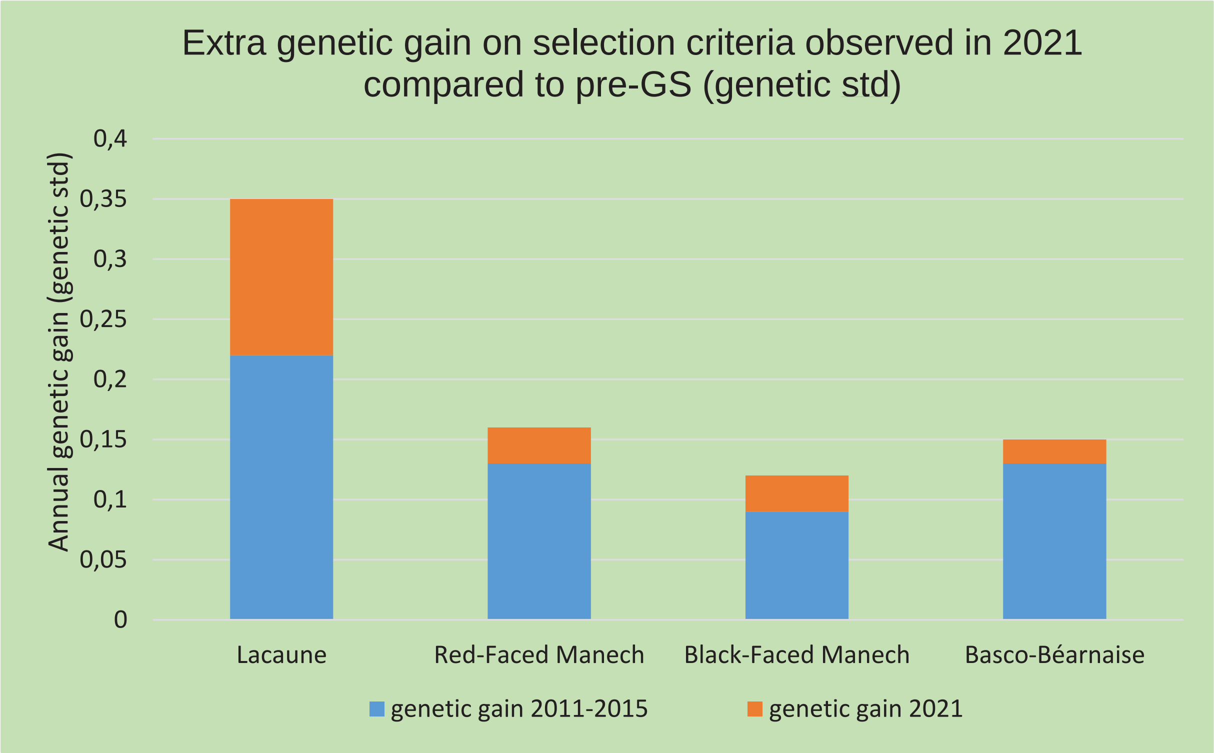

Figure 1. Genetic trends (in genetic standard deviation or σ_g) in the French dairy sheep breeds in GS (blue+orange) and comparison with pedigree-based BLUP (in blue).

longevity, resistance to parasites) and efficiency (proxies of feed efficiency) traits in the next years (Astruc *et al.*, 2022).

The Croatian situation

In Croatia, in order to provide basic information essential for designing the selection strategy in the IST breed, genetic connectedness between flocks have been examined (Kasap *et al.*, 2021). The success of across-flock genetic evaluation system depends on the genetic connectedness between flocks. When flocks are sufficiently connected, the BLUP genetic evaluation is robust, and EBVs can be fairly compared between flocks. On the other hand, limited connectedness leads to bias when comparing EBVs of animals belonging to different flocks (Kuehn *et al.*, 2008). Several statistical measures have been used to examine the degree of connectedness as follows: prediction error variance of differences in EBVs between animals belonging to different flocks, variance of estimated differences between management units, coefficient of determination of the difference between predicted breeding values, and correlation between predicted breeding values of individuals from different flocks. These statistics are useful to estimate the risk of comparing EBVs between flocks, as well as to design breeding schemes aimed at effectively linking flocks. Results showed that despite being useful in detecting flocks that tend to share breeding animals more frequently and vice versa, results (PEVD and CD) were insufficiently informative to tell us more about the bias in ranking of the animals EBVs. Connectedness based on *r*-connectedness statistic was below the acceptable level for unbiased ranking of animals belonging to different flocks ($r_{i,j}$ = 0.05). In order to increase the link between flocks in this population, some of the long-term specially designed breeding schemes need to be implemented. The best-known schemes to provide connections between different management units are the rotation of rams between herds (circle rams) and the sire references scheme.

In the Croatian case, population genetic parameters that reflect genetic diversity such as linkage disequilibrium (LD, effective population size, and inbreeding - F_{ROH}) have been examined with genomic data in IST and PAG breeds. LD was estimated because of its influence on the expected accuracy of genomic predictions (Baloche *et al.*, 2014). The estimated LD was low (r^2 Pag = 0.04, *D'*Pag = 0.28, r^2 Istrian = 0.06, *D'*Istrian = 0.39,) with a very steep LD decay (Figure 2) in both breeds (Kasap *et al.*, 2022). The results obtained are promising for conservation of these breeds, but less promising for the success of GS. To maximize benefits of genomic information, single step genomic BLUP should be applied for genomic evaluation.

Effective population size and inbreeding (F_{ROH}) have been also studied in both breeds. There was strong evidence of recent inbreeding, and the estimate of genomic inbreeding (F_{ROH}) in IST ($F_{ROH>2}$ = 0.062) was twice as high as in PAG ($F_{ROH>2}$ = 0.029) and was on the edge of acceptable levels (Ramljak *et al.*, 2024). Therefore, optimisation of mating plans is needed in the future to maintain genetic variability. Estimates of effective population size (N_e) evidenced more genetic variability in the PAG (N_e = 838) compared to the IST (N_e = 197) breed. Based on the average F_{ROH} of different size of ROH segments ($F_{ROH2–4}$, $F_{ROH4–8}$, $F_{ROH8–16}$, and $F_{ROH>16}$) a high contribution of recent inbreeding in the overall inbreeding is evident (Ramljak *et al.*, 2024). Signatures of selection were found only in IST and were associated with growth, feed intake, milk production, and immunity traits. The results of the studied population genetic parameters showed an unfavourable loss of genetic variability in the Croatian sheep population

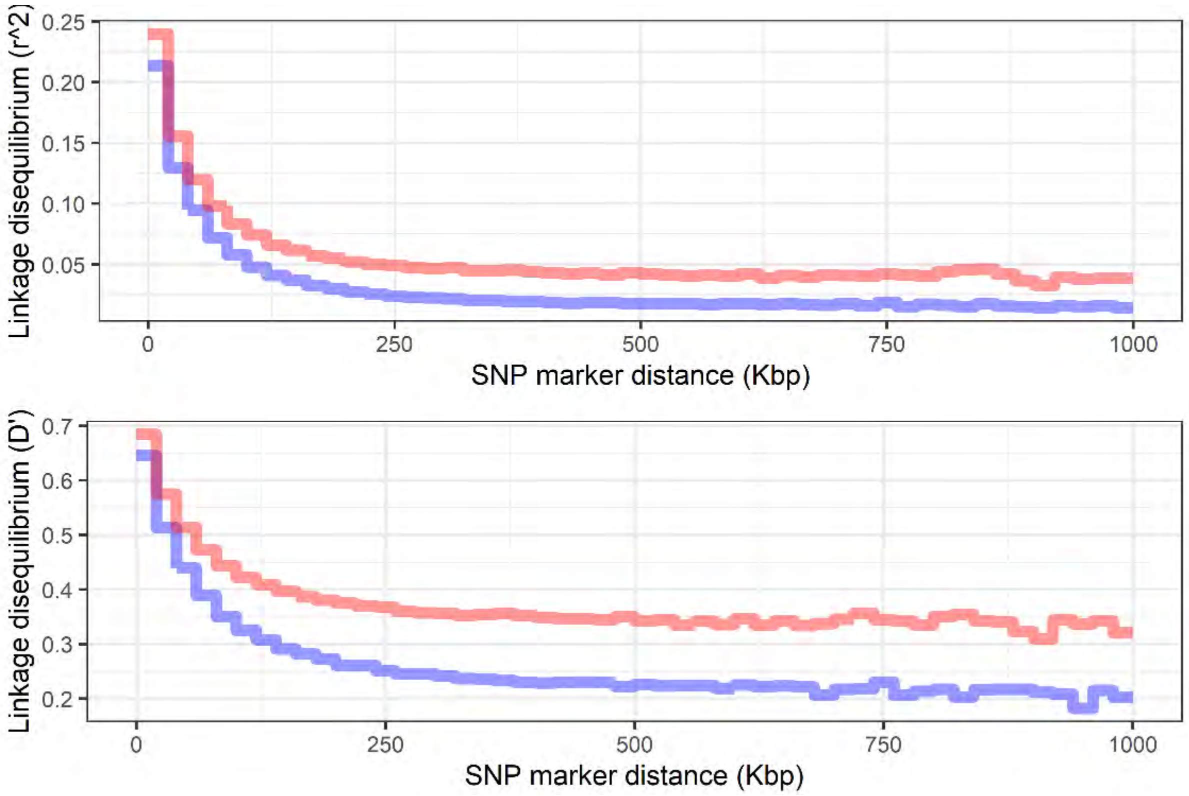

Figure 2. The linkage disequilibrium (LD) decay in IST (red) and PAG (blue) sheep

which is common in small population under selection. There is an urgent need for action to slow down this process and to create the necessary conditions (connectedness) to successfully utilize the entire genetic pool of the breed in a long-term selection.

An international initiative

It is important here to mention as well an initiative at the international level, driven by ICAR, to improve genomic evaluations in multi-breed context by establishing an EU Reference Centre (EURC) which would be responsible for performance testing and genetic/genomic evaluation in small ruminants. The EURC could offer opportunities for better valorisation of genomic data, including across country evaluation and evaluation of more sheep breeds. Across country evaluation in some multi-country breeds already exists such is the case in the French Manech and the Spanish Latxa (ARDI project). Beside evaluation, the EURC could also ensure a better exchange of genotypes for parentage verification and for share expertise across sheep and goat breeds.

Conclusion

Implementing genomic selection in sheep breeding in France has shown promising outcomes. Genomic selection not only accelerated genetic gain by allowing breeders to select animals with superior traits at an early age and based on more accurate breeding values, but also facilitated the identification of carriers of genetic markers associated with disease resistance (scrapie and mastitis) and the absence of horns. On the other hand, in Croatia, genomic selection has not yet been implemented in practice, and the population parameters that have been estimated indicate that there are some obstacles that must be overcome in order to fully exploit the potential benefits of this advanced genetic evaluation system. Following the successful example of France, it can be concluded that genomic selection has potential for improving productivity, sustainability, and profitability of Croatian sheep populations as well, but hard work is needed to strengthen connectedness between the flocks that will be included in the joint across-flock evaluation system in the future.

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Estimating the effect of kappa-casein genotype on milk coagulation properties in Israeli Holstein cows

Y. Lavon[1], J.I. Weller[1], C. Cohen[1], Y. Zeron[2] and E. Ezra[1]

[1]*Israel Cattle Breeders Association,*
Caesaria Industrial Park 38900, Israel
[2]*Sion Artificial Insemination Center, Gadara 7057102, Israel*
Corresponding Author: yaniv@icba.co.il

Abstract

In Israel, about 26% of milk production is used to produce hard cheeses and 29% for soft cheeses. Milk with preferred coagulation properties requires shorter coagulation time and yields higher curd firmness than milk with inferior coagulation properties. Studies have shown that milk from cows with the B allele of kappa casein (κ-CN) produces more cheese than milk from those with A and E alleles. There is evidence of milk from AE or EE genotype cows being unsuitable for cheese production. In the early 1990s, the proportion of the B allele in Israeli Holstein cattle was about 17%, similar to its prevalence in the Holstein population worldwide. In recent years, however, its proportion has increased to about 40%. We analyzed milk coagulation properties as a function of the cow's κ-CN genotype, including time in minutes until the beginning of coagulation, and curd firmness after 60 min in volts as measured in an optigraph device, and scored on a scale of 0–4 by the laboratory technician. Cow selection was based on their sire's genotype, so that there would be sufficient genotypes that include the rare E allele. A total of 359 cows were sampled from 15 farms: 64 with genotype AA, 142 with AB, 41 with AE, 65 with BB and 47 with BE. Data were analyzed by the general linear model procedure of SAS. We found: (a) significant differences between genotypes for optigraph measured curd firmness. In a multi-comparison test, the BB genotype gave the highest curd firmness, and AB and BE showed a significant advantage compared to AA and AE (9.4, 8.6, 8.4, 6.9, 6.8 V, respectively). Assuming a frequency of about 55% for the A allele:

- about 30% of the milk delivered to dairy plants comes from AA cows;
- there was a significant difference between the genotypes in technician-observed curd firm-ness, with BB scoring significantly higher than AA and AE;
- optigraph measured curd firmness was significantly higher for milk from primiparous cows as compared to milk from 2nd, 3rd or 4th lactation cows (8.9, 7.8, 7.9, 7.7 V, respectively).

Technician observed curd firmness was significantly higher for primiparous vs. multiparous cows. There was a clear advantage in curd firmness for genotypes that included the B allele as compared to those with AA and AE genotypes. We can increase the proportion of B allele in the population by inseminating cows with bulls of genotypes AB and BB. This factor should therefore be included in the selection index.

Keywords: kappa casein; coagulation; genotype; dairy cow.
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Introduction

Milk coagulation ability is important for cheese production. Milk coagulation and curd-firming processes have been widely studied in recent decades, and milk protein fractions have been identified as the principal factors in these processes (Bittante *et al*., 2012). In most milk-producing countries, a large and growing fraction of the produced milk is used to make cheese (Ikonen *et al*., 1999a; Wedholm *et al*., 2006; Cassandro *et al*., 2008). In Israel, around 26% of milk production is used to produce hard cheeses and 29% for soft (white) cheeses (Israeli Dairy Board, 2020). The milk's ability to coagulate (time and quality) is economically significant: milk with preferential coagulation properties will yield larger amounts of cheese with the desired contents than milk with inferior coagulation properties (Ikonen *et al*., 1999a). Milk coagulation properties are influenced by breed (Bittante *et al*., 2012), somatic cell count (SCC) and bacteriology (Leitner *et al*., 2019), milk protein composition and casein composition (Guinee *et al*., 2006; Pretto *et al*., 2013; Panthi *et al*., 2017), and stage of lactation (Tyrisevä *et al*., 2010), among other factors. In addition, the cheese-making traits can be affected by environmental factors such as feeding, udder health, season, physiological stage (e.g., parity, lactation stage), but they are also genetically influenced (Cassandro *et al*., 2008; Cecchinato *et al*., 2011; Tiezzi *et al*., 2013). Milk coagulation properties are heritable according to Ikonen *et al*. (2004) and can therefore be improved by selective breeding. In dairy products, the kappa casein (κ-CN) component in milk proteins is responsible for coagulation. κ-CN exists as variants AA, AB, BB, AC, BC, and AE in bovine milk, with allele C being more common in Jersey cows and not present in Holstein.

In the Holstein population, the gene encoding κ-CN has 3 allelic variants: A, B and E. Most studies show an advantage for the B allele in protein and CN contents. Many studies have confirmed that milk containing the BB variant of κ-CN has faster and firmer gelling ability and is more suitable for cheese production than other variants (Amenu *et al*., 2006; Ikonen *et al*., 1999b; Jõudu *et al*., 2009). According to Ng-Kwai-Hang (1998), milk with the BB variant of κ-CN shows reduced coagulation time (by 10–40%) and increased curd firmness (by 20–140%) compared to milk with its AA variant. In particular, the most consistent effect was found for CSN3 (κ-CN) variant B, which has been shown to have a positive effect on κ-CN concentration in milk (Bobe *et al*., 1999; Hallén *et al*., 2008; Heck *et al*., 2009), and to be associated with smaller average casein micelle size (Walsh *et al*., 1998). Furthermore, cheese prepared from milk containing the BB variant of κ-CN has higher fat recovery and yield than that made with milk containing the AA variant (Walsh *et al*., 1998). Thus, cows that produce milk containing the BB variant of κ-CN are economically important from a cheese-making perspective, owing to the micelle size-related benefits of this protein type.

The prevalence of allele B in Israeli Holstein cattle as tested in the early 1990s was about 17%, similar to the Holstein population worldwide (Ron *et al*., 1994). Ikonen *et al*. (1999a) concluded that allele E is associated with poor coagulation compared to the other alleles, and in some cases the milk is useless for cheese-making.

Most studies have found a significant effect of the B allele on the amount of CN in the milk and accordingly, the presence of this allele was found to result in 9.5–14% less whey protein. In a study of Jersey cows (Hunt, 2017), the prevalence of alleles E, A and B was 10, 30 and 70%, respectively, and Zepeda-Batista *et al*. (2015) found incidences of 3, 55 and 42% for these respective alleles in the Fleckvieh breed. Another study conducted in Czech Fleckvieh cattle found 5, 72 and 23% frequency of the E, A and B alleles, respectively (Bartonova *et al*., 2012). They also found that cows with the AA genotype produce the highest quantity of milk among the genotypes. Cows with the BB genotype yielded the highest amount of milk protein relative to other genotypes.

Cows with genotypes BB and BE yielded milk with the highest protein percentage. In Lithuania (Morkūnienė *et al*., 2016), frequencies of the E, A and B alleles were 6, 80 and 14%, respectively, in Black and White Holsteins; and 18, 70, and 11%, respectively, in Red and White Holsteins. In addition, that study clearly showed shorter coagulation time and firmer cheese with milk from BB cows, as compared to AA and AB cows' milk, while AE cows' milk was clearly inferior to all other genotypes in both parameters. Coagulation quality can be improved by increasing the prevalence of the B variant of κ-CN and collecting direct data, such as rennet coagulation time (RCT), or performing genetic tests to genotype the cows. However, the latter approach is rarely performed. A quicker and more efficient way of estimating the prevalence of the B allele in advanced commercial dairy populations is to use information from the cow's pedigree, especially sires, since only a very low number of sires are generally used each year. To-day, AI associations worldwide publish the bulls' genotype for κ-CN, which accounts for 11% of CN, so the farmer can also choose the inseminating bull according to its κ-CN genotype.

However, most studies have neglected to look at the effect of the different genotypes, and especially allele E, on milk coagulation parameters (MCP). Our study hypothesis was therefore that cows having the B allele will show superior MCP, whereas cows with allele E will have inferior MCP. To test this hypothesis, our objectives were to analyze milk coagulation properties as a function of the cow's κ-CN genotype, including time in minutes until the beginning of coagulation (RCT); and curd firmness after 60 min in volts as measured in an optigraph device, and as observed by a laboratory technician rated on a scale of 0–4.

Materials and methods

We analyzed two datasets. Dataset 1 was used to estimate the κ-CN allele frequency in Holstein cow populations in Israel, based chiefly on the genotypes of the sires. A total of 1447 bulls and 4430 cows were genotyped between 2011 and 2021. We routinely use the Bovine 150K chip for genotyping bulls and cows, and one of the out-puts is the κ-CN genotype. All genotyping was done by Neogen (Lansing, MI, https://www.neogen.com/about) and BeadChips v3 (Illumina Inc., San Diego, CA, https://www.illumina.com/science/technology.html). Dataset 2 was used to analyze MCP in a group of cows (n = 359). Cows were selected from the herdbook according to their sire's and grandsire's allele for κ-CN, to reach a balanced sample that includes all genotypes. We selected 391 cows with sire genotypes AE, BE and BB to have a sufficient number of cows presenting the different genotypes for the study. The selected cows were tested for their κ-CN genotypes from a hair sample (Neogen). Cows with known κ-CN genotype were sampled according to the following protocol: cows in mid-lactation (average of 148 d) and cows with SCS (Somatic Cell Score) lower than 4 (average of 2.03) were checked for clinical or subclinical mastitis using the California mastitis test (CMT) on the quarter level; If a quarter show that CMT result was positive (1 or higher) we didn't took milk sample from this specific quarter and the quarter was removed from the MCP analysis. So, when taking samples we used the quarter as the experimental unit. After we collected the milk sample the experimental unit was the cow. In total, we tested 359 cows from 15 dairy farms.

A milk sample was collected (30–45 mL of a mixture of whole udder yield) and di-vided into 2 different samples for analysis as follows: the first sample was tested for SCC with a Fossomatic 360 (Foss Electric, Hillerød, Denmark) and gross milk composition, i.e., protein, fat and lactose contents, with the MilkoScan FT6000 (Foss Electric). These analyses were performed at the Israel Cattle Breeders' Association laboratory (Caesarea, Israel); the second sample was tested for curd firmness after 60 min (CF-60) and RCT with an optigraph (Ysebaert, Frepillon, France), and for curd firmness as ob-served by the same laboratory technician after every cheese-making session, who scored the curd from 0 – weak and more liquid to 4 – hard and more stable. Samples (10 mL) were placed in wells and equilibrated at 30°C. The coagulating enzyme was Fromase 15 TL (0.5 mL, Gist-Brocades NV, Delft, The Netherlands), diluted (1:100) to achieve clotting within about 900 s in bovine milk (Leitner *et al*., 2011).

Data analysis

Dataset 2 included 359 cows. The MCP were analyzed by the GLM procedure of SAS (2009, SAS Institute Inc., Cary, NC). The analysis model was:

Yijklm = Hj + Lk + Gl + SCSijkl + Mijkl + PFijkl + PPijkl + Dijkl + eijklm,

where the dependent variables, Yijklm, were CF-60 and RCT of cow i in herd j of parity k, with genotype G; Hj was herd effect j; Lk was parity effect k (1,2,3,4+); Gl was genotype effect l (AA, AB, AE, BB, BE); SCSijkl, Mijkl, PFijkl, and PPijkl were the effects of test day SCS, milk, % fat and % protein records of cow i; D was days in milk effect (DIM) on the date of the milk sample; and eijklm was the random residual.

Multiple comparisons for significance among the genotype effectss were tested by Bonferroni procedure. All first-degree interactions were tested and found to be non-significant and were therefore excluded from the final models. Results for a level of a specific variable included in the model was based on least square (LS) mean values as presented in Lavon *et al*. (2011).

Results

The frequencies of κ-CN alleles and genotypes from dataset 1 are presented in Table 1. The allele frequencies of A, B and E were 57.1, 40.3 and 2.6%, respectively. The genotype frequencies of AA, AB, AE, BB, BE and EE were 32.47, 46.33, 2.84, 16.05, 2.28 and 0.03%, respectively.

Distribution of alleles and genotypes in the cows from dataset 2 are summarized in Table 2. Our sampling protocol resulted in >40 cows for all genotypes, except for EE, which was very rare in the population. The allele frequencies of A, B and E were 43.31, 44.43 and 12.26%, respectively. The genotype frequencies of AA, AB, AE, BB, BE and EE were 17.83, 39.55, 11.42, 18.11, 13.09 and 0.00%, respectively.

Table 1. Genotype frequency and κ-CN alleles from dataset 1.

Genotype[1]	n	Frequency (%)	Allele	n	Frequency (%)
AA	1908	32.47	A	6706	57.11
AB	2723	46.33	B	4743	40.28
AE	167	2.84	E	305	2.61
BB	943	16.05			
BE	134	2.28			
EE	2	0.03			
Total	5877	100		11754	100

[1] A total of 5,877 bulls and cows were genotyped using the Bovine 150K chip between 2011 and 2021.

Table 2. Genotype frequency and κ-CN alleles found in cows included in dataset 2.

Genotype[1]	n[2]	Frequency (%)	Allele	N	Frequency (%)
AA	64	17.83	A	311	43.31
AB	142	39.55	B	319	44.43
AE	41	11.42	E	88	12.26
BB	65	18.11			
BE	47	13.09			
EE	0	0			
Total	359	100		718	100

[1] Cow genotype was tested in a hair sample taken from each cow.
[2] Data of 359 cows from 15 different dairy farms.

Table 3. Test-day results for milk yield, milk solids contents, SCS and DIM according to cow genotype for κ-CN.

Genotype[1]	n[2]	Milk (kg)	Fat[3] (%)	Protein (%)	SCS	DIM
AA	64	42.6	3.18	3.20	1.8	135
AB	142	40.3	3.54	3.30	2.1	147
AE	41	39.7	3.41	3.30	2.2	161
BE	47	39.6	3.49	3.36	2.4	172
BB	65	43.5	3.43	3.27	1.8	140
Total	359	41.4	3.43	3.28	2.0	149

[1] Cow genotype was tested in a hair sample taken from each cow.
[2] Data of 359 cows from 15 different dairy farms.
[3] Milk solids and SCC were tested at the Israel Cattle Breeders Association laboratory.

Test-day results for milk yield, milk solids content, SCS and DIM for each genotype are summarized in Table 3. Cows with the AA genotype had lower percentages of milk fat and protein, but differences between the genotypes for the test-day parameters were not significant.

Milk coagulation traits (CF-60 and RCT) were affected by κ-CN genotype (Figure 1A and B) and by lactation number (Figure 2A and B). Cows with genotype BB had the highest curd firmness, followed by cows with the B allele in combination with A or E ($P < 0.05$; Figure 1A). Cows with AA or AE genotype (without the B allele) had the lowest curd firmness ($P < 0.05$; Figure 1a). Milk from cows with the B allele took less time to coagulate (lower RCT), especially the BB cows, but the difference was not significant (Figure 1B). Higher curd firmness was obtained from primiparous cows' milk compared to that of multiparous cows, with no difference found among the multiparous cows ($P < 0.05$; Figure 2A). The RCT was lower for the primiparous vs. multiparous cows, but the difference was only significant compared to second-lactation cows ($P < 0.05$; Figure 2B).

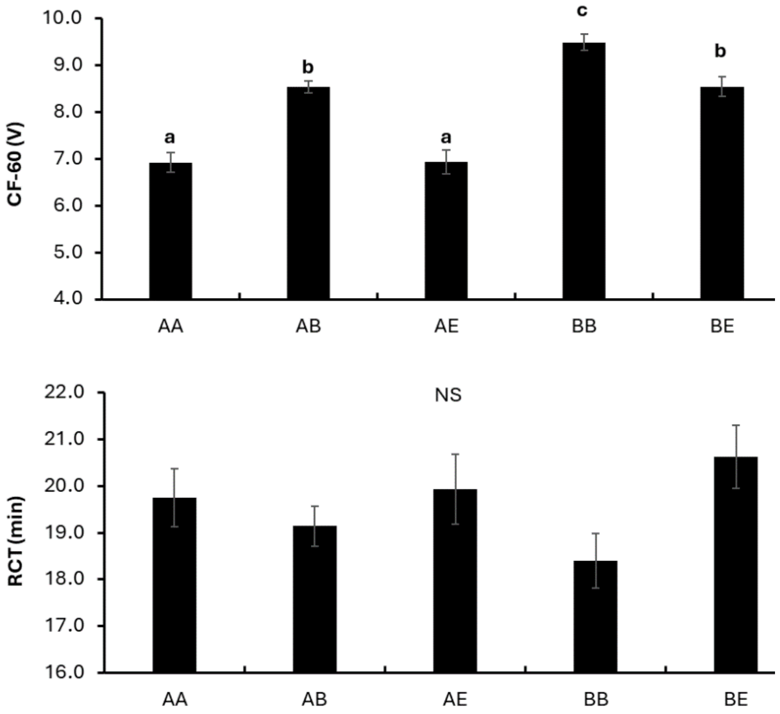

Figure 1. Genotype effect on (A) curd firmness after 60 min (CF-60) and (B) rennet coagulation time (RCT). Values are LS means ± SEM. Different letters indicate significant difference (P < 0.05).

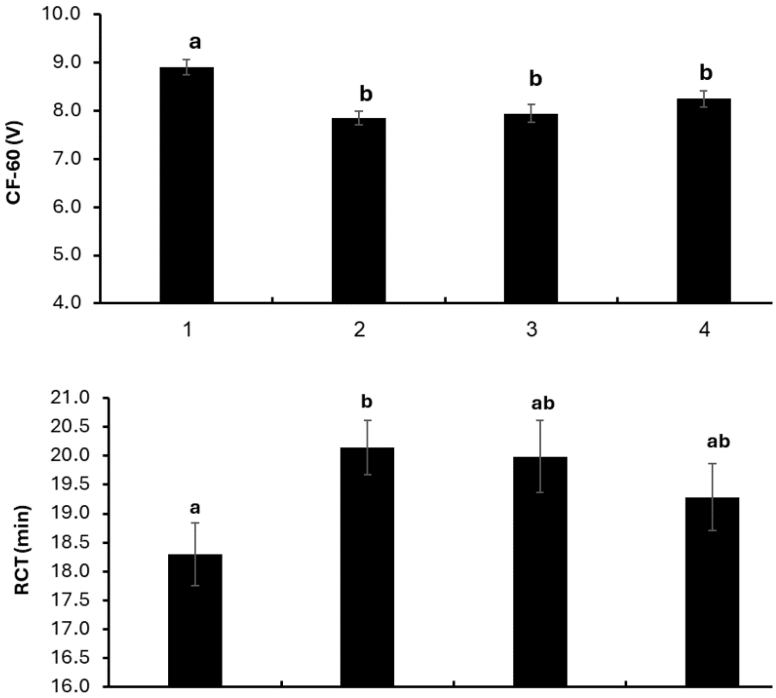

Figure 2. Effect of lactation number on (A) curd firmness after 60 min (CF-60) and (B) rennet coagulation time (RCT). Values are LS means ± SEM. Different letters indicate significant difference (P < 0.05)

Table 4. Effects of genotype, lactation number, SCS, milk yield, milk fat and protein percentages and DIM on rennet coagulation time (RCT) and curd firmness at 60 min (CF-60).

Pr > |t|	SE	CF-60 (V)	Pr > |t|	SE	RCT	Level	Factor
<.0001			0.3409				Genotype
	0.253	-2.14		0.848	1.36	AA	
	0.212	-0.85		0.711	-0.01	AB	
	0.286	-2.44		0.959	0.24	AE	
	0.271	-1.27		0.908	0.94	BE	
	-	0.00		-	0.00	BB	
<.0001			0.0006				Lactation number
	0.247	1.20		0.829	-2.73	1	
	0.214	0.21		0.720	0.23	2	
	0.241	0.05		0.810	-0.02	3	
	-	0.00		-	0.00	4	
	0.050	-0.03	0.0349	0.168	0.36		SCS
<.0001	0.012	0.05	0.0064	0.040	-0.11		Milk (kg)
<.0001	0.123	0.77	0.0132	0.413	-1.03		Fat (%)
<.0001	0.292	2.69	NS	0.979	1.67		Protein (%)
0.0237	0.001	0.0028	0.0006	0.004	0.01		DIM

Table 4 presents the model effects, SE and *P*-values for the effects of genotype, lactation number, log SCC, milk yield, milk fat and protein percentages and DIM on RCT and CF-60. With respect to the former, there was no difference among genotypes. In contrast, cows in first lactation showed reduced time to coagulation than the older cows (Table 4; $P < 0.0001$). There was significantly higher curd firmness as determined by CF-60 for cows with the BB genotype. The order of curd firmness (high to low) was: BB > AB > BE > AA > AE (Table 4; $P < 0.0001$). In addition, milk from first-lactation cows gave higher curd firmness than that of older cows (Table 4; $P < 0.0001$).

The 10 cows with the highest and lowest curd firmness scores are given in Table 5. Of the 10 cows with the highest curd firmness, 9 had the B allele and 6 had the BB genotype (Table 5). Of the 10 cows with the lowest curd firmness, only 1 cow had allele B and all of the cows had the A or E allele. This clear distinction was not apparent with respect to the RCT values.

Table 5. Effect of κ-CN genotype on milk coagulation parameters.

10 cows with the lowest CF-60 scores				10 cows with the highest CF-60 scores			
Genotype	CF-60	RCT	Visual index	Genotype	CF-60	RCT	Visual index
AE	4.2	14.3	2.8	BB	14.6	15.8	3.7
AA	4.2	21.9	1.0	BE	14.4	19.5	3.7
AE	4.1	19.1	2.5	AA	14.1	18.5	3.7
AE	4.0	17.3	3.4	AB	13.4	16.1	3.3
AE	3.8	18.6	2.6	BB	13.2	22.8	3.8
AB	3.4	42.4	0.0	BB	13.0	16.4	3.8
AE	3.4	42.1	0.0	AB	12.8	21.1	3.7
AE	3.3	12.7	3.5	BB	12.8	14.9	3.9
AA	2.8	13.7	1.9	BB	12.3	20.8	3.6
AA	0.3	31.6	0.2	BB	12.3	18.9	3.6

Discussion

This study presents the current distribution for κ-CN genotypes and alleles in the Israeli dairy population. Most publications show an advantage for the B allele in protein and CN content. The prevalence of allele B in Israeli Holstein cattle in the early 1990s was about 17% (Ron *et al*., 1994). In the current study, prevalence of the B allele has more than doubled, to around 40% in the entire population. This strong increase stems from the advantage of the B allele for protein content, which is the major objective in the Israeli breeding index (Weller *et al*., 2022). In contrast, prevalence of the E allele has decreased from about 6% to 2.6%.

Cheese-making requires milk coagulation and development of syneresis. The dairy industry pays a great deal of attention to MCP, principally because the amount of milk used for cheese production is growing worldwide (International Dairy Federation, 2020). In the last decade, the fraction of total milk destined for cheese production has increased by about 10% in the European Union and North America, and it is now slightly higher than 50% in the EU and slightly lower in North America. An increase in the amount of milk used to manufacture cheese has been reported in other European countries, Oceania, and Latin America, whereas a much lower amount is used as com-pared in Asia and Africa. The B variant of κ-CN is associated with a higher protein percentage compared to the E variant, with the A variant being intermediate between the two (Heck *et al*., 2009). Milk production is correlated with κ-CN genotypes in the order AB > AA > BB (Hristov *et al*., 2014). The order of κ-CN genotypes as they relate to protein content is BB > AB > AA (Aleandri *et al*., 1990), or AB > AE > AA (Devold *et al*., 2000).

However, Lodes *et al*. (1997) found the opposite order, i.e., AA > AE > AB. In addition, Ikonen *et al*. (1999a) reported that the EE, AE and BE variants contribute to high milk yield, but low protein percentage. The BB variant was found to be positively correlated with milk and milk-protein production during the first lactation (Mao *et al*., 1992). In the current study we did not find any correlation between genotype and milk level or milk solids content. Comin *et al*. (2008) reported that κ-CN is the most important milk protein in rennet coagulation, as it is the key to CN micelle stability, providing steric and electrostatic repulsion between micelles to prevent aggregation through the surface 'hairy' layer of micelles (Jensen *et al*., 2012). The B variant is found to be associated with high milk quality in European cattle breeds (Martin *et al*., 2002) and, compared to the A variant, B is found to be associated with shorter RCT (Lundén *et al*., 1997). Cheese formed using milk with the BB variant has higher yield, higher protein content and better quality compared to the AB variant (Ron *et al*., 1994).

The different genetic milk-protein variants and CN haplotypes have a major effect on the protein composition of milk. In general, the A allele of κ-CN has been associated with a longer RCT and weaker curd (Hallén *et al*., 2007). We found higher curd firmness in cows with the B allele as compared to those without it. Genotype BB had the highest curd firmness (CF-60), followed by AB, BE, AE, and AA with the lowest curd firmness. The RCT was lower, in agreement with higher curd strength, in cows with allele B, but this difference was not significant. Cow age (primiparous vs. multiparous) had a significant effect on MCP, where first-parity cows had higher curd firmness and lower RCT compared to older cows. A determination of MCP in the entire cow population is not practical, due to the large effort and expense required. An alternative way of improving MCP indirectly might be to favor the B allele of κ-CN in the selection of bulls for general service.

Conclusions

This study shows the result of the Israeli breeding program which favors bulls and cows with higher percentages of milk solids. We noted a steep rise in frequency of the B allele in the population, which can improve coagulation properties. The current study analyzed a large number of dairy cows with known genotypes for κ-CN. We found that the presence of the B allele for κ-CN is associated with superior coagulation parameters (CF-60 and RCT) compared to cows without the B allele. Cows with AA or AE genotype had lower curd firmness compared to cows with the BB, AB or BE genotype. We also found that primiparous cows present superior coagulation parameters (CF-60 and RCT) compared to cows in their 2nd, 3rd or 4th lactation. Inclusion of the κ-CN genotype in the Israeli selection index could further raise the frequency of the B allele in the population as a direct effect and have an indirect effect through the percentage of milk solids, leading to a faster rise in the B allele and improved MCP for the milk industry in Israel.

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Genetic and genomic evaluations of quantitative milking speed phenotypes

A.M. Miles[1], J.L. Hutchison[1], S. Toghiani[1], J.R. O'Connell[2], R.H. Fourdraine[3], P.M. Van Raden[1], K.L. Parker Gaddis[4], S. Sievert[5], S. Eaglen[6], J. Bewley[7] and J.W. Dürr[4]

[1]Animal Genomics and Improvement Laboratory, USDA-ARS, 10300 Baltimore Ave, Beltsville MD 20705, USA
[2]University of Maryland School of Medicine, 655 W. Baltimore St, Baltimore MD 21201, USA
[3]Dairy Records Management Systems, North Carolina State University, 313 Chapanoke Rd, 27603 Raleigh, USA
[4]Council on Dairy Cattle Breeding, 4201 Northview Dr, 20716 Bowie, USA
[5]National Dairy Herd Information Association, Quality Certification Services, 5940 Seminole Centre Ct, 53711 Fitchburg, USA
[6]National Association of Animal Breeders, 8413 Excelsior Dr Suite 140, 53717 Madison, USA
[7]Holstein Association USA, Inc., 1 Holstein Pl, 05301 Brattleboro, USA
Corresponding Author: asha.miles@usda.gov

Abstract

Many milking systems with inline milk meters can record the milk yield and duration of each milking for individual cows. The objective of this work was to determine the suitability of milking speed traits for genetic and genomic selection and the amount of phenotype data required to produce a reliable evaluation. Records from January 2021 to December 2022 were retrieved by Dairy Records Management Systems, comprising data from 305 herds, 9 different original equipment manufacturers and 23,201 complete lactations of 23,180 cows, including 4,246 genotyped cows.

Milking speed was defined as milk yield divided by milking duration for each individual milking. Four traits were compared:

1. Average of total lactation data for all parities.
2. Average of test days for all parities.
3. Average of total lactation data for first parity only.
4. Average of test days for parity 1.

Breed, milking frequency, parity, lactation length, and meter manufacturer were included in the genetic model along with genetic groups and permanent environment. The pedigree relationship matrix included 219,703 animals with records or descendants with records plus 96 million other animals. Variances were estimated by both Gibbs sampling and REML; estimates were very similar. Residual variance was 51% higher for test day traits compared to total lactation traits. Milking speed test day heritability was 28% vs. 37% for total lactation data; genetic correlation between them was 0.97, suggesting that even with a 99% reduction in amount of phenotypic data included they are describing the same trait. Milking speed was less stable in parity 1 compared to other parities, but high genetic correlations (> 0.92) suggest the same trait is being captured. Milking speed had a small favorable genetic correlation with milk yield but unfavorable with somatic cell score based on 756 Holstein bulls with reliability > 50%. Genomic predictions for young animals born in the last 10 years averaged 37% reliability compared to ~70% reliability for several other traits. We conclude that evaluations for milking speed are not only feasible but would have significant economic impact for producers using various milking systems. Work on implementing an evaluation for milking speed is currently underway.

Keywords: milking speed, heritability, genomic prediction, genetic selection.
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Introduction

In October of 2021, the Council on Dairy Cattle Breeding (CDCB) appointed a task force to investigate the feasibility to implementing milking speed (MSPD) evaluations in the United States. The research efforts to standardize a phenotype definition for quantitative measures of MSPD derived from electronic in-line milk meters have been previously described in Miles *et al*., (2022) and Miles *et al*., (2023). The major conclusions of this work were that milking frequency, parity, breed, and milking meter manufacturer (OEM) all have substantial effects on quantitative MSPD phenotypes. Data sparsity remains a major challenge – as phenotypes are stratified by more factors, trends become harder to elucidate and there is a significant reduction in statistical power. Thus, the research presented in this paper was conducted using only Holstein phenotypes collected from conventional milking parlors (no robotic systems). The goals of this research were to determine the heritability of various MSPD traits and develop methods for genetic and genomic evaluations that can be feasibly implemented in the USA.

Materials and methods

Records from January 2021 to December 2022 were retrieved by Dairy Records Management Systems, comprising data from 305 herds, 9 different original equipment manufacturers (OEM) and 23,201 complete lactations of 23,180 cows, including 4,246 genotyped cows. Milking speed was defined as milk yield divided by milking duration for each individual milking between 10 and 305 days in milk (DIM). Four possible traits were compared:

1. Avg_all: average of total lactation data for all parities,
    a. a hypothetical 3X cow would have 3 * 295 DIM = 885 records contributing to phenotype
2. Avg_TD: average of test days for all parities,
    a. a hypothetical 3X cow would have 3 * 10 test days = 30 records contributing to phenotype, and a ~34% reduction in data
3. Avg_all_P1: average of total lactation data for first parity only,
4. Avg_TD_P1: average of test days for first parity only.

Trait 1) represents the most complete phenotype it is possible to assemble; Trait 2) was evaluated to address the feasibility of collecting, transmitting, and storing the data required to compute Trait 1; Traits 3) and 4) were evaluated to address the potential that MSPD is a different trait for first parity animals.

Breed, milking frequency, parity, lactation length, and meter manufacturer were included in the genetic model along with genetic groups and permanent environment. The pedigree relationship matrix included 219,703 animals with records or descendants with records plus 96 million other animals. Variances were estimated by both Gibbs sampling and REML; estimates were very similar and REML was used in evaluation models.

Results and discussion

The higher residual variance observed in TD traits (Table 1) is expected because there are fewer data points. The higher heritability observed for Average_all_P1 was not expected, but there is greater standard error indicating heritabilities are less accurate with less data. Both P1 traits have higher residual variance suggesting MSPD is less stable in first parity, the very high h^2 SE for Average_TD_P1 (0.21± 0.18) may be related to the fewer number of animals but suggests this trait is less useful than others compared.

Avg_all and Avg_TD had high genetic correlation (0.97) suggesting they are describing the same trait even with a significant reduction in data (Table 2). Parity 1 traits were also highly correlated (>92%) to MSPD traits including all parities, suggesting that they can be evaluated together with other parities.

Approximately 23,000 cows had full lactations for phenotype assembly. Preliminary PTAs were generated with the fixed effects of breed, parity, and OEM. Below are descriptive stats on >50% REL HO bulls born since 2012 for each of the four MSPD traits (Trait 1 inTable 3; Trait 2 in Table 4; Trait 3 in Table 5, Trait 4 in Table 6), with comparison to SCS and NM$.

Table 1. Heritabilities, standard error, and residual variance computed for each trait in AIMREMLF90 ver. 1.148.

Trait	N	h^2 (SE)	Residual Variance
Trait 1) Avg_all	23,180	0.37 (± 0.02)	1.10
Trait 2) Avg_TD	22,227	0.28 (± 0.02)	1.66
Trait 3) Avg_all_P1	9,569	0.38 (± 0.04)	1.12
Trait 4) Avg_TD_P1	9,208	0.21 (± 0.18)	2.05

Table 2. Genetic correlations (upper diagonal) and phenotypic correlations (lower diagonal) for traits compared

	Avg_all	Avg_TD	Avg_all_P1	Avg_TD_P1
Avg_all		0.968	0.916	0.976
Avg_TD	0.821		0.944	0.991
Avg_all_P1	1.000	0.819		0.924
Avg_TD_P1	0.820	1.000	0.819	

Table 3. Predicted Transmitting Ability (PTA) and Reliability (REL) for Avg_all (n = 772 bulls, genetic correlation with somatic cell score (SCS) = 0.38, Net Merit (NM$) = 0.07).

	PTA				REL			
Trait	Min	Mean	SD	Max	Min	Mean	SD	Max
MSPD	-0.80	0.12	0.30	1.00	50.10	67.05	11.84	97.80
SCS	-0.72	-0.17	0.18	0.67	50.00	92.95	10.50	99.90

Table 4. Predicted Transmitting Ability (PTA) and Reliability (REL) for Avg_TD (n = 603 bulls, genetic correlation with somatic cell score (SCS) = 0.43, Net Merit (NM$) = 0.06).

	PTA				REL			
Trait	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**
MSPD	-0.84	0.09	0.30	1.02	50.10	65.31	11.25	97.10
SCS	-0.72	-0.17	0.18	0.67	50.00	93.28	10.55	99.90

Table 5. Predicted Transmitting Ability (PTA) and Reliability (REL) for Avg_all_P1 (n = 344 bulls, genetic correlation with somatic cell score (SCS) = 0.42, Net Merit (NM$) = 0.09).

	PTA				REL			
Trait	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**
MSPD	-0.76	0.17	0.30	1.03	50.10	67.10	11.81	94.60
SCS	-0.72	-0.18	0.19	0.67	50.00	89.10	13.68	99.90

Table 6. Predicted Transmitting Ability (PTA) and Reliability (REL) for Avg_TD_P1 (n = 198 bulls, genetic correlation with somatic cell score (SCS) = 0.51, Net Merit (NM$) = -0.01).

	PTA				REL			
Trait	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**
MSPD	-0.46	0.15	0.28	0.82	50.20	64.00	10.09	91.20
SCS	-0.58	-0.17	0.18	0.31	50.00	89.56	13.71	99.90

Conclusions

The above data suggest that producing a reliable evaluation for MSPD using quantitative inline meter data is possible. While using a significantly reduced dataset (e.g., the TD traits) appears adequate, the task force recommends adopting Trait 1) Avg_all with the highest heritability with lowest standard error and residual variance in the model. Ensuring data flow will be critical to the successful implementation of this trait, and a new data transfer Format 8 has been developed to provide the required data for delivery of a MSPD evaluation. Collection, transfer, and storage of high-resolution sensor-based data like that used in this study requires significant investment in infrastructure by both CDCB and USA data providers. Work is ongoing in this area and represents an opportunity to develop pipelines and precedent for other high-throughput phenotypes besides MSPD.

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Igenity® BeefxDairy: A genomic tool for identifying genetic merit of Beef on Dairy cross calves

R. Tait Jr., V. Pedrosa, S. Smith and J. Qiu

Neogen Genomics, 4131 N 48th Street, Lincoln, NE 68504, USA
Corresponding Author: Rtait@neogen.com

Abstract

An increasing number of dairy farms are interested in managing their replacement heifer inventories. They utilize genomic testing and sexed semen to create enough replacement heifers for their dairy herd replacement needs. From a dairy system sustainability perspective, this facilitates the opportunity to utilize beef semen on cows not destined for dairy replacement purposes to increase profitability based on beef on dairy cross calve production. In general, these beef on dairy calves have advantages over straightbred dairy calves; however, there is interest in specific animal performance insights in this growing class of beef on dairy cattle. With the large influx of beef-influenced dairy cattle into the feeder space, it is becoming more imperative to better understand the genetic differences among those calves. This need has led Neogen to develop a commercial genomic testing tool to help rank beef influenced dairy calves for terminal traits of interest. This facilitates an opportunity to choose how to manage the animals through the growth and finishing stages of production.

In a study population of 1,002 feedlot cattle, we investigated the genomic breed composition as well as the relationship between genomic prediction of performance and actual phenotypic harvest and performance data. Samples taken on 438 steers and 564 heifers were approximately 55% beef influence and 45% dairy influence. Statistical analysis (R software) demonstrated positive correlations between Igenity® scores and phenotypic performance of 0.29, 0.23, and 0.22 for marbling (MARB), average daily gain (ADG), and hot carcass weight (HCW), respectively. Furthermore, when ranking cattle according to Neogen's Terminal Index and comparing the top quartile (average = 6.71) to the bottom quartile (average = 5.22) of animals, phenotypic performance was significantly different for ADG (1.32 kg/d vs. 1.24kg/d, $P < 0.001$, respectively), HCW (390.6 kg vs. 372.2 kg, $P < 0.001$), and USDA yield grade (2.59 vs. 2.43, $P < 0.05$). Based on August 2022 grid pricing, this resulted in a ~US$78 difference in individual carcass revenue, per head between Terminal Index top quartile and bottom quartile. Igenity® BeefxDairy demonstrates a viable opportunity to manage animals relative to their genetic potential during the feeding period, representing appropriate investment of precious feedstuffs and improving the production footprint of beef production out of dairy cows.

Keywords: genetic profiling, breed verification, calf management.
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Introduction

The use of genomic information can provide more reliable phonotypic estimates, which are essential for designing or refining selection indicators in cattle breeding programs (Pedrosa *et al*, 2023). Genomic selection has been shown to increase accuracy and decrease generation interval in US dairy cattle (Guinan *et al*, 2023). Dairy farms are working to increase the value of every calf produced on the farm. Many farms are utilizing beef semen on the dairy cow inventory which they need to maintain and get more lactations out of due to development costs, but those cows do not represent the future genetic attributes for replacement female creation (NAAB, 2022). As the calves are generated from beef semen on dairy cows, many producers want to genetically confirm three key attributes of those calves:Are they really beef x dairy cross? If so, what is the percent beef and the percent dairy of the individual?

Can I sort and manage these animals based on their ability to grow?

Can I have a prediction of what the likely revenue potential of this animal will be at harvest?

Beef production from dairy cows offers a unique opportunity to utilize animals less suitable for dairy herd replacement. However, optimizing their growth and carcass quality requires efficient management strategies. This study aims to evaluate the effectiveness of Igenity® BeefxDairy, a novel genetic testing technology, in identifying the genetic potential for growth and carcass traits on dairy herds.

Materials and methods

Cattle for this study were sourced as Beef x Dairy cross cattle entering commercial feedlots in the summer and fall of 2020. Samples for Igenity® BeefxDairy (Neogen Corporation) genetic testing were collected with tissue sampling units (TSUs, AllFlex) at time of arrival into the feedlot with other feedlot entry processing activities. Cattle were managed under the feedlot's normal management system and harvest dates were chosen by feedlot management based on lot based optimum harvest date. Cattle (438 steers and 564 heifers entered the feedlot at an average of 570.0 pounds and were fed an average of 274.1 days with average hot carcass weight at harvest of 844.6 pounds (Table 1).

Table 1. Summary statistics for carcass traits, closeout data, and Igenity scores.

Variable	n	Mean	SE	Range
Enrollment wt, lb	1002	570.09	3.22	355 to 950
Days on feed, d	1002	274.11	0.80	207 to 305
ADG, lb/d	1002	2.84	0.01	0.81 to 4.06
HCW, lb	1002	844.62	2.87	511 to 1149
USDA Quality Grade[1]	1002	2.83	0.01	2 to 4
USDA Yield Grade	1002	2.52	0.03	1 to 5
Rib-eye area, sq in	1002	13.86	0.05	9.1 to 19.5
Marbling	1002	498.27	3.30	310 to 898
Fat Thickness, in	1002	0.49	0.01	0.12 to 1.16
Igenity Score				
Marbling	1002	5.73	0.04	2 to 8
Rib-eye area	1002	6.86	0.04	3 to 10
12th-rib fat thickness	1002	4.92	0.04	1 to 7
HCW	1002	6.47	0.04	2 to 9

Results and discussions

Igenity BeefxDairy report information

The average breed composition of animals in this study was 55% Beef and 45% Dairy. Igenity® BeefxDairy scores represented the majority of the expected 1 to 10 range for the traits of interest: average daily gain (ADG); marbling (MARB); and hot carcass weight (HCW). Furthermore, Igenity Terminal Index (ITI) was calculated for each animal and then animals were sorted into quartiles based on ITI for further evaluation and reporting.

Performance information

Performance of the cattle in the feedlot and carcass trait information is summarized in Table 1, which represents tremendous variability which exists within the beef x dairy cattle production systems.

Robust positive relationships were found between Igenity® BeefxDairy scores and actual phenotypic performance across both steers and heifers for average daily gain (Figure 1), marbling (Figure 2), and hot carcass weight (Figure 3).

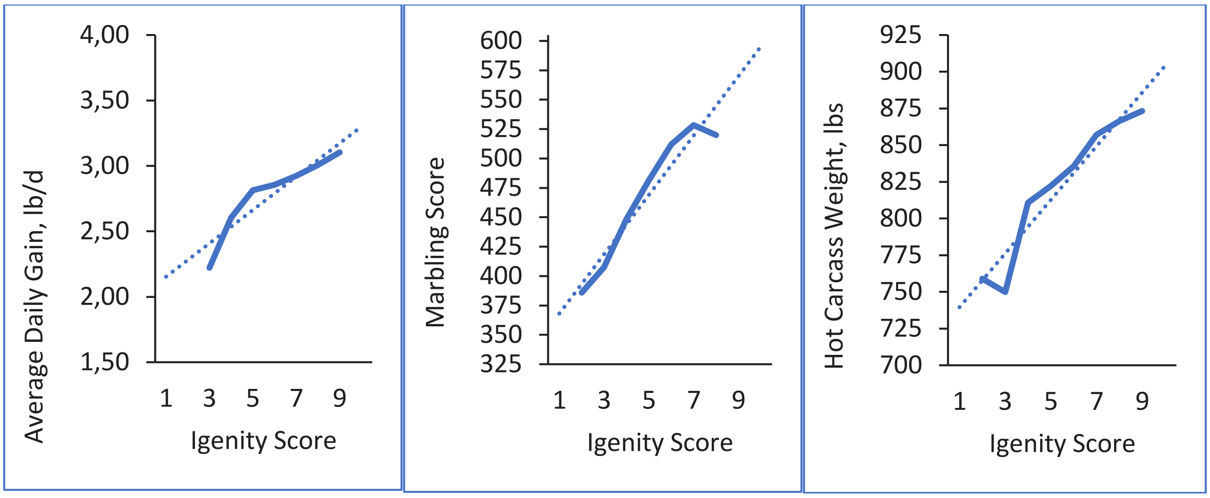

Figure A Figure B Figure C

Figure 1. Robust positive relationships were found between Igenity® BeefxDairy scores and actual phenotypic performance across both steers and heifers for average daily gain (Figure A), marbling (Figure B), and hot carcass weight (Figure C).

Igenity® Terminal Index (ITI) Quartile Analysis

Phenotypic performance for animals in the top 25% based on ITI were compared to animals in the bottom 25% for ITI and were tested for significant differences. Significant ($P < 0.05$) differences were found between top quartile ITI animals and bottom quartile ITI animals for enrollment weight, average daily gain, hot carcass weight, USDA yield grade and revenue (Table 2). Marbling score had a suggestive ($0.05 < P < 0.10$) difference between top quartile ITI animals and bottom quartile ITI animals. This is likely due to the modest emphasis of marbling in the ITI compared to weight.

Revenue for each individual animal was calculated based on the value-based pricing grid represented in Table 3. Top ITI quartile animals averaged $77.23 more per animal than the bottom ITI quartile animals.

Conclusions

Igenity® BeefxDairy is a viable tool to manage animals relative to their genetic potential during the feeding period, representing appropriate investment of precious feedstuffs and improving the production footprint of beef production out of dairy cows.

Table 2. Summary statistics and difference in revenue between the top 25% and bottom 25% of animals based on Igenity® Terminal Index (ITI).

	Top 25% N = 250	Bottom 25% N = 250	P value[1]
Average Terminal Index	6.71	5.22	P < 0.01
Enrollment Weight, lbs	586.10	564.03	P < 0.05
Days on Feed, d	271.13	272.47	P = 0.57
Average Daily Gain, lb/d	2.91	2.73	P < 0.01
Marbling Score[2]	504	489	P = 0.09
12th – rib fat thickness, sq in	0.49	0.49	P = 0.98
HCW, lbs	861.17	820.55	P < 0.01
USDA Quality Grade[3]	2.86	2.79	P = 0.11
USDA Yield Grade	2.59	2.43	P < 0.05
Revenue[4], $	1500.62	1423.39	P < 0.01
Difference per animal	**$77.23**		

[1] Calculated using a two-sided t-test, P < 0.05 considered significantly different.
[2] Marbling score: < 300 = Trace, 300 = Slight, 400 = Small, 500 = Modest, 600 = Moderate, >700 = Slightly Abundant
Grades: 1 = USDA Standard; 2 = USDA Select; 3 = USDA Choice; 4 = USDA Prime.
[4] Revenue calculated using the grid in Table 3.

Table 3. Beef carcass grid premiums and discounts used for carcass revenue calculation, $US/Carcass.

	USDA Yield Grade				
USDA Quality Grade	**1**	**2**	**3**	**4**	**5**
Prime	22.19	20.185	18.61	7.38	1.76
Choice	3.58	1.575	Base[2]	-11.23	-16.85
Select	-20.67	-22.675	-24.25	-35.48	-41.10
Standard	-22.19	-32.355	-33.93	-45.16	-50.78
Base Price/CWT: $179.28					
400-500 lb	-29.29		900-1000 lb		-1.07
500-550 lb	-22.64		1000-1050 lb		-5.00
550-600 lb	-11.57		Over 1050 lb		-16.07

[1] Prices summarized from USDA National Weekly Direct Slaughter Cattle Report August 3. 2022
[2] Premiums and discounts given $/CWT of hot carcass weight.

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Design of a medium density chip (microarray) for the genetic management of Pura Raza Española horses and related breeds

A. Encina[1]; M. Valera[2], N. Laseca[3], A. Rodríguez[1]; S. Demyda[3]; D.I. Perdomo-González[2], P.J. Azor[1], A. Gil[1], M. Ripollés[2], G. Anaya[3], I. González[1], C. Medina[3], M.J. Sánchez-Guerrero[2], J. Poyato[1], A. Molina[3]

[1]Real Asociación Nacional de Criadores de Caballos
de Pura Raza Española (ANCCE), Sevilla, Spain.
[2]Department of Agronomy, ETSIA, Universitu of Seville, Sevilla, Spain.
[3]Deparment of Genetics, University of Cordoba, Córdoba, Spain.
Corresponding Author: aencina@lgancce.com

Abstract

The GO EQUIGENOM is a project funded by Spanish Ministry of Agriculture, Fisheries and Food funds, which involves the development of an optimized medium density array for parentage control, disease diagnosis, detection of traits of economic importance and dedicated to research and development in equine genomics, specifically focused on the Pura Raza Española Horse (PRE) and other genetically related breeds. The design of this chip has been structured in several phases, currently in the last phase, validation in an independent PRE population. Firstly, various GWAS analyses were carried out to search for molecular markers associated with diseases of great importance for the PRE. In this sense, using information from animals diagnosed and genotyped with a HD array (867 animals) or MD array (738 animals) and sequenced (284 animals), 257 markers were selected. Subsequently, an exhaustive search for all genetic markers associated with equine diseases described in the bibliography, and in international databases such as OMIA, or Ensembl genome browser was carried out. To this search, markers related to other traits of economic importance, 1,240 markers have been found.

Second phase, a reference population was selected for imputation analysis to the currently available HD and MD array. For this purpose, a selection of 4,490 representative animals (1,907 males and 2,583 females) belonging to 1,718 studs has been made, based on the maximum variability available in this breed and the maximum effective contribution to increase the reliability of the assessments in the evaluations (greater number of offspring in performance control of the different selection objectives of this breed). Genotyping of these animals in HD (2,359) and MD (1,781) allowed us to select 26,017 markers for imputation to HD (99.97 % imputation) and 15,705 markers for imputation to MD (99.96 % imputation). Based on this information, a selection of markers with MAF ≥ 0.4 was carried out to fine-tune the genomic selection. Our results have demonstrated the effectiveness of this methodology for improving the accuracy of genetic assessments, especially in traits such as morphology, and to a lesser extent in others such as reproductive efficiency.

Finally, a selection of markers has been made to distribute them homogeneously over all the chromosomes of the equine genome. To this end, priority was given to the quality of genotyping of these markers (based on the information generated with previously genotyped animals) and their informative power in genomic assessment (maximum MAF). In the final design, there are 90,938 markers with an average distance of 26 k. Furthermore, 1,165 ECAY and 1,000 mitochondrial markers selected thanks to the 300 sequenced PRE animals have been included.

Once developed, the chip must be validated to ensure its efficacy and accuracy in the identification and evaluation of genetic markers associated with phenotypic traits of interest, such as morphology, sport performance and health. Final objective of the project is to integrate the information obtained through this MD array into existing selection programs for the PRE using an ssBLUP genomic assessment strategy, to improve the efficiency and accuracy of the selection of breeding stock.

Keywords: SNP, horses, genetic markers, genomic selection, equine.
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Introduction

The Pura Raza Española (PRE) horse is a native Spanish equine breed that has been officially recognized since the 15th century. Actually, the PRE is the most popular equine breed in Spain, representing the 70% of all registered equids. The total PRE population, 282,066 horses, are mainly located in Spain but also in other 67 countries (ANCCE, 2024). Recently, the Ministry of Agriculture, Fisheries and Food has awarded the innovative project GO EQUIGENOM to a consortium led by ANCCE and the Royal Spanish Federation of Selected Cattle (RFEAGAS), involving researchers from the PAIDI Group AGR-273 of the University of Seville, and AGR-158 of the University of Cordoba.

The aim of the project is to develop an economic medium density (MD) genotyping chip for the equine species, which simultaneously enables parentage control, the early diagnosis of hereditary and chromosomal diseases, the detection of economically important traits (coat colour markers, or those related to sporting ability, among others) and the development of genomic selection. Additionally, it will be complemented with the development of a robust and easy to use digital tool, which integrates the genetic information (from the breeding programme) and/or genomic information (generated by the chip) of each animal, allowing its use in a quick and easy to interpret way for the technician and for the breeder, in order to make breeding decisions, as well as the detection and control of hereditary diseases, avoiding a considerable economic expense and anticipating future problems and economic losses; with the consequent economic benefit for the farm.

The results of the project will have a direct impact, firstly, on the PRE breed, but also on the whole equine sector, both nationally and internationally.

Material and methods

Animals genotyped and sequenced

In total, 4,490 representative animals (1,907 males and 2,583 females) belonging to 1,718 studs were selected for genotyping, based on retained the maximum variability available in this breed and the maximum effective contribution to increase the reliability of the assessments in the evaluations (greater number of offspring in performance control of the different selection objectives of this breed).

Genomic DNA was isolated from blood or hair samples using a DNeasy Blood and Tissue extraction kit (Qiagen, Germantown, MD, USA). 2,359 horses were genotyped with the high density (HD) Affymetrix AxiomTM Equine 670K SNP Genotyping Array (Thermofisher, Spain), including 670,804 markers uniformly distributed across the entire genome (Schaefer *et al*., 2017). The raw genotype data were processed following the "Best Practices Workflow" procedure in the Axiom Analysis Suite package v5.0 with default parameter (DishQC $\geq$ 0.82). In the same way, 1,781 horses were genotyping with medium density (MD) GGP Equine Array (NEOGEN), including 70K SNPs distributed across the entire genome. The raw genotypic data were filtered using PLINK v1.9 software (Purcell *et al*., 2007). Only SNP markers showing a high-quality genotyping rate (call-rate >0.95), with a known genomic position located on the autosomes, mitochondrial and sexual chromosomes (XY) were kept.

Finally, 284 individuals were completely sequenced with a minimum depth of 4X. 144 of the samples were sent to NEOGEN Genomics (Lincoln, NE, USA) and 140 were sent to Psomagen, Inc. (Rockville, MD, USA) to the sequencing on the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA). After filtering by quality, the adapters were removed with the fastp software. The remaining high-quality sequences were aligned with the Equus Caballus v.3.0 (to obtain the ECA1 to ECA31, ECAX and mitochondrial sequences) and MH341179 (to obtain the ECAY sequence) using the Burrows-Wheeler Aligner (BWA) software. Variants from the ECA1 to ECA31, ECAX and mitochondrial chromosomes were called from the Equus Caballus v.3.0 reference genome while the variants from the ECAY chromosome were called from the MH341179 reference sequence. After variant calling (27.76M), SNPs were filtered out with a MAF < 0.2 and individuals which missingness was greater than 30% using PLINK v1.9. 1.6M variants were kept for whole-genome association analysis and population-based linkage analysis.

Imputation analysis

A reference population of animals genotyped at high density (2,359) and medium density (1,781) were used to select the markers that best imputed at high and medium density. Imputation and phasing were carried out using the Beagle 5.4 software (Browning *et al*., 2018; Browning *et al*., 2021). All SNPs (default) and genotype probabilities > 0.85, 0.90, and 0.95 were considered for imputed genotype calls. The accuracy of imputation was calculated by the ratio of true and imputed genotypes and the presence of missing and non-missing SNPs in the imputed population.

Bibliographic search for genomic markers associated with traits of economic importance

An exhaustive search for all genetic markers (SNPs, CNV, Indel...) associated with traits of economic importance such as diseases, coat colour and control parentage described in the bibliography, and in international databases such as OMIA, Ensembl genome browser, ISAG etc. was carried out.

Final chip design

For the final selection of the markers to be included in the array, a chromosome-to-chromosome simulation was performed trying to provide an average distance of 26k between markers and had a maximum genotyping quality. The genotyping quality was determined by Thermofisher according to internal parameters and empirical results, as well as by the type of allele detected.

Results and discussion

SNPs and traits of economic importance in the horse

A total of 1,240 markers were selected from the literature and published international databases for different traits of economic importance for the horse such as diseases, behaviour, sport performance, coat colour…

SNPs selected to improve imputation

Genotyping HD (2,359) and MD (1,781) animals allowed us to select 26,017 SNPs markers for HD imputation (99.97% imputation) and 15,705 markers for MD imputation (99.96% imputation)..

Final chip design

The number of filler markers was 45,263 SNPs. The total number of markers included in the final design was 90,938 SNP.

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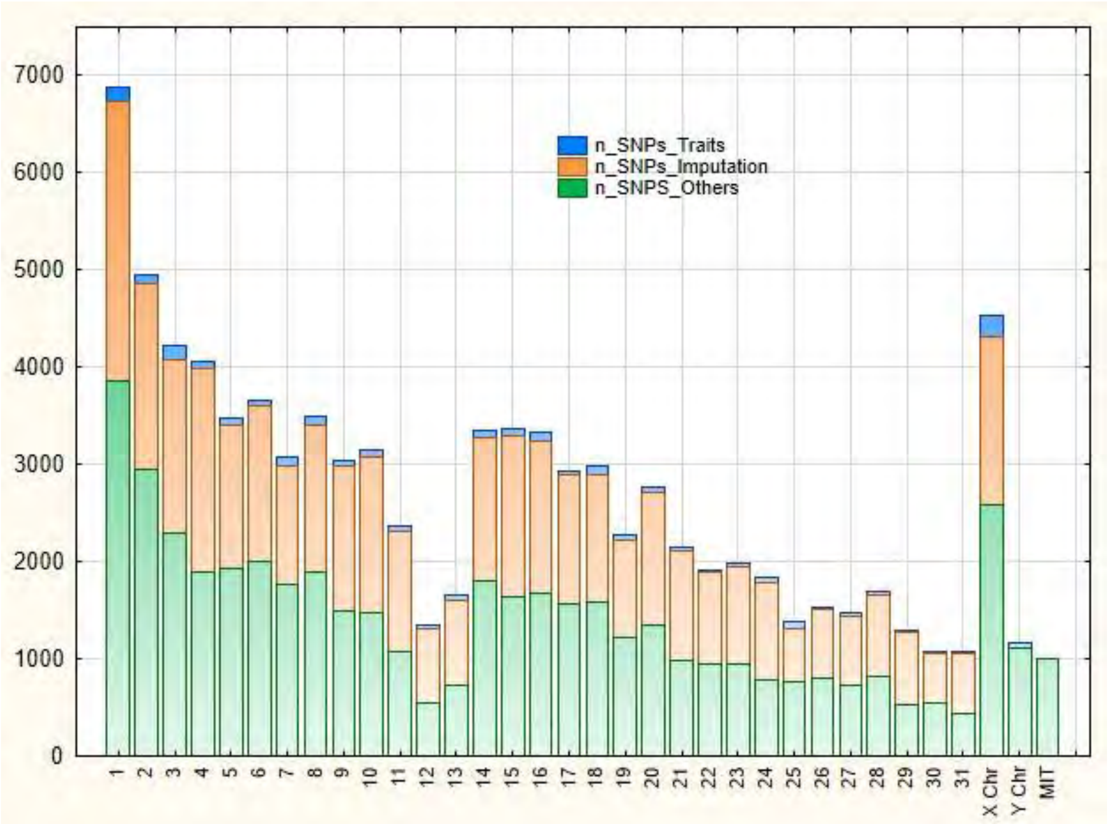

Picure 1. Total number of SNPs found on each chromosome.

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Improving animal health and welfare by using sensor data in herd management and dairy cattle breeding – a joint initiative of ICAR and IDF

C. Egger-Danner[1], I. Klaas[2], L. F. Brito[3], K. Schodl[1], J. M. Bewley[4], V. Cabrera[5],
M. Haskell[6], M. Iwersen[7], B. Heringstad[8], K. F. Stock[9], A. Stygar[10], R. van der Linde[11],
M. Hostens[12], N. Charfeddine[13], N. Gengler[14] and E. Vasseur[15]

[1]ZuchtData EDV-Dienstleistungen GmbH, Dresdner Str. 89, 1200 Vienna, Austria
[2]DeLaval International AB, Gustaf de Lavals Väg 15, 14721 Tumba, Sweden
[3]Department of Animal Sciences, Purdue University, West Lafayette, IN, 47907, USA
[4]Holstein Association USA, 1 Holstein Place, PO Box 808,
VT 05302-0808 Brattleboro, USA
[5]University Wisconsin-Madison, 1675 Observatory Dr., WI53706 Madison, USA
[6]SRUC (Scotland's Rural College), West Mains Road, Edinburgh EH9 3JG, UK
[7]Centre for Veterinary Systems Transformation and Sustainability, Clinical Department
for Farm Animals and Food System Science, Veterinärplatz 1, 1210 Wien, Austria
[8]Norwegian University of Life Sciences, P. O. 5003, 1432 Ås, Norway
[9]IT Solutions for Animal Production (VIT),
Heinrich-Schroeder-Weg 1,27283 Verden, Germany
[10]Bioeconomy and Environment, Natural Resources Institute Finland (Luke),
Latokartanonkaari 9, 00790 Helsinki, Finland
[11]ICAR, Arthur van Schendelstraat 650, 3511 MJ Utrecht, Netherlands
[12]College of Agriculture and Life Sciences, Cornell University,
272 Morrison Hall, Ithaca, New York, USA
[13]Conafe, Ctra. de Andalucía , Km 23,600- Apdo. 31, 28340 Valdemoro, Spain
[14]TERRA Teaching and Research Center, Gembloux Agro-Bio Tech,
University of Liège, 5030 Gembloux, Belgium
[15]McGill University. Ste Anne de Bellevue, H9X 3V9, QC Canada
Corresponding Authors: egger-danner@zuchtdata.at; ilka.klaas@delaval.com

Abstract

Digitalization is advancing with rapid developments in farm technologies, which has the potential to revolutionize dairy production and to improve its long-term sustainability. Farmers are increasingly using sensors and other technologies that monitor various parameters on their farms. Large amounts of data are collected, but just a small fraction is currently used along the dairy value chain. This has motivated the International Committee of Animal Recording (ICAR) and the International Dairy Federation (IDF) to start a joint initiative aiming at providing guidelines and best practices for using data from sensors across systems and applications, with a focus on functional traits such as health and animal welfare. The key partners are the ICAR Functional Traits Working Group and the IDF Standing Committee of Animal Health and Welfare who have formed a network of representatives from various stakeholders and leading scientists. Research and approaches to improve the usability of data are discussed to promote knowledge transfer and practical implementation in the dairy industry. Experiences and best practices are exchanged, and recommendations for the use of sensor data are being elaborated. The results will be broadly disseminated through ICAR and IDF avenues. Furthermore, the collaboration among multidisciplinary experts is enabling a holistic approach to the current challenges faced by the worldwide dairy industry and will facilitate cutting-edge research and innovation. The initiative will be presented, with

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a progress report on reference standards, harmonized definitions, and terminology, as well as recommendations and best practices regarding data cleaning and editing and definition of novel traits using data from sensor technologies in herd management and genetic evaluations.

Keywords: interdisciplinary network, wearable sensor, animal welfare, animal health, rumination, standardisation, precision dairy, smart farming.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 10: New approaches in the field of functional traits for management and breeding

Introduction

Recent advancements in sensor technologies have significantly enhanced the capacity to technically support farmers and their advisors in monitoring the health, performance, and welfare of dairy cattle. As presented in the systematic review by Stygar *et al*. (2021) and in other focused reviews (e.g., Hogeveen *et al*., 2021), a wide range of commercially available sensor systems exists and promise significant gains in the understanding and improvement of welfare in livestock. The technologies cover the spectrum from wearable devices with multiple functions (e.g., tracking of physiological parameters) to environmental sensors that monitor housing and climatic conditions, and collectively aim to provide actionable information about animal health, reproductive status and well-being. Most wearable sensors rely on 3D accelerometers, which measure acceleration or motion to quantify cow behaviour. Manufacturers use algorithms and pattern recognition to enhance the raw accelerometer data and produce sensor systems which recognize rumination, eating, lying, standing, and other behaviours using the data from sensors on the cow's leg, neck, ear, or tail. The integration of sensor systems in livestock farming presents numerous opportunities to enhance animal health, performance and welfare, supporting farmer decision making on individual cow and group level and farm efficiency. However, while large amounts of sensor data are being collected, only a small fraction is currently used on farms, in genetic evaluation and breeding programs, or along the dairy value chain. To maximise the use and potential of data derived from sensor systems for herd health, production management, genetic and genomic selection, and welfare quality assurance programs, we need to address several challenges. Combinating sensor-derived data with routinely recorded data may be a key factor for more confidence in the use of data from advanced technologies and sensor-based herd management systems among stakeholders, including farmers, consultants, authorities, dairy processors, breeding companies, and consumers. The currently low fraction of commercially available sensor systems with independent validation (14%; Stygar *et al*. 2021) shows the clear need for more extensive validation of sensor systems in diverse farming environments, across different farm and management systems and geographical locations. Comparing different sensor systems poses a significant challenge due to the lack of standardized and agreed on criteria for evaluation and validation.

This has motivated the International Committee of Animal Recording (ICAR) and the International Dairy Federation (IDF) to start a joint initiative aiming at improved usability of data across sensor systems and applications. The initiative leaders are the ICAR Functional Traits Working Group (ICAR FTWG) and the IDF Standing Committee of Animal Health and Welfare (IDF SCAHW) in collaboration with international experts from academia and industry organizations. The primary aim of this initiative is to promote the integrated use of sensor data and derived novel traits along the dairy value chain. Standardisation and harmonisation will be supported through guidelines that include basic definitions and recommendations regarding data processing and use. Topics addressed include reference methods, data cleaning and validation, trait definitions for herd management and selective breeding, genetic improvement, and quality assurance.

This paper's main objective is to present the methods and working strategies that the joint initiative will use to achieve its aim, using rumination as a case study.

Definitions and reference methods

With the increasing adoption of sensor systems in dairy farming, the number of different technologies provided by different manufacturers has increased. While the diversity offers farmers the opportunity to choose a system that best suits their own farming set-up, it has increased the variation in type of measurements, type of data generated, algorithms, sensor output, attention lists and monitoring key performance indicators. This poses challenges to advisors, researchers and other stakeholders when interpreting sensor outputs across technologies and brands and comparing herd performance. The joint ICAR/IDF initiative will provide definitions for health or behaviour situations and conditions that can be measured by sensor systems. Those definitions will be based on the primary premise that the condition of interest should be detected or quantified irrespective of the applied technologies and with a level of accuracy that enables users to make meaningful decisions based on the supplied information. To promote the use of data from sensor technologies along the dairy value chain, the collection and use of reliable data is key, emphasizing the need for validation or at least documentation of the performance against a reference standard, i.e. to show that the technology measures what it is supposed to measure. Agreement on a reference standard method is crucial as is common understanding on how to perform comparisons and report results. Furthermore, establishing a minimum level of accuracy might be helpful. Irrespective of the use of sensor data (management support, research, genetic evaluation), the processes of validation and standardization are key to ensuring that the data collected from diverse sources are comparable, reliable, and usable.

While developing these standards and recommendations we chose a participatory approach to address stakeholder concerns such as intellectual property considerations and achieve a common understanding with manufacturers. If, for instance, a product is offered as rumination sensor, the output should be easy understandable and reflect the actual rumination behaviour of a cow.

Harmonized terminology is fundamental to the standardization process, which comprises the development of common definitions and descriptions. The very positive experiences with the ICAR Claw Health Atlas (ICAR, 2020), presenting different lesions in the distal limb of bovine with harmonized names and descriptions, illustrates the potential for success of the current initiative on sensor data relating to health and welfare of cattle, with first results to be expected for rumination. The IDF action team on sensor-based health management recently published guidance for udder health management with sensor systems (Hogeveen *et al*, 2021). ICAR and IDF have experience in leading the standardization efforts with strong engagement to provide the crucial framework and promote a more integrated and forward-thinking approach to dairy farming technology.

Achieving agreement on a ‘gold standard’ which is appropriate across measurement approaches, is difficult for a biological phenomenon such as rumination where interactions and influences on its expression must be considered (e.g., farm environment, nutritional input, other management factors). In this case, rumination time may be the most suitable biological parameter to allow quality assessment and comparison of sensor outputs for the rumination process. Here, knowledge on the major sources of variability in rumination time (which include diet composition, breed, and health status) exists and facilitates defining standard criteria and conditions for validation. The target for a rumination sensor may be, for example, a correlation of 80% between sensor-recorded rumination time and the visually recorded rumination time in healthy cows of a given breed on a given diet. Because certain layers of complexity or specific sources of variability across herds and animals may or may not be accessible in any environment, reference standards need to be adaptable and robust enough to cope with that. A deep understanding of animal physiology and behaviour within the range of farming settings, is needed to establish baselines and thresholds for deviations that are both scientifically valid and practically applicable across diverse farming conditions.

Such agreed references and standards are indispensable for the validation and calibration of sensor outputs, because they help to ensure that accurate and actionable data is used across the dairy value chain, that products and services within the industry are comparable, and uniform approaches to data interpretation and decision-making can be used. Thus, the efforts of ICAR and IDF to harmonize definitions and develop industry-wide standards and guidelines are not merely procedural but are vital for the advancement and sustainability of dairy production on a global scale. The interdisciplinary approach and wider exchange with stakeholders will contribute to acceptance and adoption of standards despite the operational challenges which may be substantial. ICAR is offering tests for milk (recording) devices according to agreed standards, which are described in the ICAR guidelines (www.icar.org). Devices that pass such test receive an ICAR certificate. One of the aims of this initiative is to elaborate and recommend options for validation of sensor systems.

Data cleaning and quality of sensor data

Sensor data can be noisy, and the output variables that are presented are the result of the use of an algorithm that transforms raw sensor values into variables such as rumination time or activity level. Typical errors in sensor data include outliers, missing data, bias, drift, noise, constant value, uncertainty, and stuck-at-zero conditions (Teh *et al*, 2020). Examples for cleaning sensor data can be found in Schodl *et al* (2022). Proper data integration and interpretation is dependent on reliable assignment of records to individual animals and on coordination of times of recording. Furthermore, whenever working with sensor data, it must be considered that the processes used for cleaning, procedures and transformation of raw sensor data into variables such as rumination time or activity values are usually proprietary to the sensor companies, which means that these procedures cannot normally be shared or used by others. Analyses of data structure and quality require an understanding of the type of measurements, i.e. single-shot measurement at a certain point in time versus already summarized or averaged figure (Bouchon *et al*, 2019). Visualisation of the data is helpful in understanding the type of distribution, patterns, gaps, outliers, etc. (Unwin, 2020). Detecting and removing outliers is a very important step in the data cleaning process. Another important aspect is the detection of technology -related noise such as measurement drift (Giannoni *et al*, 2018; Munirathinam 2021). The same is the case for sensor calibrations or replacement and software updates and ideally this information should be available from the sensor companies. Finally, all data cleaning steps should be documented and reported. The integration and availability of other data sources (calving date, breed, events like in heat etc.) is beneficial for data cleaning and validation.

Use of sensor technology for herd management and welfare assurance

Rumination behaviour data from sensors has been used successfully on working dairies around the world, implying availability of mature technology with proven results across multiple manufacturers. Changes in rumination are linked to feed intake, and a reduction may indicate health issues (including metabolic and non-metabolic disorders, like mastitis or pneumonia). Identification of drops in rumination can help identify individual cows which require manager or veterinary intervention. Especially in early lactation, monitoring of rumination patterns is of high value for timely disease detection and proper intervention and treatment. At the group level, rumination data is a useful tool to monitor and manage feeding during the lactation or to identify stressful situations in the herd. The particular value of rumination data from early lactation is obvious due to the increased risk of metabolic diseases. Early identification of affected cows can facilitate a successful intervention through therapeutic treatment. Whereas research often focuses on the sensitivity and specificity of sensor-based alerts for identifying disease, high false positive rates (where unaffected animals are identified as a positive case) may have practical benefits in larger operations through reducing labor costs.

As rumination decreases around the time of an estrus event, the accuracy of estrus detection can be improved by including rumination data. In general, sensor-based alerts rely on deviations from each individual cow's baseline. Thus, consistency within a technology from the farmer's perspective may be at least as important, or even more important than the relationship to a 'gold standard'. However, direct comparisons of rumination times across technologies can lead to false conclusions. Studies with multiple rumination technologies attached to the same cows have shown that differences in rumination time across technologies can be large (Zambelis *et al*., 2019). Guidelines, video tutorials and protocols for visual assessment of rumination are available and support collection of observation data.

To date, sensor technologies are rarely used for welfare assessment in quality schemes (Stygar *et al*, 2022). However, research efforts have been made to develop algorithms which classify dairy cow welfare into good, moderate, and poor using data from various sensors (Stygar *et al*, 2023). Standardization of rumination definitions across manufacturers is necessary for the development of a global welfare assessment algorithm from a technical point of view. Common implementation practices will also be beneficial in terms of reducing the cost of sensor-based welfare assessment.

However, wider use of the rumination data as for welfare quality programmes, breeding purposes require interoperability of data from various sensor systems, such that definition of the rumination trait(s) can be performed across data sources. Agreement on standards and common definitions is prerequisite.

Use of sensor data for genetic improvement of novel traits

The large number of variables recorded by sensor technologies can be integrated with routinely collected variables for deriving novel welfare indicators for management and breeding purposes (Brito *et al*., 2020). Ideally, the derived traits should capture the biological mechanisms of interest, be heritable and repeatable, and be clearly defined and evaluated in a standard way across breeding programs. After identifying the variables of interest (e.g., rumination time), statistical models need to be developed for the genetic analyses. Modelling systematic effects (e.g., parity, lactation stage, herd, season, diet, reproductive stage, housing time) that significantly influence the target trait is enabled by integrating sensor data into the established data infrastructure for dairy cattle. Comprehensive genetic analyses of the target trait(s) and possible correlated traits provide the genetic parameters for setting up genetic and genomic evaluations: additive genetic (co)variances, heritability, repeatability, genetic correlations. In the context of sensor-based measures of health and welfare, multiple trait analyses may include, e.g., direct health traits, feed efficiency, methane emissions, milk production and composition, productive life, reproductive performance. Additional genome-wide association studies and functional genomic analyses can contribute to better understanding the genomic background of the target trait.

Functional traits are integral parts of the breeding goals and breeding programs of dairy cattle. However, the main challenge for health- and welfare-related traits is the often limited availability of phenotypic data (e.g., veterinary diagnoses, farmer observations, records from hoof trimming) and mostly low heritabilities. The continuous data collection provided by sensor technologies offer new possibilities for multiple trait solutions with the potential for integrated data usage in future genetic evaluation routines. Key challenges to be overcome include access to data, protocols for integrating data from different data sources, many different data providers, tools with different information from different data providers and different resolution of the information, a lack of background information on the data, regular updates with respective needs to adjust the data pipelines for genetic analyses, and the large amount of data (Egger-Danner *et al*. 2022).

However, research on the use of sensor data for breeding is ongoing, with publications on fertility (Heringstad and Wethal, 2023), resilience traits (Poppe *et al*. 2022) and others. With regards to rumination, focus has been mainly on 'Rumination Time (RT; min/day)' which represents the time per day a cow spends ruminating. Derived variables that have been proposed include daily average RT, 2-hour averages of RT (Hut *et al*., 2022), changes in RT during specific time periods as well as trait definitions based on deviations and more recently, longitudinal measurements of RT have been suggested as measure of overall resilience. For RT, heritability estimates of 0.14 - 0.45 have been reported (Byskov *et al*., 2017; Lopes *et al*., 2022). Future research will show whether breeding and management purposes will benefit from combined use of several sensor-derived traits (e.g., rumination together with activity).

Conclusions

Both ICAR and IDF see great benefit in providing standards and recommendations for the use of sensor data to improve cow health and welfare to the benefit of farmers, manufacturers, dairy herd improvement and breeding organizations, consultants, researchers, dairy processors and consumers. Leveraging the synergies of both organizations will facilitate communication, collaboration between stakeholders and implementation along the dairy chain, e.g. herd management, breeding and welfare quality assurance programs. A participatory approach involving researchers and industry is key to understanding the interests, needs, concerns, and expertise of different stakeholders and develop standards that would benefit all. Expanding our discussion to all stakeholders will bring together the different aspects and approaches, lessons learned and recommendations. The aim is to develop harmonized definitions and terminology that are feasible, acceptable, beneficial, and implementable for the different stakeholders. Improving the quality and comparability of data will encourage use beyond current applications and the development of more accurate tools, thereby contributing to increased sustainability through productivity and economic performance, improved animal health and welfare, and better use of resources and reduced environmental footprint.

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A Gigacow example, weather data in longitudinal studies on heat stress

T. Klingstrom[1], J.I. Ohlsson[1], P. Ask-Gullstrand[1], Lena-Mari Tamminen[2] and D.J. de Koning[1]

[1]Department of Animal Biosciences, Swedish University of Agricultural Sciences,
Box 7023, 750 07, Uppsala, Sweden
[2]Department of Clinical Sciences, Swedish University of Agricultural Sciences,
Box 7054, 750 07, Uppsala
Corresponding Author: tomas.klingstrom@slu.se

Abstract

Large and varied datasets from modern precision livestock farming equipment can be used to tackle increasingly complex research questions such as defining a phenotype of heat stress resistance in dairy cattle milk production. Heat stress causes drops in production and changes in behavior that are difficult to detect unless the cow in question is monitored over longer periods and more variable conditions than conventional experimental designs may allow. We present here how the SLU Infrastructure for dairy data collection, Gigacow support complex data-driven dairy research using heat stress as a case study. SLU Gigacow gathers daily data updates from a set of Swedish commercial dairy farms with a digital farm management system (FMS) overseeing either a robot milking system or milking parlor, and links the data per-cow to individual 50k SNP genotypes and national animal database information including trade history, pedigree and health events. The way SLU Gigacow support the project *From Sensitive to Robust Athlete – Exploring the Opportunities of Genomic Selection to Help Dairy Cows Cope With Increasing Temperatures* provides a good case study on how the data and knowledge generated by:

- Validating an external dataset from the Swedish Meteorological and Hydrological Institute.
- Collecting daily milking data from farms over multiple lactations.
- Providing cost coverage to Gigacow farms for genome analysis services and collect genetic data generated from the genome analysis in collaboration with Växa Sverige and Nordic Cattle Genetic Evaluation.
- Continuously evaluating, and including new data analysis models to support researchers and ensure that lessons learned in different projects can be included in new studies.

The collection of high quality longitudinal datasets was a key motivation for the establishment of SLU Gigacow and the infrastructure actively tries to prioritise data collection efforts to support future research and industry needs.

Keywords: data collection, infrastructure, heat stress.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 10: New approaches in the field of functional traits for management and breeding.

Introduction

As the digital transformation of society progress it is apparent that agricultural research institutions must adapt and ensure that a mixture of competency in data science and agriculture is maintained within the organization. The rapid pace of development also makes it difficult for researchers to keep up to date on new technology or data sources becoming available. Researchers affiliated with a data collection infrastructure can therefore have an important role in evaluating technology and ensuring that colleagues have access to new technologies to generate data for their research.

The SLU Infrastructure for dairy data collection, Gigacow (SLU Gigacow) is a data collection infrastructure at the Swedish University for Agricultural Sciences previously presented at ICAR 2023 (Ohlsson et al. 2023). The infrastructure collect data from a wide variety of sources and its role in the project *From Sensitive to Robust AthleteExploring the Opportunities of Genomic Selection to Help Dairy Cows Cope With Increasing Temperatures* provide an example of how a data collection infrastructure can support dairy research.

A SWOT analysis of SLU Gigacow

Working with a data-driven approach to develop a research infrastructure requires both patience and trust as the start-up process of a general data platform require more time and consideration than ad-hoc data collection for a single project. Looking at the Strengths, Weaknesses, Opportunities and Threats to the Gigacow infrastructure highlights the following table:

Strengths	**Opportunities**
Reduced technical overhead in projects	Integration of new data sources
Continuous improvement of data collection	Standardised APIs make big data accessible
Provides a platform for development	Support research on data models
Can integrate results in multiple projects	Systematic gap-analysis for new technologies
Threats	**Weaknesses**
Slow start before first results are generated	Prioritisation of data sources
Little funding for indefinite projects	Lack of data specialists in agriculture
Risk of obsolescence	False negatives are hard to detect

Timeline of the project and SLU Gigacow

The project application was written in the early spring of 2022 and funding began on 1 January 2023, data collection from the project however began already in 2020 as climate change and warming was identified as an area where researchers at SLU where likely to wish to use SLU Gigacow. In Sweden (Figure 1)

In this project data from the herd management system of farms participating in the SLU Gigacow network is combined with genotypes from the Nordic Cattle Genetic Evaluation and meteorological analysis data collected from the open data collection of the Swedish Meteorological and Hydrological Institute (SMHI).

To evaluate the need for on-farm temperature sensors SLU Gigacow has cross-referenced the Mesoscale Analysis (MESAN) model from the Swedish Meteorological and Hydrological Institute (SMHI) with historical measurements from SLU Lantmet, which collates climate data from weather stations distributed across the country. The MESAN model interpolates climate data from SMHI's national network of weather stations to model conditions across Sweden on a 11x11 km grid. Measurements from MESAN on temperature and humidity were deemed to be of

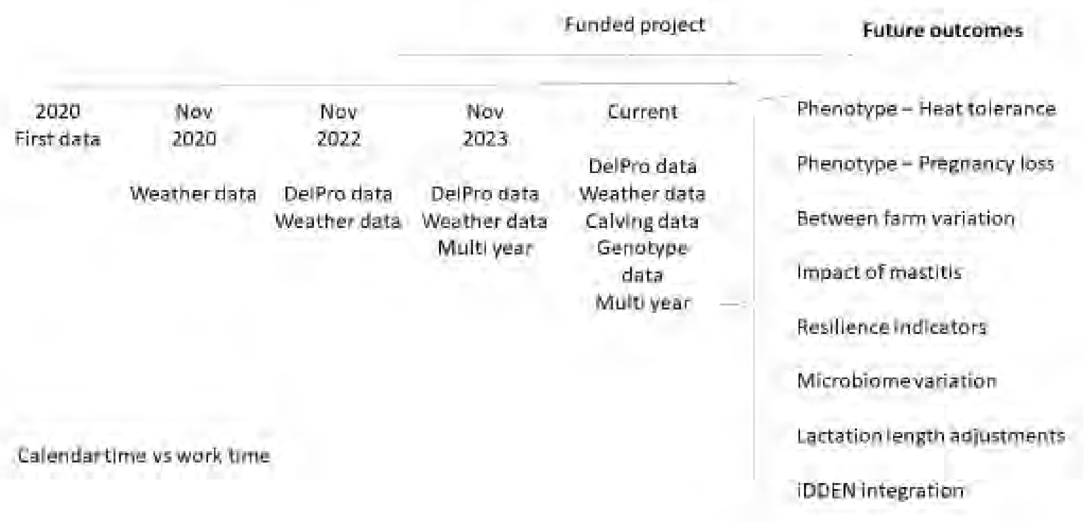

Figure 1. Timeline of the project and SLU Gigacow.

sufficient quality while wind and precipitation could differ significantly when comparing on-site measurements with the MESAN model. MESAN model temperatures from each farm's matching grid square was therefore used to infer the Temperature-Humidity index at each farm in the project.

Strengths

- Faster per project
- Continuous improvements to data collection
- Provides a platform for development
- Provide an integration platform.

Opportunities

- Integration of new data sources
- API:s "only" legal issues limit data.
- Methods development
- Gap analysis
- Model building.

Threats

- Slow start
- Long term funding
- Obsolescence

Weaknesses

- Prioritisation
- Recruiting
- Missing data
- Dedicated staff
- Data separation in "data lakes"

Performance monitoring in the cattle sector innovates with 3D imagery

T. Dechaux[1], A. Lebreton[1], Y. Do[1], M. El Jabri[1], L. Delattre[2], C. Gillé-Perrie[4], C. Allain[1] and M. Bruyas[3]

[1]Institut de l'Elevage, 149 Rue de Bercy, F-75595 Paris, France
[2]3D Ouest, 5 Rue Louis de Broglie, 22300 Lannion, France
[3]Eliance, 149 Rue de Bercy, F-75595 Paris, France
[4]Races de France, 149 Rue de Bercy, F-75595 Paris, France

Abstract

Performance recording and phenotyping of beef cattle are on the verge of a new era. Indeed, Bruyas *et al*. (2023) have recently shown that it is possible to collect three-dimensional images of beef calves at weaning using a 3D device suitable for high-throughput phenotyping and to automatically extract morphological parameters (heights, widths, volumes, surfaces, etc.). The aim of this new study, which is part of the PHENO3D project, was to develop models based on artificial intelligence to estimate Body Weight (BW) and morphological linear scores based on previously estimated body measurements. To achieve these objectives, 1194 Charolais calves aged 4 to 12 months and weighing from 90 to 620 kg were scanned on 14 commercial farms. Most of them were scanned twice, allowing a total of 2210 3D images to be acquired. Reference measurements were collected on these same animals: each calf was weighed on an electronic scale (BW) and scored by 3 experienced technicians. Scoring resulted in the estimation of 10 elementary scores, which then allowed for the calculation of 2 synthetic scores (ratings from 1 to 100) used ultimately for genetic selection: 1. the muscular conformation (MUS), relating to the musculature of the animal and 2. the size (SKE) of the animal relating to skeletal development. To predict BW, MUS, and SKE, various Machine Learning (ML) algorithms such as Extreme Gradient Boosting, Random Forest, and Elastic Net Regression were trained using 70% of the images and tested on the remaining 30%. The models were evaluated using Mean Absolute Error (MAE) and Spearman's correlation (rs). The repeatability of predictions was also assessed by Spearman's correlation between estimates made for the 1st image and the 2nd (when available). For BW, 1462 images were used for the learning model and 356 images for testing. For the best model, BW was predicted with a rs of 0.97 and an MAE of 12.1 kg (4.2%). The repeatability rs was 0.98 between the two images. For MUS and SKE, 1267 images were used to train the model and 308 images for testing. For the best model, MUS and SKE were predicted with respective rs values of 0.78 and 0.75, and MAEs of 7.1 (14.5%) and 6.5 (11.9%). The repeatability rs for these predictions for MUS and SKE were respectively 0.81 and 0.87. The Spearman's correlation for prediction and repeatability of MUS and SKE were higher than the average results obtained by experienced scorers during annual certification sessions. These results show that automating the scoring process using a 3D scanner combined with ML models is possible and allows for more accurate and repeatable estimates than those obtained by long-term scorers. The performances achieved on the Charolaise breed allow us to consider multiplying our models on the 9 other beef cattle breeds scored today (Limousine, Blonde d'Aquitaine, Salers, Aubrac, Parthenaise, Rouge des Prés, Blanc Bleu, Gasconne des Pyrénées, and Bazadaise) and to project towards the industrialization of the PHENO3D solution.

Keywords: Phenotyping, Calves, Weaning, 3D imaging, Artificial intelligence, pheno3d.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 10: New approaches in the field of functional traits for management and breeding

Introduction

In the French beef cattle sector, genetic selection heavily relies on the monitoring and phenotyping of a diverse animal population (Griffon *et al*., 2017). This crucial process for breeding organizations, is predominantly conducted through a network of affiliated farmers and involves initial phenotyping usually conducted around the calves weaning. Technicians from either the Eliance network or breeding organizations of the Races de France network undertake on-farm data collection, encompassing animal weighing and morphological traits assessment. The morphological evaluation encompasses 19 scores, evaluating both muscular and skeletal development, as well as functional traits. Trained technicians visually perform this linear scoring, following the detailed methodology outlined by Lajudie *et al*. (2014) (Section 3 - ICAR Guidelines for Beef Cattle Production Recording). Despite the effectiveness of visual scoring, it requires extensive training and is susceptible to subjective biases. Hence, there is a pressing need in the beef sector to automate scoring processes to reduce training costs and minimize the impact of human biases on measurements.

To tackle these challenges and modernize the phenotyping process, the PHENO3D project was launched, representing a collaboration between Eliance (the French federation of breeding advising and service companies), Races de France (French federation of breeding organizations), and Idele (the French Livestock Institute). The project aims to harness 3D imaging technology and artificial intelligence to streamline phenotyping by automating weight measurement and morphological scoring of beef calves (Bruyas *et al*., 2022). An initial milestone of PHENO3D involved the development of a 3D scanning device capable of accurately capturing the three-dimensional profiles of weaning-age beef calves and extracting relevant morphological data from these images. The validation of this technology, following a methodology similar to that described by Le Cozler *et al*. (2019), compared live animal measurements with those derived from 3D images, yielding promising results (Bruyas *et al*., 2023). This successful validation marked a significant advancement, reinforcing the project's trajectory and paving the way for subsequent phases of development and implementation.

Material and methods

Technology and image processing

The 3D scanner utilized in this investigation was previously detailed by Bruyas *et al*. (2022). It comprises a modular gantry with dimensions of 3 x 2.5 x 0.7 meters and incorporates ten depth sensors (see figure 1a). These sensors synchronize their data acquisition processes to produce comprehensive 3D images of the entire body of beef calves. Animals are scanned while in motion, passing beneath the device by walking or trotting, thereby enabling high throughput phenotyping. Integrated algorithms automatically enhance the images, streamlining the process for immediate image analysis. Through preprocessing and new feature extraction techniques, hundreds of indicators are automatically extracted to estimate body traits from 3D images. The developed methodology facilitates the automatic extraction of key body traits (Do *et al*., 2024), such as hip width (HW), chest depth (CD), wither height (WH), sacrum height (SH), body volume (BV), body surface (BS), and other measurements across numerous body slices (see figure 1b). All these body measurements were subsequently utilized to construct the prediction models developed in this investigation.

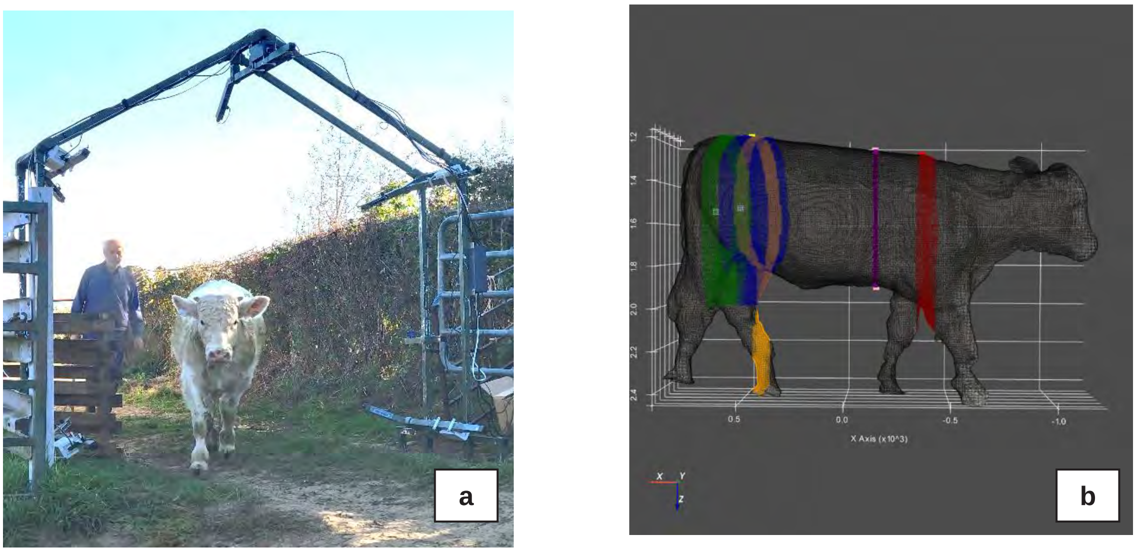

Figure 1. 3D scanner used for the trial (a) and automated image analysis (b).

Animals and reference data

To conduct our study, we scanned a total of 1194 Charolais calves, aged 4 to 12 months, and ranging in weight from 90 to 778 kg, across 14 commercial farms. Most of these calves underwent two scans, resulting in a total of 2210 3D images. All captured images were securely stored in the Microsoft Azure cloud platform.

Concurrently, reference measurements were obtained from these same animals: each calf was individually weighed on an electronic scale to determine its body weight (BW) and assessed by three experienced technicians. The visual scoring process led to the estimation of 10 elementary scores, each rated on a scale from 1 to 10, where a lower score indicates lower values and a higher score indicates higher values, based on frame and muscularity traits. The assessed traits are detailed in figure 2 below.

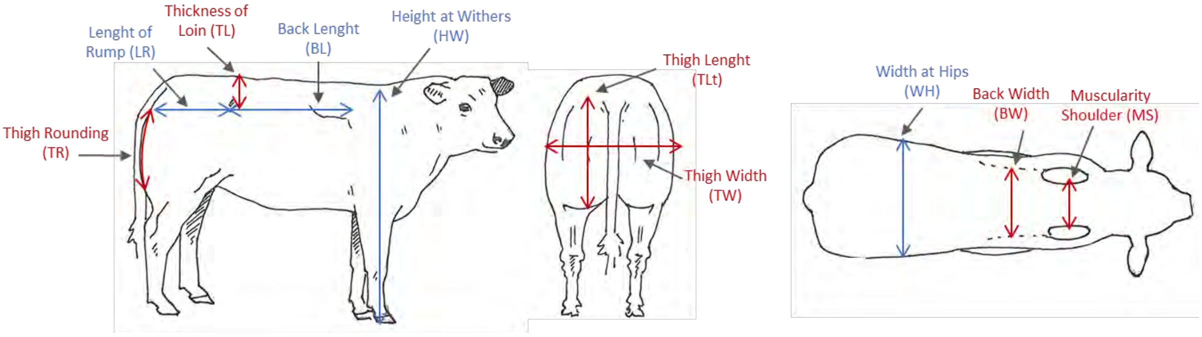

Figure 2. Frame traits (blue) and muscularity traits (red) used for linear scoring.

The 10 elementary scores were subsequently utilized to compute 2 synthetic scores, each rated on a scale from 1 to 100, which are ultimately employed for genetic selection purposes:

1. Muscle development (MUS), which pertains to the overall musculature of the animal.
2. Skeletal development (SKE), which relates to the body frame of the animal.

Figure 3 below displays images of calves exhibiting extreme morphologies for MUS and SKE. The four images depict calves of roughly the same age but with notable variations in size and muscularity.

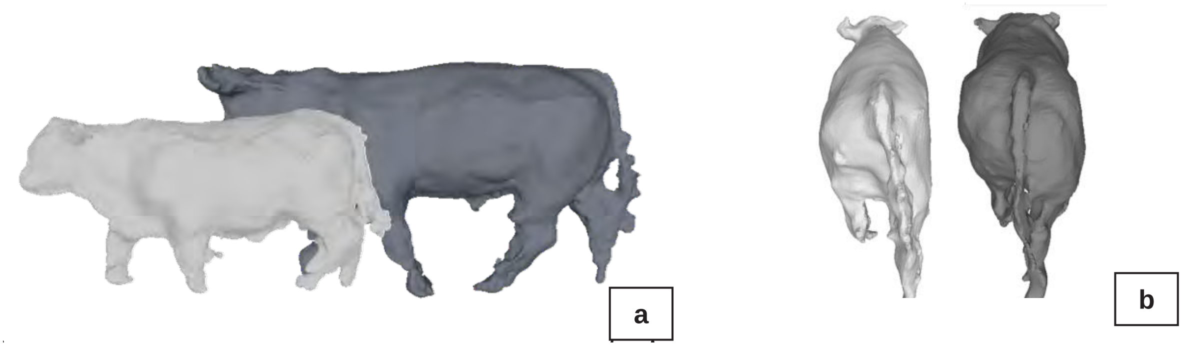

Figure 3. 3D image examples of extreme morphologies for calves around 6 months age.

The details concerning the animals' characteristics used for the trial are presented in table 1 below.

Machine learning models and data analysis

In our database, a total of 2210 3D images were initially available for analysis. However, after careful consideration, a subset of these images was excluded from the study due to factors impacting image quality and data reliability. These factors encompassed issues such as insufficient image clarity, inappropriate animal positioning during imaging (e.g., jumping or kicking), absence of duplicate images necessary for calculating repeatability, and errors in animal identification. The exclusion of these images was crucial to uphold the integrity of our study's findings. Ultimately, we utilized 1818 images for predicting body weight (BW) and 1575 images for predicting muscle (MUS) and skeletal (SKE) development. For both predictions, the models were trained using 80% of the images and tested on the remaining 20%, ensuring no overlap between train and test sets.

For BW prediction, 173 features were initially extracted from the 3D images. To enhance predictive performance and reduce dataset dimensionality, we employed

Table 1. Animals characteristics.

n=1194	Age	Weight	MUS	SKE
Average	221	287	56,2	59,1
SD	61,2	80,1	15,1	14,1
Min	44	130	10	12
Max	559	568	95,7	94

the Recursive Feature Elimination (RFE) method. RFE iteratively eliminates the least important features from the dataset, resulting in 61 selected features after training on the Random Forest estimator.

Subsequently, four machine learning models (Extreme Gradient Boosting, Random Forest, SVM Linear, and Lasso Regression) were trained on 1462 images from the learning dataset. To mitigate overfitting, models underwent training using a 4-fold cross-validation method with 5 repetitions. Following training, each model was evaluated on a test set comprising 356 images.

Similar methodologies were applied for predicting MUS and SKE synthetic scores. The RFE algorithm was used to select the most important features, resulting in 16 features for SKE score prediction and 51 features for MUS score prediction. The same four algorithms were trained on 1208 images using a 4-fold cross-validation method, repeated 5 times, and evaluated on a test set of 367 images.

Model evaluation metrics included Mean Absolute Error (MAE), Root Mean Squared Error (RMSE), and Spearman correlation (rs). Additionally, the repeatability of predictions was assessed by calculating the Spearman correlation between estimates from duplicate images when available.

Results and discussion

Weight prediction

Table 2 below illustrates the performance of the ML models. Among the models evaluated, Extreme Gradient Boosting emerged as the top performer in terms of predictive accuracy following feature selection. On the training set, BW was predicted with an RMSE of 15kg (5.2%) and a MAE of 11.3kg (4.0%). This resulted in a high correlation between BW and the model's predictions, with a rs of 0.97 and an average R2 of 0.98.

The model's performance remained consistent across both datasets, with an RMSE of 15.6kg (5.4%) and a MAE of 12.1kg (4.2%). This consistency suggests that our model has effectively captured the underlying data patterns without overfitting to the training set, enhancing its reliability for real-world applications.

While Random Forest (RF) also demonstrated competitive performance, it slightly trailed behind Extreme Gradient Boosting. These ensemble methods excel in capturing intricate data interactions. Interestingly, SVM Linear and Lasso regression, despite their reputation for excellence in prediction tasks, exhibited relatively lower performance in terms of MAE and RMSE.

Figure 4 illustrates the relationship between estimated weight and ground truth values for both the train and test sets. With an overall R2 of 0.964, predictions and actual weights are distributed around the line of perfect prediction. With such high-performance levels across both train and test sets, the model demonstrates exceptional accuracy and reliability in predicting weight, rendering it suitable for practical applications.

The model's repeatability was assessed to confirm the consistency of BW predictions when different images of the same individual were provided. This was determined by calculating the Spearman correlation between two images of the same cattle when two 3D images were available. Our findings demonstrate a high level of repeatability of the model across two images of the same animal, with a rs of 0.98 for 738 cattle.

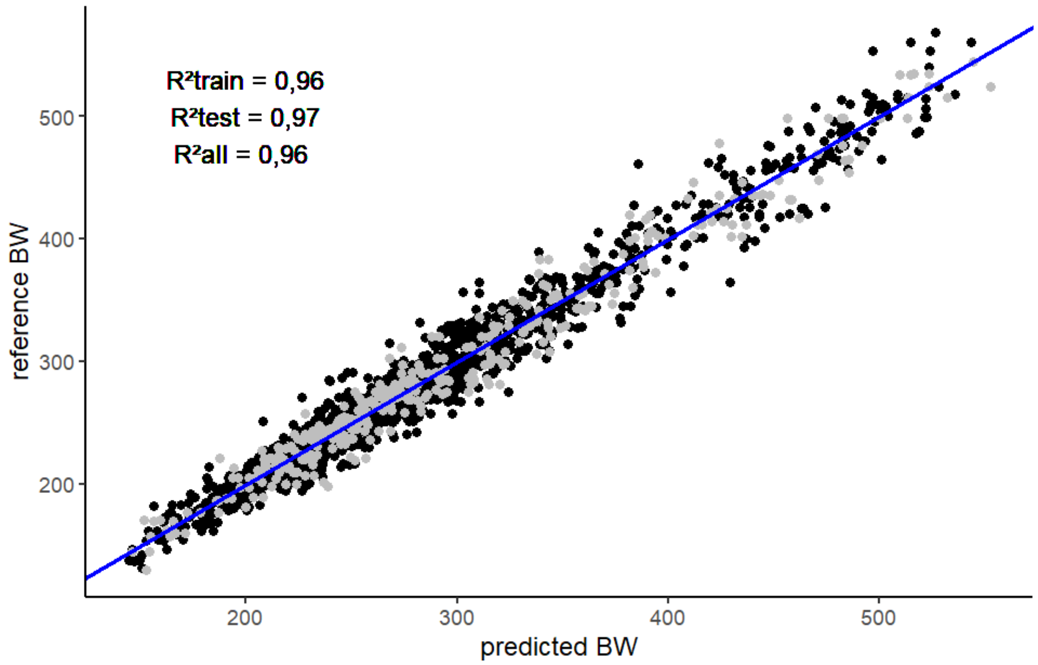

Figure 4. Predicted BW vs real BW for training set (black) and test set (grey). The blue line indicates the optimal fitting.

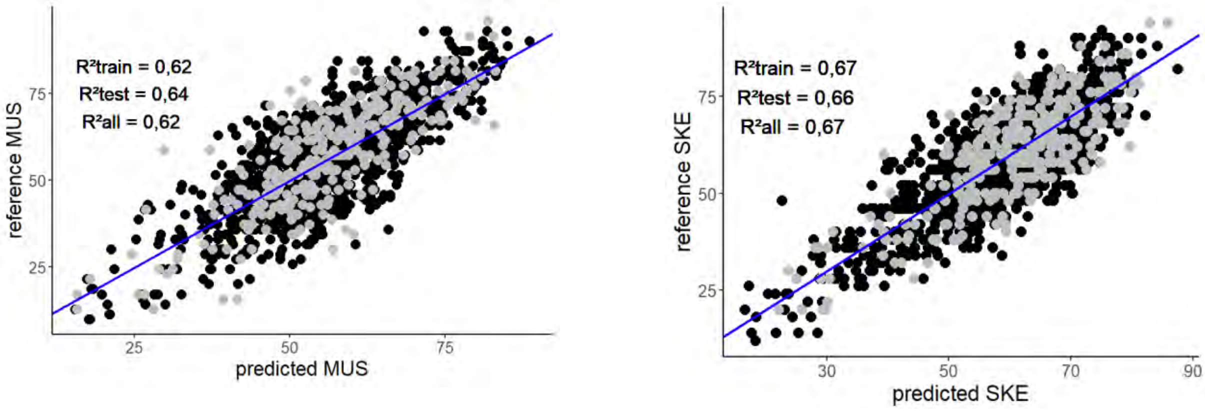

Figure 5. Predictions vs reference MUS and SKE scores for training set (black) and test set (grey). The blue line indicates the optimal fitting

Table 2. Models' performances on BW and synthetic scores prediction.

Predicted traits	Data sets	ML models / Statistics	Random Forest	Extreme Gradient Boosting	Lasso Regression	SVM Linear
BW	Train data set n=1462	MAE (kg)	11.7	11.3	12.9	12.8
		MAPE (%)	4.2%	4.0%	4.6%	4.6%
		RMSE (kg)	15.4	15.0	16.9	16.7
		r_s	0.97	0.97	0.96	0.96
		R^2	0.96	0.96	0.95	0.95
	Test data set n=356	MAE (kg)	12.4	12.1	13.6	13.3
		MAPE (%)	4.3%	4.2%	4.7%	4.5%
		RMSE (kg)	16.2	15.6	17.7	17.7
		r_s	0.97	0.98	0.97	0.97
		R^2	0.96	0.97	0.95	0.95
MUS	Train data set n=1267	MAE	7.6	7.1	7.6	7.6
		MAPE (%)	15.6%	14.5%	15.8%	15.5%
		RMSE	9.4	9.0	9.6	9.7
		r_s	0.73	0.75	0.72	0.73
		R^2	0.58	0.62	0.56	0.56
	Test data set n=308	MAE	7.4	7.1	8.0	7.9
		MAPE (%)	15.5%	14.5%	16.9%	16.7%
		RMSE	9.4	9.1	10.0	10.0
		r_s	0.77	0.78	0.74	0.74
		R^2	0.64	0.65	0.58	0.58
SKE	Train data set n=1267	MAE	6.3	6.1	7.2	7.2
		MAPE (%)	11.9%	11.3%	13.9%	14.0%
		RMSE	7.8	7.7	9.0	9.0
		r_s	0.78	0.79	0.74	0.73
		R^2	0.67	0.68	0.56	0.55
	Test data set n=308	MAE	6.3	6.5	6.9	7.0
		MAPE (%)	11.3%	11.9%	12.9%	13.1%
		RMSE	7.7	8.0	8.5	8.5
		r_s	0.78	0.75	0.76	0.75
		R^2	0.69	0.67	0.62	0.62

Synthetic scores prediction

To predict the synthetic scores, we utilized a dataset comprising 1575 images, with predicted weight included as a predictive variable. Employing the same methodology, we trained models using a 4-fold cross-validation and evaluated their performance on the test set. The results across the entire dataset are presented in Table 2.

Both Random Forest and Extreme Gradient Boosting emerged as the top-performing algorithms. For SKE score predictions, Extreme Gradient Boosting exhibited greater precision, with an MAE of 6.2 (11.4%) and an RMSE of 7.7 (15.2%). Figure 5 illustrates the relationship between reference SKE and the predicted SKE of this model, with an R2 of 0.67 suggesting a correct linear relation between predictions and reference. Similar performances were observed in both the train and test sets, indicating good generalization of the model. To enhance this performance further, it may be advantageous to include a certain proportion of extreme SKE values, particularly those below 40, where the number of animals in our study is limited. Moreover, the model demonstrated good repeatability, with a rs of 0.87, significantly surpassing the repeatability target of 0.78.

For MUS score predictions, Extreme Gradient Boosting also emerged as the top model, achieving an MAE of 7.1 (14.5%) and an RMSE of 9.0 (21.6%). Figure 5 illustrates a strong correlation between predictions and references, with an R2 of 0.62 for the train set, 0.64 for the test set, and 0.62 across the entire dataset. Additionally, the model exhibited good repeatability, with a rs of 0.84, notably exceeding the repeatability target of 0.75.

Conclusion

In conclusion, this study underscores the feasibility of employing three-dimensional imaging in conjunction with artificial intelligence methods to accurately estimate body weight (BW) and linear scores in calves. By leveraging machine learning models, we achieved robust predictions for BW, muscle development (MUS), and skeletal development (SKE), surpassing the accuracy of experienced human scorers. The high repeatability of these predictions underscores the reliability of our approach, promising improved phenotypic assessment in livestock breeding programs..

Acknowledgments

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HappyFeet: Hoof Health Project in Italian Holstein cattle

M. Marusi[1]*, F. Testori*[2]*, L. Benzoni*[1]*, R. Finocchiaro*[1] *and M. Cassandro*[1,3]

[1]*Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana*
(ANAFIBJ), Via Bergamo 192, 26100 Cremona (CR), Italy.
[2]*DVM, FA.MA. Services, Milano (MI), Italy*
[3]*Department of Agronomy, Food, Natural resources, Animals and Environment*
(DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy.
Corresponding Author: lorenzobenzoni@anafi.it

Abstract

Hoof lesions are a significant issue in dairy herds, with a prevalence ranging from 40% to 70%. In Italy, hoof lesions are the second leading causes of culling cows, following fertility and reproductive disorders. Hoof health is also related to economic and social consequences, resulting in increased labour expenses, reducing milk production, longevity, fertility, health and welfare. Management practices and genetic selection are crucial for reducing hoof lesions in dairy cows.

Italian Holstein, Brown and Jersey Breeders Association (ANAFIBJ) in collaboration with a group of hoof-trimmers is collecting data on hoof lesions through the HappyFeet Project. Data are collected using an Android App developed by FA.MA Services (FA.MA Services, Milan (MI), Italy) and installed into a portable device (e.g., tablet) according to ICAR Atlas. A total of 18.826 hoof lesions records are stored into ANAFIBJ database collected on 10.583 Italian Holstein lactating cows across 78 herds in Italy. Most frequent lesions are dermatitis (18%), sole ulcer (14%) and sole hemorrhage (9%). Further descriptive statistics related to hoof lesions prevalence are studied based on season, including year, semester, quarter and trimester as well as factors like parity and lactation stage.

The objectives of the HappyFeet Project are to streamline data from hoof-trimmers to database, to provide a benchmark reference both for farmers and hoof trimmers and to set up a genetic and/or genomic evaluation for hoof health.

Keywords: hoof lesions, data-collection, hoof health.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 10: New approaches in the field of functional traits for management and breeding

Introduction

Hoof lesions are a significant issue in dairy herds, with a prevalence ranging from 40% to 70%. In Italy, hoof lesions are the second leading causes of culling cows, following fertility and reproductive disorders. Hoof health is also related to economic and social consequences, resulting in increased labour expenses, reducing milk production, longevity, fertility, health and welfare. Management practices and genetic selection are crucial for reducing hoof lesions in dairy cows.

Material and methods

Italian Holstein, Brown and Jersey Breeders Association (ANAFIBJ) in collaboration with a group of hoof-trimmers is collecting data on hoof lesions through the HappyFeet Project. Data are collected using an Android App developed by FA.MA Services (FA.MA Services, Milan (MI), Italy) and installed into a portable device (e.g., tablet) according to ICAR Atlas.

First data have been collected from September 2022 with a total of 380 visits in 99 Italian farms. 27.085 hoof lesions records are now available in ANAFIBJ database collected on 14.401 animals.

Results

Descriptive statistics at National level related to hoof lesions prevalence based on season, including year, semester, quarter and trimester as well as factors like parity and lactation stage are reported in this article.

The majority of visits (75%) are carried out in northern Italy, while others are carried out in the south and in the islands (Figure 1).

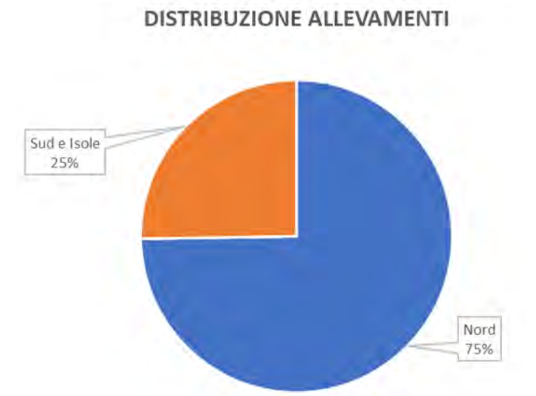

Figure 1. Geographical distribution of visits.

Figure 2. Kind of visits distribution.

Regarding the distribution of visits, 65% are ties, 27% time-trimming, 6% visits on request and 2% emergencies (Figure 2)

Healthy animals are over 45%. the most frequent lesions are digital dermatitis (18%), soleal ulcer (11%), hemorrhage (7%), wall abscess (7%). Other lesions are less relevan (Figure 3).

In addition to the cumulative descriptive statistics, the evolution of hoof lesions per year from 2022 to 2024 (Figure 4), and the evolution of hoof lesions per quarter from 2022 to 2024 are also reported (Figure 5).

Descriptive statistics relating to cumulative hoof lesions are also available, by year and quarter, depending on the order of calving and stage of lactation (Figure 6 to Figure 11).

All these descriptive statistics are provided also to farmers at herd level.

Figure 3. Hoof lesions frequency.

Figure 4. Evolution of hoof lesions per year (2022-2024).

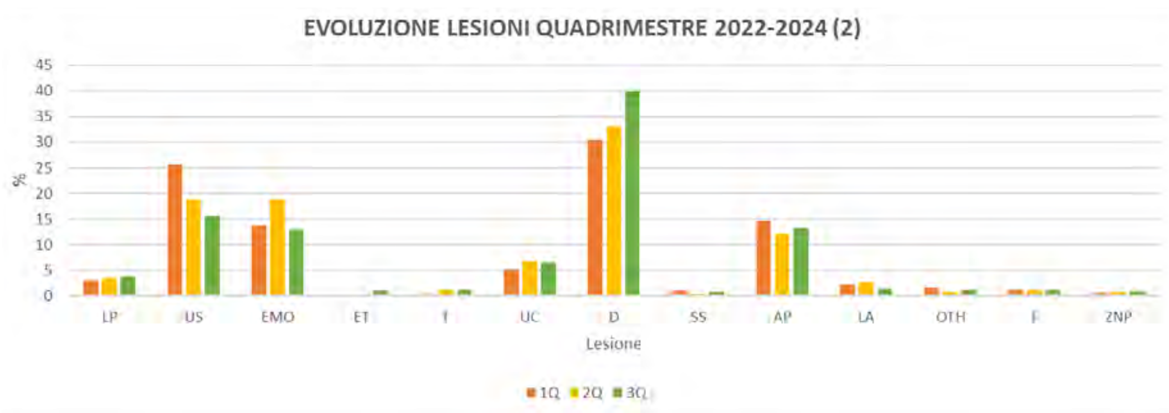

Figure 5. Evolution of hoof lesions per quarter (2022-2024).

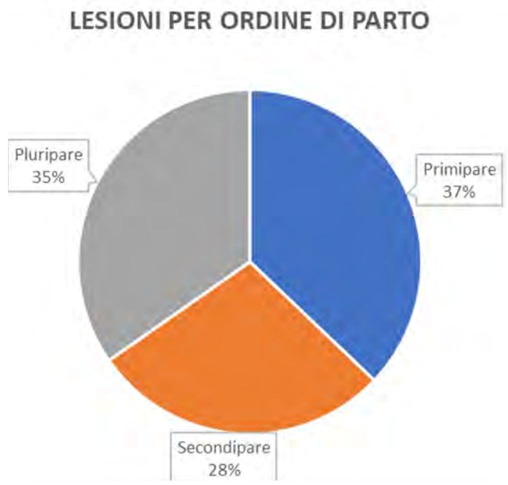

Figure 6. Hoof lesions and order of calving.

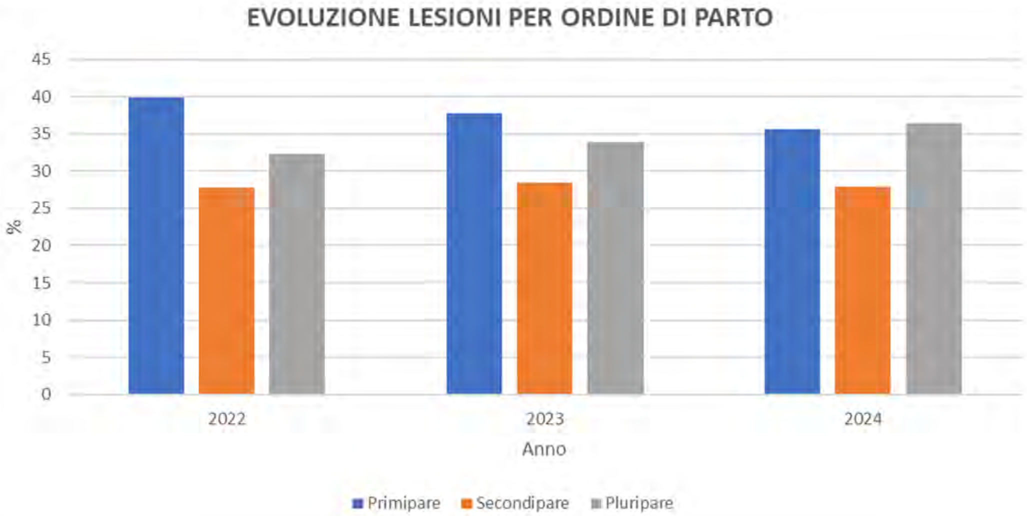

Figure 7. Hoof lesions, order of calving per year.

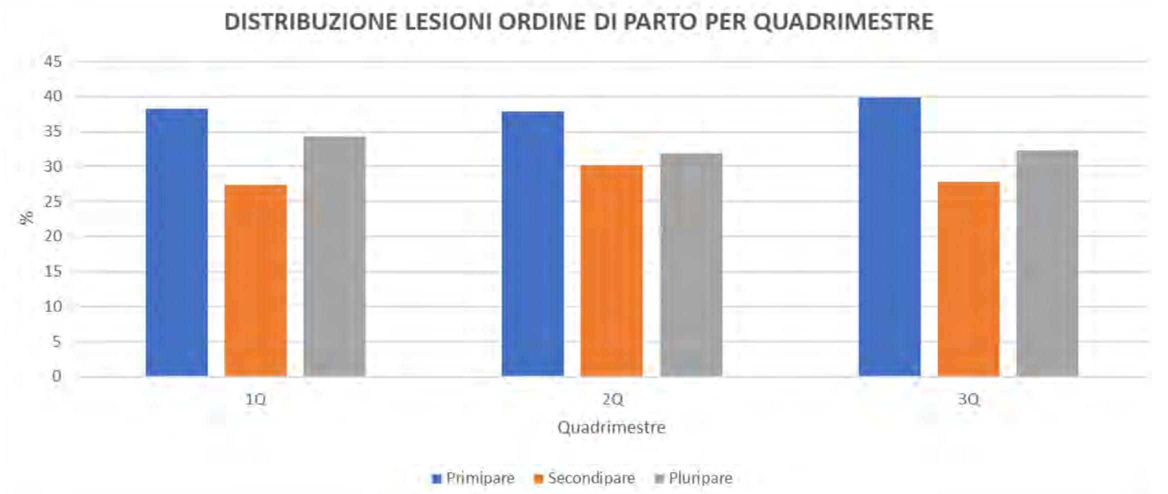

Figure 8. Hoof lesions, order of calving per quarter.

Figure 9. Hoof lesions and stage of lactation.

Figure 10. Hoof lesions, stage of lactation per year.

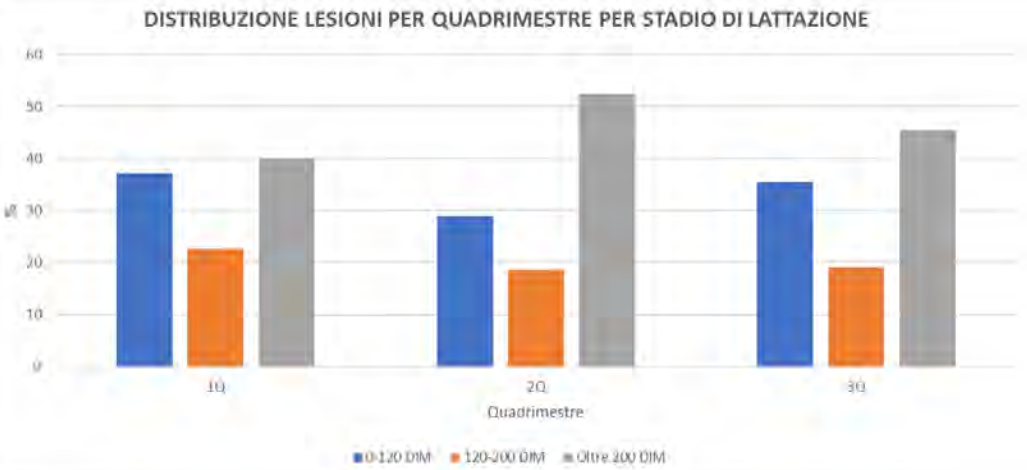

Figure 11. Hoof lesions, stage of lactation per quarter.

Conclusion

HappyFeet project is at starting stage and, of course, data collection is going to be enhanced. Further objectives of the Project are:

1. To provide benchmark reference at national, regional and herd level.
2. To perform an Italian economical evaluation for hoof lesions.
3. To set up genetic evaluation for hoof health.

Body Conditions Scoring: a first proposal for recommendations for recording and use for herd management, genetic improvement and welfare assessment

A. Gyawali[1], C. Egger-Danner[2] and N. Gengler[3]

[1]University of Natural Resources and Life Sciences, Vienna, Austria
[2]ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria
[3]University of Liege - Gembloux Agro-Bio Tech, Gembloux, Belgium
Corresponding Author: nicolas.gengler@uliege.be

Abstract

Body Condition Scoring (BCS) is a widely used and subjective method of assessing the amount of metabolizable energy stored in fat and muscles in live animals. It provides a rapid indication of levels of body fat reserves, e.g., which are crucial in early lactation to buffer cows against negative energy balance as they prioritize energy towards milk production. However, the rapid mobilization of body fat reserves at early-lactation, but also over-condition at late-lactation or dry period can lead to fertility and health issues. Therefore, regular monitoring of BCS is essential for maintaining optimal body condition, health, and productivity in dairy herds. This paper proposes first ideas for standardized ICAR guidelines for BCS recording, emphasizing its applications in herd management, genetic improvement, and welfare assessment. We highlight the diversity of BCS scales used in the different BCS systems and suggest approaches to overcome challenges in comparing responses across different systems. The contributions to ICAR guidelines reported here are a direct continuation of the ICAR-IDF webinar on “Recording and evaluation of BCS and its relationship with health and welfare” and the work done by the “Joint Expert Advisory Group for BCS Guidelines” organized by the ICAR Functional Traits Working Group.

Keywords: animal health and welfare, bovine scoring, energy balance, genetic improvement, herd management, ICAR guidelines, welfare assessment, Presented at the ICAR Annual Conference 2024 in Bled at the Session 10: New approaches in the field of functional traits for management and breeding

Introduction

Body Condition Scoring (BCS) is considered today an essential tool for evaluating the health and metabolic status of dairy cows by estimating their body fat reserves, particularly during early lactation. BCS is widely accepted as the most practical method for assessing body fat content, mobilization and changes in energy reserves in dairy cattle (Bewley *et al.*, 2008). By estimating their status, it assesses the pivotal role that fat plays in buffering cows against negative energy balance while they partition energy primarily toward milk production. This rapid mobilization of fat reserves but also being over conditioned may lead to reproductive and health problems, including fertility issues as cows, that are either too thin or too fat, are prone to these disorders (Garnsworthy, 2006). Optimal body condition is important and requires frequent monitoring of BCS which can be used to detect and to correct problems and improve the health, welfare,

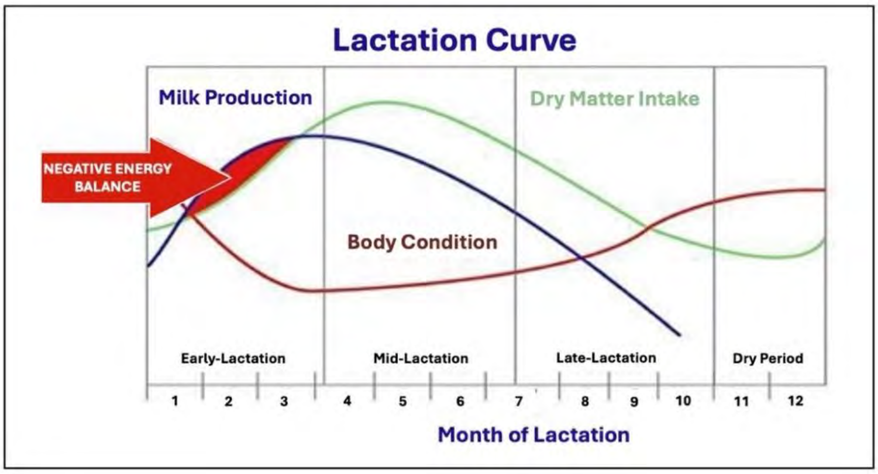

Figure 1. Lactation curves comparing milk production, intake and body condition showing reaction of body condition in early and potential over condition at the end of lactation.

fertility and productivity of dairy cows and herds (Domecq *et al.*, 1997; Roche *et al.*, 2007). Figure 1 shows the lactation curves comparing milk production, intake and body condition.

The practice of visual and tactile appraisal of BCS began in the 1970s, with one of the earliest developments by Jefferies (1961) of a BCS system for ewes and has evolved into various numerical systems with multiple scales, depending on the country, organization, and intended purpose. While some scales focus on welfare assessment and are simple, others are more detailed to optimize feeding strategies by detecting early changes in body condition. Despite these advancements, different scoring systems can cause confusion when comparing targets and results across farms and programs. Moreover, while automated BCS recording is becoming more common, it remains challenging to achieve the same accuracy as manual palpation in dairy cattle. This contribution to ICAR guidelines is a direct continuation of the ICAR-IDF webinar on “Recording and evaluation of BCS and its relationship with health and welfare” and the work done by the “Joint Expert Advisory Group for BCS Guidelines” organized by the FT-WG. In the following document we will call BCS system the use of a given BCS scale in a specific context (e.g., group of animals scores, frequency of scoring, use of scores).

Defining Body Condition Score (BCS)

BCS as an indicator of fat content

There were no simple measures of a cow’s energy reserves or condition prior to 1970s (Stockdale, 2001). Because cows of a given weight might be tall and thin, short and fat, or both, body weight (BW) alone was not a reliable measure of body reserves. Energy storage in cows with comparable body weights differed by as much as 40%, according to Andrew *et al.* (1994) and Gibb *et al.* (1992), demonstrating the unreliability of using BW as the only indicator of body condition. Furthermore, because increased feed intake coincides with tissue mobilization during early lactation, reductions in body tissue weight may be masked by increased gut fill, meaning that increases in BW may not correspond to changes in adipose and lean tissue weight. A strong positive correlation ($r^2 = 0.86$) between BCS and the proportion of physically dissected fat in Friesian cows as reported by Wright and Russel in 1984 has been used as visual or tactile (palpation) appraisal of cow condition or BCS. This provides a good assessment of body fat reserves, ignoring, or minimizing the influence of frame size and intestinal contents (Wright and Russel, 1984). This traditional subjective appraisal has been rationalized into various numerical BCS systems using many different scales. This diversity in scales is based on the purpose of recording BCS and different scales employed in different circumstances by different countries or organizations (i.e., BCS Systems).

BCS scales and their diversity

In contrast to some other traits with very precise definitions, there are wide variety of scales that have been used to measure body condition. This variety of scales is also based on the purpose of recording the BCS and on different scales used in different circumstances by different countries or organizations, resulting in different BCS systems.

BCS scales across countries

A large variety of BCS scales exist today. Some are reported in Table 1. It is important to notice that the number of classes available to the assessors is the important feature allowing fine scoring, not the numerical boundaries. Jefferies (1961) initially developed a BCS system for ewes which involved palpation of backbone and lumber processes, feeling the sharpness, and covering of the bones. He developed a scale from 0 to 5 (here after called 0-5 scale), where 0 was extremely thin, i.e. no longer viable, and 5 was extreme obese. His technique was adapted for scoring beef cattle by Lowman et al. (1973) which involved palpation of the lumbar vertebrae and around tail head. Table 1 give some other relevant reference methods. Subsequently a similar system with 0-5 (11 classes) was proposed by Mulvany (1977) in the UK but introduced adjustment factors if the scores in the tailhead and the loin areas differed. In Australia, an 1-8 system (15 classes) of scoring dairy cows was developed by Earle et al. (1977) and a similar 1-10 system (19 classes) developed in New Zealand (Roche et al., 2004). Both scoring systems used photographs of individual cattle to define condition scores. Body condition scoring of dairy cows in the US is generally performed according to the scale 1-5 (Wildman et al., 1982). This method, like those used in the UK, involves palpating cows to access the amount of tissue under the skin. As for many scales different variants were proposed. Ferguson et al. (1994) also used a 1-5 scale but 0.25 interval leading to 17 classes. Body condition score being an optimal intermediate trait, and, in all scales, lower values indicate a leaner body conditioning in cows, whereas higher values indicate greater obesity level. Several scales used in various countries are summarized in Table 1.

Table 1. Various BCS scales used in different countries along with the method of assessment.

Country	Scale	Interval (classes)	Method	References
United Kingdom, Ireland	0 to 5	0.5 (11)	Palpation	Mulvany (1977),
New Zealand	1 to 10	0.5 (19)	Palpation	Roche et al. (2004)
Australia	1 to 8	0.5 (15)	Visual	Earle et al. (1977)
United States	1 to 5	1 (5) 0.25	Palpation/Visual	Wildman et al. (1982)
	1 to 5	(17)	Palpation/Visual	Ferguson et al. (1994)

Diversity in BCS scoring systems

Developments of various BCS scoring system were based on circumstances and purposes such as breeding, herd management, and welfare. Within each system factors like granularity of scoring (i.e., scale and intervals used), population evaluated, timing, and frequency various within different systems. Recent advancements in technology-assisted or even technology-driven BCS systems are also leading to other BCS systems. This leads to challenges as this variation among systems can lead to confusion when comparing and difficulty exists in interpreting the literature. Especially putting together data across different herds and different BCS systems and can require transformations of scales.

Using Body Condition Score (BCS)

Description of some commonly used scoring system

BCS system associated to linear scoring and genetic evaluations (1-9 scoring scale)

Currently in many countries BCS is scored in routine once in first lactation inside the linear scoring system used for genetic evaluation for conformation. Therefore, a 1-9 scoring scale (9 classes) is taken as a linear scale although BCS is not a true linear trait. The covering of fat over the tail head and rump is taken as the reference point and is scored as described in Figure 2. For the score ranging from 1-6, the accessor has to look at the loin, while the tail implant is important with the higher scores (7-9).

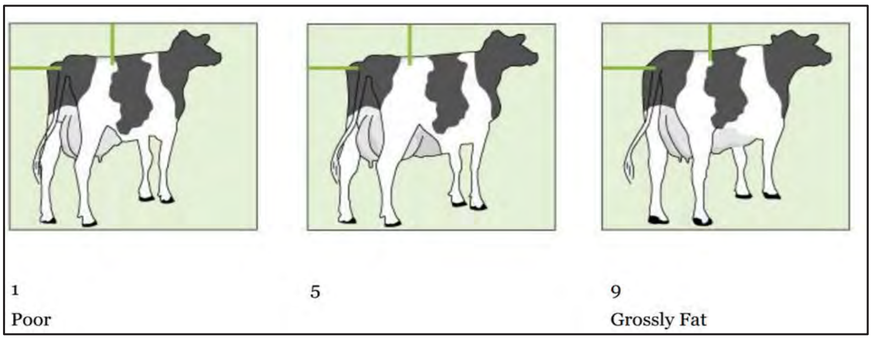

Figure 2. 1-9 BCS scale (adapted from ICAR).

BCS based on a 1-5 scoring scale

Detailed information describing the way the 1-5 scoring scale with 0.25 intervals (17 classes) are assigned are given by Edmonson *et al. (1989).* In Figure 3, the major elements for assigning the 5 major steps are given as an example.

How to assess BCS

Manual assessment

Manual assessment of BCS involves palpating key body regions (e.g., ribs, spine, hips) to estimate fat and muscle reserves. Figure 4 shows the anatomical features associated with body condition scoring. This method remains reliable but is subject to assessor variability. Consistency in training assessors is crucial to reduce this variability.

Digital tools

Automated BCS recording using digital technologies, such as 3D imaging systems, is becoming more widespread. These tools offer a more objective and consistent assessment of BCS, minimizing human error. However, technological limitations still

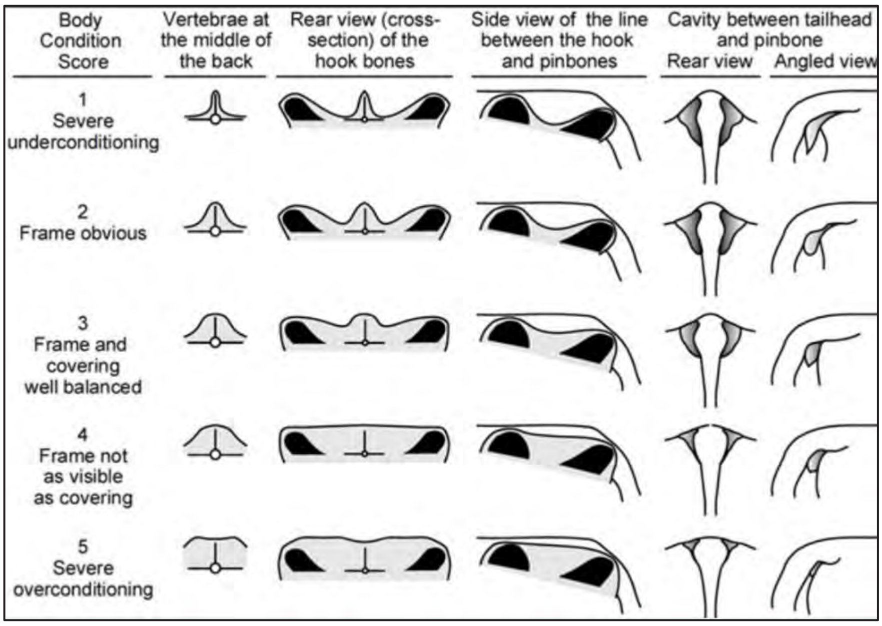

Figure 3. Example of an 1-5 BCS scale chart (Modified from Edmonson et al., 1989).

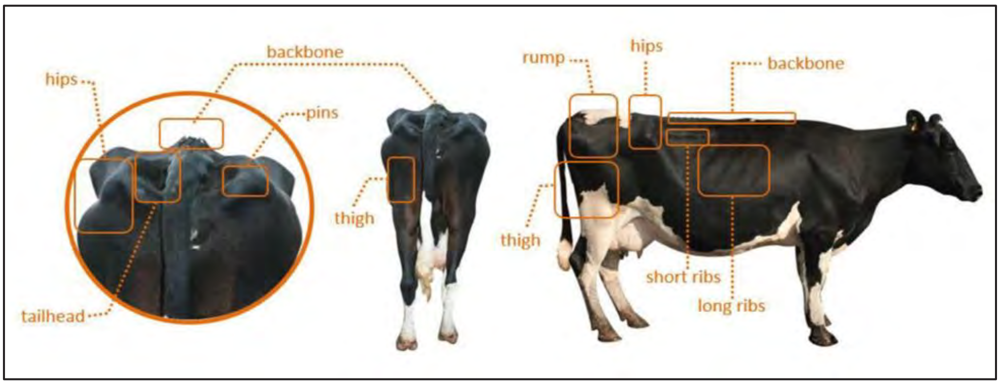

Figure 4. Anatomical features associated with body condition scoring in cattle. (Huang et al., 2019).

make it challenging to achieve full accuracy, particularly when compared with manual palpation. Three levels were identified and can be recommended:

1. Use of digital tools to document and do easy recording of visual assessments.
2. Technology-assisted assessments: Human assessors are still doing the scoring but devices providing support to the manual assessment replacing the human eyes. These tools should be easy to use, resilient to environmental disturbance, and allow easy identification of animals and data transfer.
3. Technology-driven assessments: These are purely automatic sensor-based technology driven assessment that should be more reliable, allowing more frequent

on-farm BCS assessment. Technologies are diverse, generally digital images of the rear aspect of the cow based or 3D body condition scoring using fixed position optimized camera systems.

Recommendations for use of BCS scales

Conversion between BCS scales

Mathematical conversion

Although Garnsworthy (2006) and others highlighted the common practice of BCS systems to assess similar body parts and to establish links between scores and levels of adiposity, there are concerns about the reliability of simple mathematical conversions between different scales. These might not be accurate because scales may use the range of conditions not linearly (Garnsworthy, 2006). Therefore, we recommend only using these equations with caution, and only when no other information is available. As example here are the proposed transformations towards a 1-5 scale:

- 1-4 scale: BCS x 4/3 – 1/3
- 0-5 scale: BCS x 4/5 + 1
- 1-8 scale: BCS x 4/7 + 3/7
- 1-9 scale: BCS / 2 + 1/2
- 1-10 scale: BCS x 4/8 + 5/9

Conversion based on simultaneous scoring

An alternative way to develop conversion equation was presented by Roche *et al.* (2004). In their method simultaneous scoring is required (Figure 5). Still enough variability at the extremes is needed and local scales may be adapted to local populations (e.g., breeds) making conversion more difficult.

Conversion based on distributions of scores

Under the hypothesis that all scales describe the same underlying trait, the adiposity of animals in a given population, the distribution of attributed scores using each scale

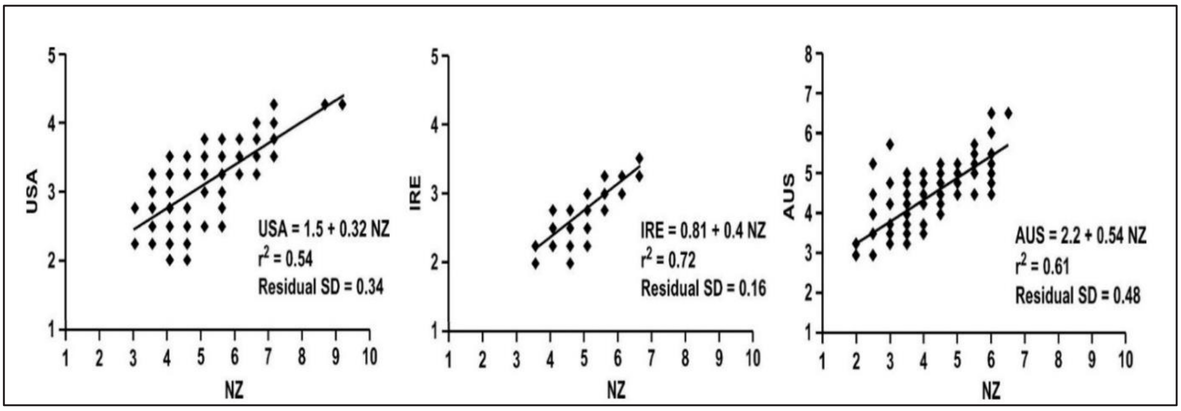

Figure 5. Conversion equations based on simultaneous scoring in the USA, IRE and AUS of local cows using local and NZ scales (Roche et al., 2004).

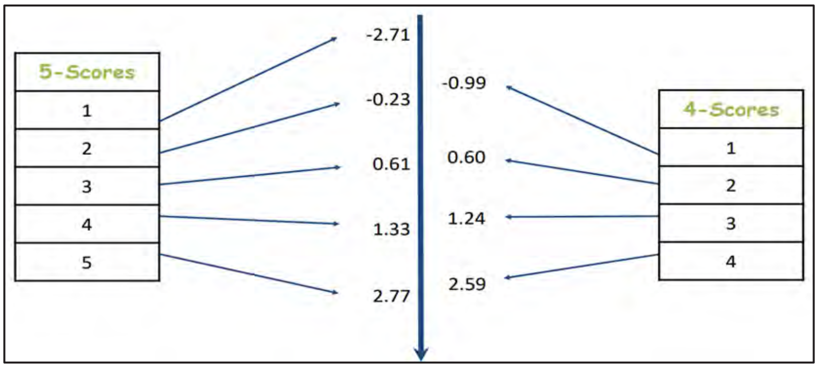

Figure 6. Matching scores taken on 5- and 4-class scales using Snell Scores (Snell, 1964).

can be mapped a posteriori to this underlying normal distribution (i.e., z-scores which are the standardized distances from the zero of a normal distribution) using appropriate methods (e.g., Snell Scores). Figure 6 shows how a posteriori 5-class respectively 4 -class score scales can be mapped to a common scale or even common classes. In this example scores 1 and 2 of the 5-class match approximately to 1 of the 4-class scale, as do 3 to 2, 4 to 3 and 5 to 4. The z-scores can obviously also be used directly as a common measure of adiposity.

Under the hypothesis that both scoring scales are used on animals representing the same population with large enough sample sizes this method does not require simultaneous scoring.

Recommendations for herd management

BCS plays a vital role in managing dairy herds, allowing farmers to adjust feeding strategies and monitor metabolic health. Frequent BCS assessments help identify cows that are either losing or gaining condition too quickly, which may indicate underlying health or nutritional issues. In general, five BCS classes are usually sufficient to capture significant BCS variability, but can be increased for specific purposes (e.g., feeding optimization, early detection of health problems) or reduced for welfare assessments aiming only to detect the general status of the cows (too thin – normal – too fat) (Table 2).

Table 2. Various BCS scales proposed for specific purposes

Purpose	BCS Scale	Frequency	Remarks
Feeding advice	5 classes	Frequent and longitudinal	Identification of cows with BCS change indication potential health problems and optimization of feeding
Detection of metabolic disturbance	5 classes	Before and after calving and at least 2 time before peak of lactation (~50 DIM)	Enables BCS change in the herd
Welfare assessment	3 classes		Detect general status of cows (thin-normal-fat)

Table 3. Timing of BCS assessments.

Moment	Recommended frequency
Pre-calving:	Conduct BCS evaluations approximately 3 weeks before calving to ensure optimal condition.
Early-lactation:	Closely monitor BCS during the peak of lactation to detect metabolic imbalances early.
Dry off period:	Assess BCS 7-8 weeks before calving to adjust feeding as needed

Table 3 outlines the recommended frequency for BCS assessment depending on key stages in the cow's lactation cycle:

With an optimal recording scheme could be (in bold recommended): Dry off, Pre-calving, Calving, Early lactation/Pre-service, 1st Service, Pregnancy Check, and Late lactation.

It should be noted that a representative random stratified sample of cows representing all lactations should be measured at least at the beginning (pre-service), at the end of the lactation (drying-off), and before calving to ensures effective assessment. In herds at risk of transition cow issues, more frequent recording of all at-risk cows is required.

Recommendations for individual cow management

In individual cow management, BCS can be used as a troubleshooting tool to adjust feeding programs or identify health concerns. For example, cows that drop below a certain BCS threshold may require increased energy intake, while those with higher-than-recommended scores might benefit from a restricted diet. These measures are essential for improving not only productivity but also fertility, feed efficiency, and overall wellbeing in dairy herds.

Detection of extreme BCS animals is required for individual cow management, therefore finer scales than only 5 classes and repeated recordings to enable detection of body condition changes are recommended.

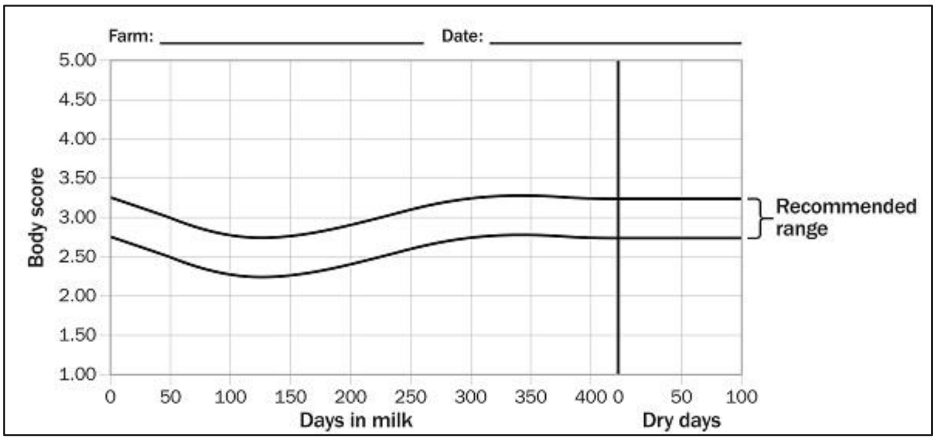

Figure 6. BCS chart used in Ontario, Canada. (https://www.ontario.ca/page/body-condition-scoring-dairy-cattle).

We recommend developing optimal BCS lactation curves based on breeds and management system. Figure 6 shows the chart developed in Ontario, Canada, that allows farmers to plot individual cows on this chart according to stage of lactation. This optimal BCS lactation curves can be used to profile a herd at one point time or to monitor changes over a lactation for an individual cow.

Recommendations for genetic evaluation

BCS is recognized as an intermediate optimum trait in genetic selection. Incorporating BCS data into genetic evaluations enhances breeding programs, particularly for selecting cows with a more favorable balance between milk production and metabolic health. The use of BCS as auxiliary trait is common in many genetic evaluation systems (e.g., for fertility). Regular, accurate BCS data collection allows for better herd selection and ultimately contributes to long-term herd sustainability. Current practice is organized in parallel to linear scoring which involves in most systems recording BCS once in a lifetime done during 1st lactation using the same 1-9 scale as for linear scores.

Our recommendations are that for genetic evaluation BCS should be recorded on all cows on a frequent basis throughout the cow’s life with at least a 5-class scale. Obtaining repeated records of BCS can also be useful for the derivation of novel traits such as resilience and resource allocation. Weakness of single recording on a cow level can be partially compensated by appropriate modeling of BCS changes on a sire level through its progeny. Even if this single measurement does not capture the BCS variation throughout the cow’s lactation, on the level of the offspring of a sire we recommend the use of random regression models to assess the heritable changes observed in the progeny of a given sire.

Recommendations for welfare monitoring

As explained previously, current practice in welfare monitoring BCS systems is the use of a 3-class scale which is sufficient in this context. Because assessment is only conducted once at a specific point in time, a critical element is sampling a representative group of animals, including recording of relevant elements to ascertain this (e.g., parity, lactation stage).

As for other BCS systems, to maximize synergies, for example with herd and individual cow management, and breeding, it would be beneficial if all animals were assessed even in a welfare monitoring. This would also allow the detection of individuals with specific welfare issues.

Important considerations

Additional data to be recorded

In addition to the recorded BCS, also to allow further use the following information is recommended to record: unique Animal ID, Herd ID, breed, date of recording, assessor-ID, BCS Scoring System (linked to a comprehensive description of the system), days to / from calving in relevant parity and parity number.

Training of the accessor

An important element is the training of accessors. First, they need to have a clear understanding of and training on the BCS Scoring System. Standard Operating Procedures (SOP) along with a scoring chart and ensuring comprehensive and regular training on utilizing these resources effectively need to be developed. Frequent harmonization between assessors is needed. Best practice is that different assessors score the same farm(s) and grouping of data across different farms is done e.g. the data is used for benchmarking. Finally frequent evaluation of inter- and intra-assessors' repeatability is important especially for use in research studies.

Benchmarking and use for herd management

For herd management information on individual cows could be of less importance. But to effectively benchmark, manage herds, and genetically evaluate animals, it is crucial to centralize the collected information into a database. Benchmarking enables comparisons among farms and the identification of areas for improvement. However, for meaningful comparisons between herds, factors such as assessment frequency, lactation stage, and recording must be considered and therefore recorded (see point "Additional data to be recorded"). Furthermore, the data should be representative of the population and the distribution of BCS is more relevant rather than just means.

Conclusion

Body Condition Scoring is a key method for assessing the health and wellbeing of dairy cows, providing a practical measure for managing herd nutrition, productivity, and welfare. Standardizing BCS scales and recording methods is crucial to improve data consistency across regions and systems. While technological advancements, such as automated scoring, offer promise, manual assessments remain important. Regular BCS monitoring, along with harmonized guidelines for recording, will support better decision-making in herd management, genetic selection, and welfare assessment. Here we presented the first proposal draft of guidelines for the scoring of body condition in dairy cattle. This should lead to comprehensive guideline aiming at standardizing BCS methods and includes considerations and recommendations for improved BCS recording in the context of a herd management, animal welfare, and genetic evaluation maximizing also synergies between BCS systems.

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Incorporating genetic information about methane into the Dutch Annual Nutrient Cycling Assessment

C.I.V. Manzanilla-Pech[1], B. Gredler-Grandl[1], M. de Haan[2], H. van Laar[3], N. Meijer[4], A.E. van Breukelen[1], G. de Jong[4], and R.F. Veerkamp [1].

[1]Animal Breeding and Genomics, Wageningen Livestock Research, Wageningen
University and Wageningen University and Research,
6708 PB Wageningen, The Netherlands
[2]Wageningen UR, Livestock Research, PO Box 338 Wageningen,
6700AH, The Netherlands
[3]Animal Nutrition Group, Wageningen Livestock Research, Wageningen University and
Research, PO Box 338, 6700 AH Wageningen, the Netherlands
[4]Cooperation CRV, Animal Evaluation Unit, P.O. Box 454, 6800AL Arnhem, Netherlands
Corresponding Author: coralia.manzanillapech@wur.nl

Abstract

Since 2016, using the Annual Nutrient Cycling Assessment (ANCA, Kringloopwijzer) tool has been mandatory for all Dutch Dairy farmers. ANCA determines the farm-specific environmental performance. This includes:

- Efficiency of feeding (conversion of N and P from feed into milk and meat).
- Crop yields for N, P, C, energy (kVEM).
- Efficiency of fertilisation (conversion form fertiliser and manure into crop yields).
- Production of manure, excretion of N and P.
- Surpluses of N, P on farm balance and soil balance.
- Carbon sequestration.
- Ammonia emissions.
- Green House Gas emissions (CH_4, N_2O, CO_2).

Therefore, one of the primary objectives of the Kringloopwijzer is to evaluate the methane (CH_4) and carbon dioxide (CO_2) emissions associated with milk and meat production. Approximately 75-80% of methane emissions on dairy farms stem from fermentation in the gastrointestinal tract, with the remainder originating from manure storage. The CH_4 emissions from rumen enteric fermentation in dairy cattle are derived from methane emission factors (EF) for different feedstuff, which is a linear application of the Dutch Tier 3 method (IPCC). While this approach is robust, it does not include genetic variation among cows with on different farms, assigning the same emission value to all cows on all farms based solely on their dietary composition and feed intake. Genetic selection is considered one promising way to reduce methane emission, given that its effects are cumulative and permanent. Consequently, there has been growing interest in incorporating genetic information into the calculation of CH_4 emissions for the entire dairy herd. In this project we evaluated the differences in average breeding value for CH_4 between farms, thus without any selection practised yet. The results demonstrate that differences between farms represents up to 3.7 to 5.1% of the CH_4 and adding the breeding value has potential in refining the existing

ANCA tool. The ambition is to use breeding value (EBV) for CH_4 emissions for Dutch cows in the coming years.

Keywords: methane emissions, farm-level, life cycle analysis.
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Introduction

Since 2016, the Dutch dairy sector has utilized the Annual Nutrient Cycling Assessment (ANCA) tool to evaluate farm-specific environmental performance indicators. These indicators encompass feeding efficiency, crop yields, fertilization efficiency, manure production, nutrient surpluses, ammonia emissions, and greenhouse gas emissions. The primary goal of the ANCA tool is to quantify methane (CH_4) and carbon dioxide (CO_2) emissions associated with milk and meat production, adhering to IPCC guidelines while integrating national emission factors. This initiative is funded by the Ministry of Agriculture, Nature and Food Quality and ZuivelNL.

Methane emissions from dairy cattle are calculated based on estimated feed intake and diet composition. Each feedstuff has 3 emission factors (EF) for methane (g/kg dry matter) for when that feedstuff is fed in diets with 3 levels of maize silage (vs grass silage) in the roughage part of the diet: EF0, EF40 and EF80, where the number indicated the percentage of maize silage. These EF factors have been derived by using the IPCC Tier 3 method (Van Dijk *et al*., 2022).This method employs a dynamic mechanistic simulation model to determine emission factors based on the chemical composition and digestion characteristics of specific feed ingredients. The ANCA tool interpolates methane emissions based on the proportion of maize silage in the diet and adjusts for variations in feed intake and emissions from young stock. While this approach allows for accurate assessment and potential mitigation of methane emissions in dairy farming, it overlooks genetic variations among cows within a single farm, assigning the same emission value to all cows based solely on their diet.

Genetic selection is considered a promising method to reduce methane emissions, as its effects are cumulative and permanent. Consequently, there is growing interest in incorporating genetic information into the calculation of CH_4 emissions for the entire dairy herd. The ambition is to utilize the breeding value (EBV) for CH_4 emissions in Dutch cows in the coming years. The initial proposal involves integrating data on the average EBV of a farm's cows to identify potential differences among farms. Therefore, the objective is to investigate how to incorporate individual genetic information into the calculation of CH_4 emissions for the entire dairy herd.

Material and methods

Data descriptions

The data included 8,858 Dutch Holstein cows with 152,172 records of CH_4 concentration (CH_4c in parts per million, ppm). These records were collected in primiparous and multiparous cows during 2019 to 2023 in 72 commercial farms in the Netherlands. Parities were grouped into categories of 1, 2, 3, and 4+, and records up to lactation week 59 were included (406 DIM).

Statistical model

Variance components and EBV were estimated with an univariate repeatability (animal) model in ASReml 4.0 software. The general model used to estimate the variance components for CH_4c was:

$$y = Xb + Z_1a + e \qquad (1)$$

where y is the vector of phenotypes (CH_4c); b represent the vector of fixed effects (herd, year-season interaction, week of lactation and the interaction of lactation number with age of cow at calving). X is the incidence matrix relating observations with fixed effects; a is the vector of direct additive genetic effects; Z_1 is the incidence matrix relating observations with random genetic effects; and e is the vector of residual effects. Distributions of the random effects are var(a) = $A\sigma^2$,where A is the pedigree relationship matrix and σ^2 is the additive genetic variance, and var(pe) = $I\sigma^2pe$, where I is an identity matrix of an order equal to the number of observations and σ^2pe is the permanent environmental variance., and var(e) = $I\sigma^2e$, where I is an identity matrix of an order equal to the number of observations and σ^2e is the residual variance. The pedigree included 98,324 individuals, with maximum 14 generations.

Sensitivity aanalysis

As part of the proposal to include genetics in the annual nutrient cycle assessment, mean EBV for CH_4c per farm were calculated, divided in quantiles and plotted to detect differences among farms. Differences among quantiles were calculated to determine the maximum difference in CH_4c between them. The data was divided into four equal groups (called quantiles) based on the values of the mean EBV per farm. Then, the dataset was split into 4 quantiles which assigns each observation to one of four equally sized groups according to the distribution of the mean EBV per farm. Subsequently, a mean of each quantile group was calculated, to allow us to see how the average value in each group compared to the overall average. Finally, we measured how much this group's average differed from the overall average.

Results and discussion

Descriptive statistics

The daily average for CH_4c was 552 parts per million (ppm), whereas, the standard deviation was 272. Genetic variance was 5,434, phenotypic variance was 14643, and the heritability was 0.12 (SE=0.01), whereas, the permanent environmental ratio was 0.33 (SE=0.01). Number of cows per farm (n=72) varied between 38 and 245. This average is consistent with values previously reported for Holstein cows (Difford *et al*., 2020; Manzanilla-Pech *et al*., 2022; van Breukelen *et al*., 2022).

Average breeding values per farm and quantiles

Average breeding values per farm varied between +41 to -5 for the 72 farms (Figure 1). Standard errors ranged between 1 to 8. However, when EBV were grouped per quantile (Figure 2) the difference between quantile 1 and quantile 4 is 20.6 points. This showed a difference between farms present in the first quantile compared to the farms in the fourth quantile. By increasing the number of quantiles this difference will increase too (e.g. 10 quantiles will lead to a difference of 28 points between farms.

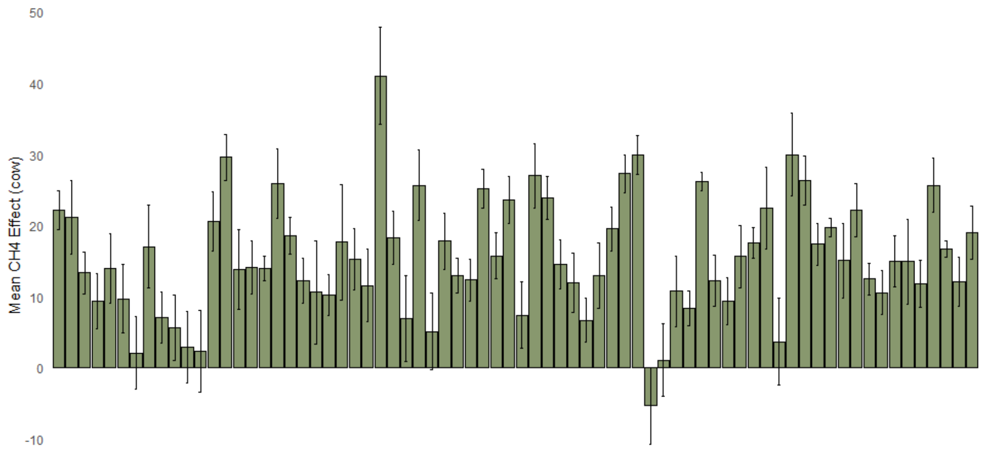

Figure 1. Average EBV for CH4c (ppm) per farm (n=72) with SE.

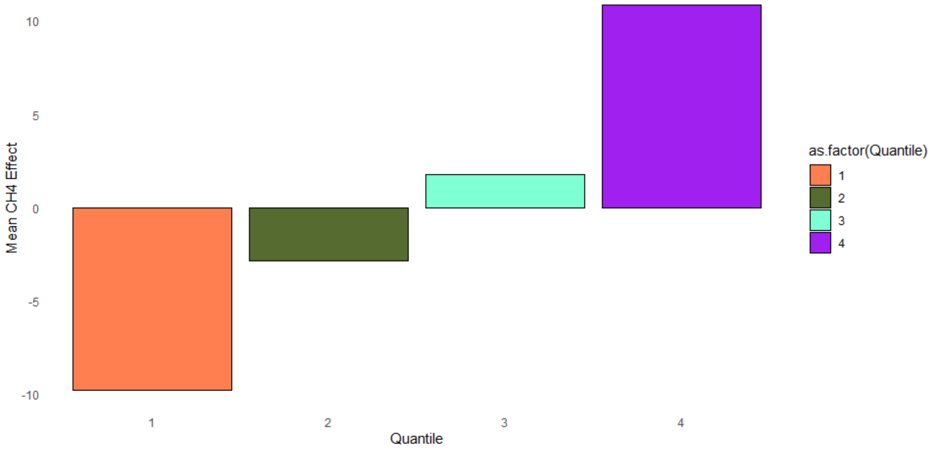

Figure 2. Quantile differences (25%) between farms for average EBV for CH$_4$ ppm.

Applications

The results of this study demonstrate that differences between farms can be detected by averaging their breeding values. This method has potential applications in refining the existing ANCA tool by incorporating a correction factor. Although still in development, the final correction factor will be based on CH4 g/d. The EBV for CH$_4$ g/d will utilize the genetic correlation (0.76; van Breukelen *et al*., 2023) between CH$_4$ concentration in ppm measured by sniffers and CH4 g/d determined by the GreenFeed system. Finally, the correction factor would adjust for the average genetic merit of the animals present on each farm, leading to more accurate evaluations and comparisons.

Conclusion

This pilot study aimed to assess the feasibility of incorporating genetic information into the calculation of CH_4 emissions from ANCA for the entire dairy herd. This study showed that it is feasible to detect differences among farms when averaging the CH_4 breeding values of the cows per farm. The difference between extreme farms was 20 points between the top and bottom 25% and up to 28 points (CH_4c ppm) between the top and bottom 10%, which represents up to 3.7 to 5.1% of the enteric CH_4c per farm. These results are promising and will be used as first step to build the new additions around the ANCA formulation that will involve genetic information.

Acknowledgments

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Machine learning for predicting environmental impacts in intensive livestock farming, overcoming data challenges

L. Foschi[1], D. Pennino[1], M. Marconi[1], M. Barbanera[1], G. Grossi[2], F. Cesarini[2], C. Rossi[2], L. Bava[3], A. Vitali[2] and N. Lacetera[2]

[1]Università degli Studi della Tuscia, Dipartimento di Economia, Ingegneria, Società e Impresa, Largo dell'Università, Viterbo, 01100, Italy
[2]Università degli Studi della Tuscia, Dipartimento di Scienze Agrarie e Forestali, Via San Camillo de Lellis, Viterbo, 01100, Italy
[3]Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali, via Celoria 2, Milano, 20133, Italy
Corresponding Author: lorena.foschi@unitus.it

Abstract

Life Cycle Assessment (LCA) is a crucial methodology for evaluating environmental impacts, and its integration with machine learning (ML) regression offers promising new applications. While LCA is widely used, its combination with ML can significantly enhance predictive accuracy. However, the importance of robust datasets is often overlooked, and LCA datasets frequently suffer from issues such as missing values, which compromise the accuracy and reliability of the assessments. This study addresses these challenges by leveraging ML regression not only for final predictions but also as a tool for cleaning and preparing datasets. We propose a systematic approach to identify and select the most suitable regression algorithm for a dataset with missing values. This approach involves analyzing dataset characteristics and applying different ML regression techniques to find the best fit. Our method was validated by applying it to three distinct datasets, each with unique data quality issues. The results demonstrate that using ML regression for both prediction and data cleaning can significantly improve the robustness and reliability of LCA assessments..

Keywords: life cycle assessment (LCA), carbon footprint, machine learning regression, environmental impact prediction, intensive dairy cattle farming
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Introduction

The livestock sector accounts for about 12% of total anthropogenic greenhouse gas (GHG) emissions, with dairy production contributing 30% of this total (FAO, 2022). In recent years, consumers have increasingly demanded environmental information, demonstrating a significant interest in the environmental impacts of the agri-food sector (Potter *et al*., 2022). The sustainability of animal-based products is a highly topical issue, with environmental concerns leading to conflicting perceptions among stakeholders (Leroy *et al*., 2022). Therefore, it is becoming necessary to provide adequate information to make society aware of the environmental impacts of agriculture and livestock production (Stygar *et al*., 2022). Life Cycle Assessment (LCA) is a valuable and standardized methodology (ISO 14040:2006; ISO 14044:2006) used to estimate the emissions per unit of product and identify the sources of environmental burden along the supply chain (Rossi *et al*., 2024; 2023). LCA provides a holistic approach to account for both direct and indirect environmental emissions and has been effectively applied in numerous studies focusing on food of animal origin and the majority of which considered emissions of GHGs (Thumba *et al*., 2021).

In conducting LCA studies, one of the most significant challenges is the acquisition of a sufficiently extensive and accurate dataset of primary data. This difficulty is inherent to the methodology itself, as the accuracy of environmental impact assessments hinges greatly upon the availability and quality of primary data. However, obtaining such data can be arduous and resource-intensive, requiring meticulous data collection efforts across various stages of the product lifecycle. In instances where primary data collection proves impractical or unfeasible, it may be necessary to consider leveraging existing databases or literature data, commonly referred to as secondary data. While secondary data can offer a valuable alternative, their use introduces complexities and uncertainties, as they are inherently reliant on the assumptions and methodologies employed in previous studies. Consequently, the reliability and accuracy of the results obtained from secondary data depend heavily on the validity and applicability of these assumptions, underscoring the need for careful consideration and scrutiny. Recognizing these challenges, researchers have increasingly explored the integration of Machine Learning (ML) techniques to augment traditional LCA methodologies. The use of ML algorithms offers a potential solution to address common limitations encountered in LCA studies, particularly pertaining to inventory compilation and completeness, as well as environmental impact calculation or estimation. ML offers the potential to automate and optimize data processing tasks, enhance the accuracy of predictive models, and uncover complex patterns and relationships within large datasets. As such, the integration of ML holds promise in overcoming data-related challenges and advancing the capabilities of LCA methodologies in assessing environmental impacts comprehensively and effectively..

LCA critical data aspects in dairy farms

However, the application of this methodology has encountered some challenges when applied to animal-based products, primarily due to difficulties in assessing emissions arising from biological processes (Lanzoni *et al*., 2023). The rationale behind these limitations stems from the methodology's original design, which was primarily intended to evaluate environmental emissions in industrial processes before being adapted for use in various fields, including agriculture (Caffrey and Veal, 2013). The challenge of adapting the LCA framework to farming systems lies in identifying additional key aspects that must be considered to use this approach effectively for livestock production and achieve an appropriate environmental evaluation. Applying LCA to agricultural systems is challenging due to their varied factors and dynamic connections. Particularly difficult is the large amount of data required for the LCA, which may have an impact on the quality of the data and the predictability of the results. (Nirmala *et al*., 2023). Seasonal conditions and long-term processes involving field and herd management make it challenging to gather reliable information, requiring significant time to develop a comprehensive understanding of farm activities and interactions (Caffrey and Veal, 2013). Additionally, the low accuracy and limited accessibility of primary data collection can result in a lack of representativeness, insufficiency, or even a complete absence of necessary input data for LCA analysis at farm level (Pradeleix *et al*., 2022).

Machine Learning contribution to data prediction

The contemporary landscape unambiguously demonstrates the pervasive influence of data-driven paradigms, as evidenced by the fact that an enormous amount of data is continuously being generated and collected every second by almost every electronic device currently in use.

Being able to manage and use this large amount of data is quite complex, but in recent years thanks to research on artificial intelligence, the valorization and use of this data is becoming possible. This field of research is itself divided into numerous subcategories, including *Machine Learning* (ML). Machine learning is a field of research that concerns the study and implementation of mathematical algorithms capable of learning from data and interpolating or predicting unseen data. The ability to analyze huge amounts of data and extract insights has become a crucial requirement in many use cases.

Machine learning approaches can be divided into three main categories, which correspond to the ways in which the system can be trained.

- **Supervised learning:** The system is trained with tuples of inputs and the desired outputs. The objective is to learn a general rule that maps inputs to outputs.
- **Unsupervised learning:** The system is trained by providing only input data, letting it independently search for a structure in it. This type of learning is widely used when you want to discover hidden patterns within a dataset.
- **Reinforcement learning:** The system is trained using a reward strategy. As the system searches for patterns within the data, as with the previous category, it is provided with feedback on its learning. These feedbacks are a sort of reward and the system tries to maximize them.

In general, when the term “data prediction” is employed, it is understood to refer to a machine learning model that performs a regression analysis with a supervised approach. This type of analysis follows a statistical process to estimate a mathematical function between input and output data. These predictive models are used in numerous fields, from engineering sciences to social networks, agriculture, finance, security, etc.

Machine learning has become a tool capable of providing information that allows those who are using it to make choices based on statistical models rather than on intuitions, conjectures, or suppositions. Moreover, the scalability and automation enabled by machine learning algorithms facilitate process optimization, reduced operational costs, and increased productivity, thus promoting organizational growth and sustainability in an environment characterized by increasing dependency on data. To streamline the discussion, the term “Machine Learning” will be used throughout the remainder of the paper to refer to supervised regression models.

Problem formalization

The need to make the Life Cycle Assessment methodology increasingly accurate in predicting environmental impacts is leading to its conjunction with Machine Learning techniques. Current research in this field is mainly focused on improving LCA models through the application of ML algorithms, with the aim of improving the accuracy and efficiency of environmental impact assessments. A growing literature shows how machine learning is enhancing the precision of predictions derived from LCA studies. For instance, Romeiko *et al*. (2024) state that ML applications are concentrated on those stages of LCA that necessitate a substantial investment of effort, such as inventory, impact assessment, and result interpretation. With regard to the initial stage, ML was employed to estimate the overall life cycle inventory (LCI) and to predict the product properties required for the realization of the final LCI. The main application of ML to support LCA studies is the use of algorithms to predict environmental impacts. The agricultural sector represents the most extensively analyzed sector in this regard, with algorithms employed to estimate yield, energy use, and life cycle impacts. Lastly, in the interpretation stage of the results, ML was employed in a variety of ways. These included the minimization of assessed impacts, the identification of patterns and the main drivers of life cycle impacts, the understanding of uncertainty and the sensitivity of the results and the classification and assessment of relationships between indicators and environmental impacts. In light of the aforementioned considerations, there are only a few works that exploit ML to optimize the quality of the dataset. While machine learning can significantly aid in predicting environmental impacts, the reliability of these predictions depends on the availability of high-quality data. Consequently, there is a clear need for reliable data to support more accurate ML and LCA modeling. The complexity and uncertainty of environmental data require careful consideration of the datasets used in ML models to ensure that predictions are robust, with low error rates, and therefore applicable to real-world scenarios.

One of the most significant challenges in LCA is the presence of missing data within datasets. This can result from data incompleteness, measurement errors, or data collection limitations. Traditional approaches for handling missing data, such as mean- or median-based inferences, are often limited in their effectiveness and may lead to biased or unreliable results. In contrast, machine learning techniques present a compelling solution to this challenge, offering sophisticated algorithms for data completion and imputation. ML models can infer missing values with remarkable accuracy by leveraging the inherent structure and patterns within LCA datasets. This enhances the efficiency and reliability of LCA analyses. Furthermore, ML systems can capture complex relationships and dependencies within the data, enabling more robust assessments of environmental impacts.

In conclusion, although the integration of ML into LCA is a promising development for predicting environmental impacts, the current research tends to overlook the importance of good datasets. Ensuring the quality and relevance of the data used is crucial for the accuracy and reliability of the predictions made by ML-LCA models. This work wants to reduce this gap by focusing on the creation of high-quality datasets that can support the predictive capabilities of ML algorithms within the LCA framework.

Material and methods

In this section, we present our methodology, which consists of steps leading to the choice of a regression machine learning model for predicting missing data from a given dataset.

As mentioned in the previous sections, there are various regression models, each with advantages and disadvantages that make it more or less suitable for a specific use case.

The following is a list of the main regression algorithms involved in our approach, with a description of their modell.

1. **Linear regression** is one of the most frequently used regression algorithms in Machine Learning. It finds the linear relationship between the input variables and output variables using a best-fit straight line. Basically, the linear regression algorithms assume that there is a linear relationship between the inputs and the outputs. Formula 1 shows a simplification and generalization of the mathematical equation on which the computation of the linear regression is performed.

   $$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_1 + \ldots . + \alpha_n x_n \qquad (1)$$

   In the above formula,
   y is the output variable of the model
   x_i is the i-th input variable
   α_i is the i-th coefficient that is estimated by the model

2. **Ridge regression** is a variant of the linear regression that use penalty terms to minimize the error between the predicted and actual values.

   Referring to the previous equation, the penalty term is the sum of the squares of the coefficients. This regularization is called L2 regularization and it shrinks the coefficients but doesn't set any of them to zero.

   It is particularly useful to mitigate the problem of multicollinearity in linear regression, which commonly occurs in models with large numbers of parameters. Multicollinearity occurs when an input variable is highly correlated with one or more of the other input variables in a regression. Multicollinearity is a problem because it undermines the statistical significance of an input variable.

3. **Lasso regression** is also a variant of linear regression that uses penalty terms to minimize the error between the predicted and actual values. Unlike the previous technique, it uses a regularization called L1 regularization. In this technique, the penalty term is the sum of the absolute value of the coefficients. It can reduce some coefficients to zero, effectively performing input selection. It is particularly useful to reduce the overfitting of the model.

4. **Polynomial regression** extends linear regression by fitting a polynomial equation to the data, allowing for non-linear relationships between input and output variables. Formula 2 shows a simplified and generalized example of a polynomial function of n-th degree.

   $$y = \alpha_0 + \alpha_1 x_1{}^1 + \alpha_2 x_1{}^2 + \ldots.. + \alpha_n x_n{}^n \qquad (2)$$

5. **Decision tree regression** is a non-linear regression model. The main function of this technique is to split the dataset into smaller sets. The splitting of the data results in a tree-like structure. A decision tree is like a flowchart where each prediction starts from the root node and, based on some criteria, moves along a path of internal nodes called decision nodes until reaching the leaves that contain the result.

6. **Random forest regression** is a collection of multiple decision trees. Each tree is independently trained so that each tree turns out to have a slightly different structure from each other. When we run a prediction using this model, we are essentially asked for the prediction at each individual tree, then their predictions are aggregated to identify the most suitable result.

7. **Support vector regression** is a technique that aims to find a hyperplane that best fits the data points in a continuous space. This is achieved by mapping the input variables to a high-dimensional feature space and finding the hyperplane that maximizes the distance (margin) between the hyperplane and the closest data points, while also minimizing the prediction error.

8. **Gradient boosting regression** is a technique that builds a sequence of weak learners, typically decision trees, with each new learner correcting errors made by the previous ones. In each iteration, the algorithm computes the residual error, which is equal to the actual value minus the predicted value. This error is then used to train a new weak model, with the objective of minimizing it. The process is repeated until a stopping criterion is met.

Our approach

The approach presented below is designed to assist in the selection of an optimal machine learning algorithm for prediction purposes, while also facilitating the incorporation of missing values from a given dataset.

For simplicity and usability, our approach is presented as a decision tree.

As represented in Figure 1 the methodology guides the selection of a model based on key considerations derived from a dataset. The initial inquiry concerns the presence of missing values within the dataset, directing to either proceed with further analysis or conclude that no regression model is required. The subsequent step is to interrogate the linearity of the input-output relationship, in case a linear relationship exists, the Linear Regression approach should be selected. Conversely, an additional evaluation is necessary to determine whether the relationship exhibits non-linearity. If this is the case, then complex interactions of high-order relationships between inputs should be revised. This process can be accomplished by either Polynomial Regression if there are such interactions, or Decision tree Regression if not there are not. In the event that the relationship is found to be non-linear, further investigation should be conducted to ascertain the necessity for effective handling of outliers or noise. If these are present, an evaluation regarding the size and dimension of the dataset is necessary. With a small to medium-sized and high-dimensional dataset, the Support Vector regression model should be used. While with another type of dataset, the Random Forest regression would be the ideal solution. In the event that there is no necessity to address outliers or noise, it would be beneficial to determine whether the input selection is a priority or not. If so, it would be advisable to identify any instances of multicollinearity and select Ridge Regression if the latter is present or Lasso Regression if not. In the case where the input selection is not a primary concern, an assessment of the importance of achieving a high degree of predictive accuracy should be conducted.

Results and discussion

In this Section, we present and discuss the results of our approach applied to a real dataset.

Our dataset consisted of information from approximately 100 dairy cattle farms in Italy, primarily from studies conducted over the past decade. The majority of these farms utilize intensive farming practices. The data collection focused on key inputs for Carbon Footprint analysis, such as production metrics like Fat and Protein Corrected Milk (FPCM) produced, herd composition, cultivated land area, and diet details, including the amount and origin of forage and concentrates, energy and fuel used, as well as the amount of CO2 eq. as output. By using our approach, we were able to increase the number of rows used for training by an order of magnitude, resulting in a significant reduction in prediction error.

Due to the complexity of the study, the dataset was decomposed into subdatasets to make it easier to manage and apply our approach. Specifically, the three subsets of data on soy meal, heard size, and total feed are used in the following to show the accuracy of our approach.

The characteristics of each dataset are listed below:

- **soy meal dataset:** Since the value of soy meal is a component that is influenced by use case components such as herd size, lactating cows, concentrate values, etc., this dataset results in a large number of heterogeneous inputs. It is also important to consider that some of the inputs have noise, due to the inherent limitations of the data collection methods employed.

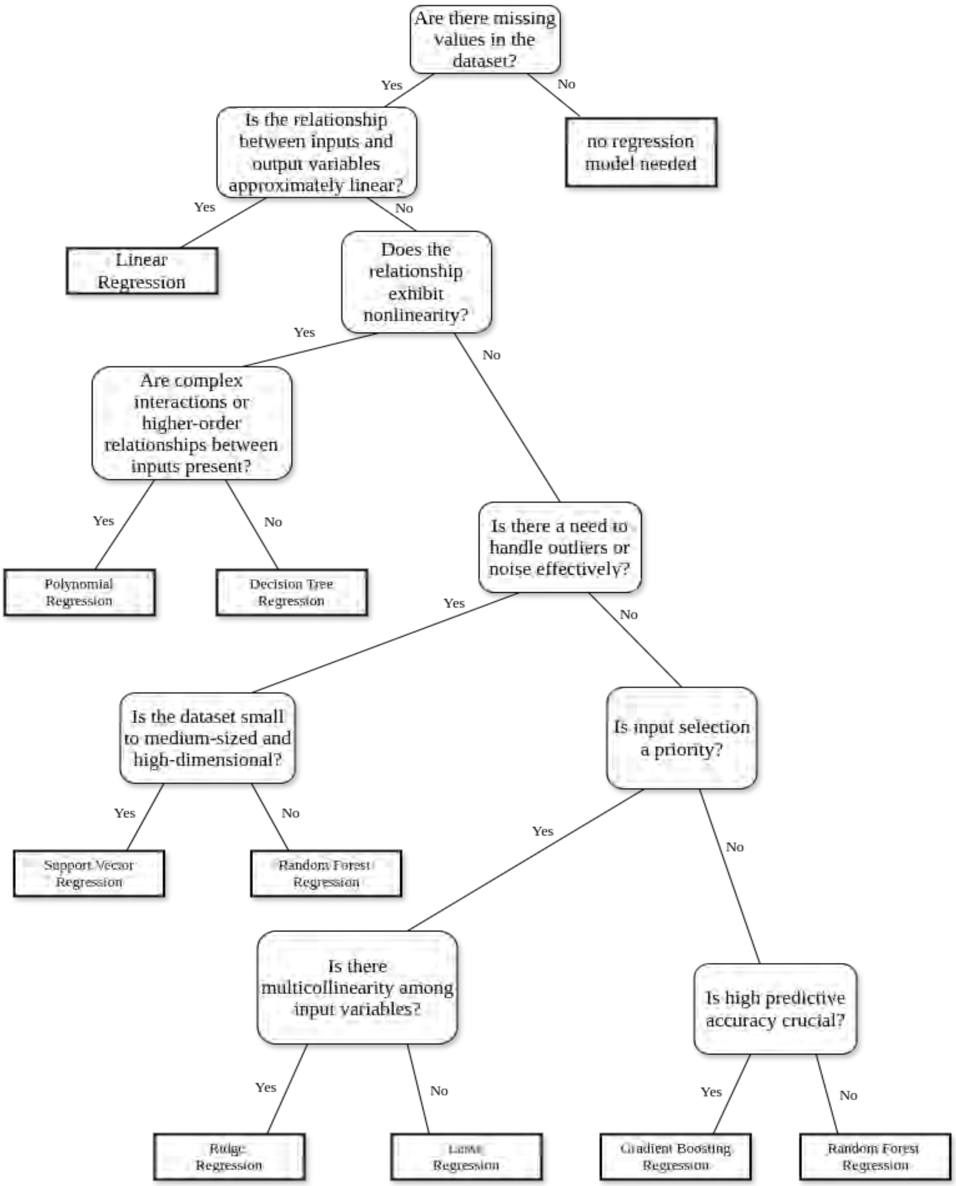

Figure 1. Decision tree representing our approach.

- **heard size dataset:** This value does not depend on an overly complex mathematical calculation and depends very much on the herd farm in question, so we use a dataset with many parameters that can help characterize the type of farm and thus estimate the possible herd size. These parameters are numerous as in the previous case, but they are easier to collect. In this dataset, elements such as the size of the farm, the cultivated area, the type of the farm, etc. are used as inputs.
- **total feed dataset:** This dataset is very similar to the previous one, there is not a complex mathematical relationship between the inputs and the output, it depends

a lot on the way the farm is managed. However, unlike the previous dataset, many inputs are correlated with each other such as soy meal, concentrates, cultivated area, etc.

To evaluate a regression model, various techniques can be used.

In this work, we decided to use the Root Mean Square Error (RMSE). The RMSE represents the standard deviation of the prediction errors. These errors are a measure of the distance of the actual data from the regression line; RMSE is a measure of the spread of these residual values. In other words, it indicates the concentration of actual value around the line of best approximation.

Table 1 shows the RMSE calculated on the three datasets. In order to facilitate a more comprehensive understanding and comparison of performance across the different datasets, the RMSE was normalized between 0 and 1.

In the following, we apply our approach to each dataset and can see that the resulting regression algorithm is equal to the best-performing one as shown in the table.

Application of our approach to the SoyMeal dataset:

1. Are there missing values in the dataset? Yes.
2. Is the relationship between inputs and outputs approximately linear? No, the inputs of the dataset are many and there is no linearity between them and the output.
3. Is there a need to handle outliers or noise effectively? Yes. Since many inputs to the dataset come from measurements that by their nature may contain errors, this dataset definitely has presence of noise within it.
4. In the dataset small to medium-size and high-dimensional? No. In general, a dataset is considered “high-dimensional” when it has a large number of variables relative to the number of observations.
5. Our approach suggests to use a Random Forest Regression model.

Application of our approach to the Herd Size dataset:

1. Are there missing values in the dataset? Yes.
2. Is the relationship between inputs and outputs approximately linear? No, the inputs of the dataset are many and there is no linearity between them and the output.
3. Is there a need to handle outliers or noise effectively? No. Since the inputs come from simpler and therefore more accurate data collection and gathering methods.
4. Is input selection a priority? Yes. It is not easy to choose appropriate inputs to predict this value, so it is preferred to have the regression algorithm do the input selection.
5 Is there multicollinearity among input variables? No. The inputs are different from each other, and although there is a slight correlation among some of them, we can consider the dataset unaffected by multicollinearity
6. Our approach suggests to use a Lasso Regression model.

Application of our approach to the Total Feed dataset:

1. Are there missing values in the dataset? Yes.
2. Is the relationship between inputs and outputs approximately linear? No, the inputs of the dataset are many and there is no linearity between them and the output.
3. Is there a need to handle outliers or noise effectively? No. Since the inputs come from simpler and therefore more accurate data collection and gathering methods.
4. Is input selection a priority? Yes. It is not easy to choose appropriate inputs to predict this value, so it is preferred to have the regression algorithm do the input selection.
5. Is there multicollinearity among input variables? Yes. Many inputs are correlated with each other such as soy meal, concentrates, and cultivated area. We can consider this dataset affected by multicollinearity,
6. Our approach suggests to use a Ridge Regression model.

Table 1. Normalized RMSE of three different datasets.

Dataset/ ML model	Linear	Ridge	Lasso	Polynomial	Decision tree	Random forest	Support vector	Gradient boosting
Soy Meal	0.118	0.116	0.116	0.114	0.114	**0.105**	0.121	0.115
Heard Size	0.023	0.019	**0.020**	0.033	0.088	0.091	0.023	0.081
Total Feed	0.066	**0.058**	0.061	0.086	0.09	0.08	0.059	0.076

Conclusion

In this paper, we introduced a novel approach to address the challenge of predicting missing values within datasets, particularly within the context of environmental impact assessment in the livestock sector. Our approach was designed to select the most appropriate algorithm for predicting missing values in a dataset, taking into account its specific characteristics and requirements. By leveraging Machine Learning (ML) techniques, we sought to develop a systematic framework that could effectively handle missing data and enhance the completeness of the dataset.

To assess the effectiveness of our approach, we conducted testing using three distinct datasets, each representing different variables that influence the environmental impacts of cattle milk farming. Through rigorous testing and validation procedures, we assessed the performance of our approach across these datasets, measuring its ability to accurately predict missing values and improve dataset completeness. The results of the testing demonstrated the versatility and effectiveness of our approach, showcasing its capability to address missing data challenges across diverse datasets. Furthermore, to demonstrate the practical applicability of our approach in real-world scenarios, the three individual datasets were consolidated into a single comprehensive dataset tailored for environmental impact prediction, which enables us to explore and analyze environmental impacts across various domains. The application of the approach to the unified dataset resulted in a significant increase in the size of the dataset by an order of magnitude, thereby enhancing its utility for environmental impact prediction.

The integration of ML techniques with LCA methodology represents a promising avenue for addressing data-related challenges in environmental impact assessment. The use of ML algorithms to predict missing values and enhance dataset completeness represents a promising avenue for overcoming common limitations encountered in LCA studies, such as incomplete or unreliable data. This synergistic approach enables more robust and comprehensive environmental impact assessments, thereby facilitating informed decision-making and sustainable practices across diverse sectors and industries.

In summary, this study underscores the potential of combining ML techniques with LCA methodologies to address data-related challenges and enhance the completeness and accuracy of environmental datasets.

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Towards breeding for lower enteric methane emissions of dairy cows in the Netherlands

A.E. van Breukelen[1], M.N. Aldridge[2], Y. de Haas[3], C. Schrooten[4], J.M.L Heck[5], M.H.P.W. Visker[5] and R.F. Veerkamp[1]

[1]Wageningen University and Research, Animal Breeding and Genomics, 6700AH Wageningen, the Netherlands
[2]Animal Genetics and Breeding Unit, A joint venture of NSW Department of Primary Industries and University of New England, University of New England, Armidale, NSW 2351 Australia
[3]Animal Health and Welfare Group, Wageningen University and Research, 6700AH Wageningen, the Netherlands
[4]Cooperative CRV u.a., 6842 BD Arnhem, the Netherlands
[5]FrieslandCampina, Stationsplein 4, 3818 LE Amersfoort, The Netherlands / PO Box 1551, 3800 BN Amersfoort, the Netherlands
Corresponding Author: anouk.vanbreukelen@wur.nl

Abstract

Various strategies have been proposed to reduce enteric methane (CH_4) emissions from ruminants, focusing on areas such as management, feeding strategies, feed additives, vaccination, and animal breeding. Among these, animal breeding currently shows the largest long-term potential, due to its low implementation costs, and permanent and cumulative effect. Nonetheless, implementing CH_4 mitigation in breeding programs is still in its infancy. An important limitation to practical application has been the lack of phenotyping of CH_4 emissions on individual cows, to estimate sufficiently reliable genetic parameters, which are required for breeding decisions. However, recent innovations have accelerated the collection of CH_4 phenotypes. We describe the outcomes of a four year project in the Netherlands, that aimed to record CH_4 emissions on 100 dairy farms, to be used in genetic parameter estimations. This dataset will be the basis for future national breeding value estimations for enteric CH_4 of Holstein dairy cows, which will be implemented by the cooperative cattle improvement organization CRV. In the project, enteric CH_4 emissions were measured by 'sniffers' that sample air from the feed bin of milking robots. The latest dataset included 110,188 weekly mean CH_4 concentration (ppm) records on 7,749 cows from 72 farms, but data recording is ongoing. Several analyses have been performed already, with the following objectives:

1. Define a CH_4 trait from the raw concentration measurements.
2. Estimate heritabilities and repeatabilities.
3 Investigate different recording schemes.
4. Investigate the relationships between CH_4 and other breeding goal traits.

To date, the research output of the project showed that the phenotype for weekly mean CH_4 concentration has a moderate heritability of 0.17 ± 0.04 and a repeatability of 0.56 ± 0.03. As the sniffers only measure concentrations, and not the total grams of CH_4 emitted by breath, genetic correlations were estimated between the weekly mean CH_4 concentration phenotype and a weekly mean CH_4 production (g/day) phenotype. The latter was recorded by GreenFeed units on 797 cows from 16 farms (4 overlapping with sniffers). The genetic correlation was 0.76 ± 0.15, indicating that selection for lower concentrations will result in a reduction of total CH_4 production output in g/day.

Furthermore, with the novel dataset we confirmed that the genetic variance changed over a lactation. This has implications for recording schemes, and we showed that short recording of CH_4 during the first or last weeks of recording can result in lower genetic gains than predicted from the reliability, when modelled without using random genetic and permanent environmental regressions over the lactation. In the last stage of the current project, genetic relationships among CH_4 concentration, DMI, bodyweight, and milk yield traits were estimated, which are required to set up the selection index that includes CH_4 mitigation. The results show low genetic correlations between CH_4 and: dry matter intake (0.06 ± 0.10), body weight (-0.04 ± 0.10), and milk yield (-0.04 ± 0.08). The developed dataset and models, are currently used to set up national breeding value estimation for CH_4 emissions of dairy cows in the Netherlands. Furthermore, the dataset will be used in additional research projects in the coming years, that aim to

1. Investigate the relationship with the microbiome.
2. investigate the impact of selecting using the developed phenotype(s).
3. Investigate incentives for farmers to use the CH_4 breeding values.
4. Proof the impact of change in breeding values at farm and national level.

This comprehensive approach not only improves our understanding of breeding for lower enteric CH_4 emissions, but also integrates this knowledge into practical breeding strategies for sustainable dairy farming.

Keywords: methane emissions, sniffers, dairy cows, quantitative genetics, animal breeding.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 11: Methane Emission-Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

Introduction

In the Netherlands, a pledge has been made to reduce greenhouse gas emissions by 55% in 2030 compared to the year 1990, and to be carbon neutral by 2050 (Communication from the Directorate-General for Climate and Energy, 2022). A large contributor to national emissions is agriculture, from which a part of the emissions stem from ruminants, such as dairy cows (Van Bruggen *et al*., 2023). Dairy cows produce CH_4 during enteric fermentation of feed in the rumen, which is released through breath and belching. That animal breeding can play a role in reducing the environmental impact of dairy farming is becoming increasingly recognized. Here, we describe the outcomes of a four year project in the Netherlands, that aimed to record CH_4 emissions on 100 dairy farms, to be used in genetic parameter estimations. This dataset will be the basis for future national breeding value estimations for enteric CH_4 of Holstein dairy cows, which will be implemented by the cooperative cattle improvement organization CRV.

To enable selective breeding, phenotypes for CH_4 were recorded with 'sniffers'. Sniffers are a comparatively cheap method to record large numbers of cattle, that are increasingly used to phenotype cows for genetic improvement (Garnsworthy *et al*., 2019; Madsen *et al*., 2010). Nonetheless, because CH_4 recorded with sniffers is a relatively new trait, many questions remain on how to apply the recorded emissions in genetic evaluations. For example, the measurements are faced with inaccuracies, which should be accounted for by taking repeated measurements and appropriate modelling (Bovenhuis *et al*., 2018; Wu *et al*., 2018), and the relationships with other economically important breeding goal traits are still unclear (Hossein-Zadeh, 2022).

In our four year project, we have collected over 100,000 weekly mean CH_4 concentration (ppm) measurements on 7,749 cows from 72 farms. Here, we summarise the genetic parameters that were estimated from this dataset, including genetic correlation estimates with other breeding goal traits. This demonstrates the progress made towards accomplishing our objective, which is to provide meaningful breeding values for methane mitigation in the Netherlands.

Material and methods

The latest dataset included 110,188 weekly mean CH_4 concentrations (CH_4c) records from 7,749 cows from commercial dairy 72 farms in the Netherlands. Data were collected between 2019 and 2023, and previously described in (van Breukelen *et al*., 2024). In short, non-dispersive infrared sensors, called sniffers (WD-WUR v1.0 and v2.0, manufactured by Carltech BV), were installed with a tube leading into the feed bin of the milking robot (AMS), and continuously recorded CH_4 (0 to 2,000 ppm) and CO_2 (0 to 10,000 ppm) concentrations. The recorded data was filtered to exclude biologically improbable records, and then matched with AMS visit information to determine the cows' IDs. Thereafter, the data was averaged per AMS visit, including records from the first up to the fifth minute of milking..

Genetic analyses

Various phenotypes defined from the sniffer concentration measurements were analysed, including visit, daily, and weekly means, on: the mean, median, log, and CH_4/CO_2 ratio. From the data, genetic parameters were estimated using univariate fixed repeatability models and a random regression repeatability model with ASReml 4.2 (Gilmour *et al*., 2015), and genetic correlations were estimated using pairwise bivariate models. All cows used for the genetic analyses had pedigree information, and most cows were genotyped. Several genetic analyses were performed, with the following objectives: 1) estimate heritabilities and repeatabilities (from various CH_4 traits), 2) investigate different recording schemes, including changes in genetic parameters over a lactation, and 3) investigate the phenotypic and genetic relationships between CH_4 and other breeding goal traits.

The estimated heritabilities and repeatabilities were used to calculate the reliability of breeding values, which was used to determine the number of daughter records needed to reach breeding value publication thresholds. For details on the datasets and methods used see van Breukelen *et al*. (2022) and van Breukelen *et al*. (2024).

Genetic correlations with GreenFeed recorded cows

To determine the applicability of CH_4c as an indicator for the reduction of total CH_4 emissions in grams/cow/ day, genetic correlations were estimated with CH_4 measurements from the more accurate GreenFeed, as GreenFeed units are considered the gold standard for on-farm recording of individual cow CH_4. Methane was recorded by GreenFeed units at 16 farms (of which four also had sniffer recording), and the analysed dataset included GreenFeed measurements in 822 cows [of which 184 cows also had sniffer records, for details see van Breukelen *et al*. (2023)].

Genetic correlations with other breeding goal traits

In addition, genetic correlations were estimated for first parity cows between CH_4c, and: milk yield (MY), protein yield (PY), fat yield (FY), protein percentage (P%), fat percentage (F%), body weight (BW), and dry matter intake (DMI). The number of weekly records and cows for each trait were: 8,891 cows with 179,469 records for MY, 8,889 cows with 139,295 records for PY, 8,889 cows with 139,328 records for FY, 5,919 cows with 119,523 records for BW, and 4,998 cows with 87,306 records for DMI. The traits P% and F% were calculated as PY or FY, respectively, divided by MY. The genetic correlations were estimated using pairwise bivariate models, including fixed effects for: an interaction between farm, year and week of measurement for the CH_4 and CO_2 traits only, an interaction between farm and experimental treatment for all traits except CH_4 and CO_2, a second order Legendre polynomial on age at calving in days, a third order Legendre polynomial on DIM, and an interaction between the second breed with a second order Legendre polynomial on the fraction of the second breed, and a random genetic and permanent environmental effect. The residual and permanent environmental covariances were fixed to zero for the runs including DMI and a CH_4 or CO_2 trait, because of the small number of cows with records on both DMI and a greenhouse gas trait.

Results and discussion

Genetic parameter estimates

The research output of the project showed that the phenotype for weekly mean CH_4 concentration has a moderate heritability of 0.17 ± 0.04 and a repeatability of 0.56 ± 0.03. As the sniffers only measure concentrations, and not the total grams of CH_4 emitted by breath, genetic correlations were estimated between the weekly mean CH_4 concentration phenotype and a weekly mean CH_4 production (g/day) phenotype. The latter was recorded by GreenFeed units on 797 cows from 16 farms (four overlapping with sniffers). The genetic correlation was 0.76 ± 0.15, indicating that selection for lower concentrations will result in a reduction of total CH_4 production output in g/day. Other phenotypes that were defined from sniffer measurements (i.e. median concentrations, log transformed concentrations, and traits defined from the maximum concentrations or peaks in concentrations) had high genetic correlations with mean CH_4 concentrations ($\geq$0.78), apart from the genetic correlations with the CH_4/CO_2 trait, which was negative.

Recording schemes

Furthermore, a comparison was made between genetic parameter estimates for CH_4 emission from a fixed regression repeatability model and a random regression (RR) model. The RR model, allowed for varying genetic variances and covariances over a lactation. The results showed that the heritability was highest mid lactation (on average 0.17 ± 0.04), and genetic correlations between lactation stages were high (0.34 ± 0.36 to 0.91 ± 0.08). Permanent environmental correlations deviated greatly over a lactation and ranged between -0.73 ± 0.08 and 1.00 ± <0.01, which highlights that it is most appropriate to model CH_4c with a RR model including a random permanent environmental effect. With a large number of full-lactation daughter CH_4 records for each bull, the reliability was similar for the fixed and RR models. However, when data were only available for shorter recording periods at the beginning and end of lactation, using the fixed regression model led to up to a 28% reduction in reliability for bulls. Assuming the fixed model when the true (co)variance structure is reflected by the RR model, more than twice as long recording from the start of lactation was required to achieve maximum reliability for a bull. Therefore, applying an overly simplistic model could lead to insufficient recording and lower than predicted genetic gains based on the estimated reliability. If all cows would only be recorded for a small period of time, for example three weeks, it is preferred to record cows in mid lactation as this would yield the highest reliabilities. In addition, to reach the Dutch breeding value publication threshold of 50% for sires, it is recommended to record at least 25 visits or 5 weeks, from 10 daughters per sire.

Genetic correlations with breeding goal traits

In the last stage of the current project, genetic relationships among CH_4c and important breeding goal traits were estimated. Results show low genetic correlations between CH_4c and: MY (-0.04 ± 0.08), PY (<0.01 ± 0.08), FY (0.12 ± 0.08), P% (0.10 ± 0.09), F% (0.21 ± 0.08), BW (-0.04 ± 0.10), and DMI (0.06 ± 0.10). This indicates that it is possible to breed for reduced CH_4 concentrations, while continuing to improve, for example, milk production or fat yield.

Continuation

The results coming from this project, will be used to set up national breeding value estimation for CH4 emissions of dairy cows in the Netherlands, from which first breeding values are expected in 2025. Furthermore, the dataset will be used in additional research projects in the coming years, that aim to 1

1. Investigate the relationship with the microbiome.
2. Investigate the impact of selecting using the developed phenotype(s).
3. Investigate incentives for farmers to use the CH4 breeding values.
4. Proof the impact of change in breeding values at farm and national level.

Conclusion

Animal breeding is an effective approach to reduce agricultural emissions, that can contribute to reaching greenhouse gas targets. To phenotype large numbers of cows on commercial dairy farms for breeding programs, sniffers provide a cost-effective method of measuring gas concentrations. In our four year project, we have shown that CH_4 concentration traits have a moderate heritability, have a favourable high genetic correlation (0.76) with CH_4 production recorded by Greenfeed units, and the CH_4 concentrations measured with sniffers have low genetic correlations with milk production traits, BW and DMI. The results coming from the project not only improve our understanding of breeding for lower enteric CH_4 emissions, but are also used to integrate this knowledge into practical breeding strategies for sustainable dairy farming.

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A holistic approach for monitoring the environmental sustainability of the Italian Holstein cattle population

L. Benzoni[1], R. Finocchiaro[1], G. Visentin[2], M. Dorigo[3], F. Tiezzi[4], M. Marusi[1], J. Layton[1], A. Bracchi[5], G. Bonacina[5] , M. Zucali [6], G. Gislon [6] and M. Cassandro[1,7]*

[1]Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ), Via Bergamo 192, 26100 Cremona (CR), Italy
[2] Department of Veterinary Medical Science, University of Bologna, Ozzano dell'Emilia (BO), Italy
[3]Nutristar SpA, Via del Paracadutista,9, 42122 Reggio Emilia (RE), Italy
[4]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine 18, 50144 Firenze (FI), Italy
[5]Tecnosens SpA, via Vergnano, 16 25125 Brescia (BS), Italy
[6] Department of Agricultural and Environmental Sciences, University of Milan, Via Celoria 2, 20133 Milano, Italy
[7]Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
Corresponding Author: lorenzobenzoni@anafi.it

Abstract

This work aims to describe the holistic approach for monitoring the environmental sustainability that the Italian Holstein, Brown, and Jersey Breeders Association (ANAFIBJ) is implementing. Since 2015, ANAFIBJ has been setting up a wide range of environmental strategies to record data and to develop tools that meet community and farmer needs on mitigation climate change. In 2018, ANAFIBJ started collecting innovative data for each young calf housed at the experimental farm of ANAFIBJ. Several phenotypes, for over 200 young bulls, were recorded using advanced technologies, including the GreenFeed system (C-Lock Inc., Rapid City, SD, USA) and the Roughage Intake Control system (Hokofarm Group, Marknesse, The Netherlands). A new pipeline was developed to incorporate these new traits into the routine database maintained by ANAFIBJ, which is updated daily. At the population level, the Association formed a Consortium with various stakeholders, including University Experimental Farms, Commercial Farms, Universities, and Private Companies for recording routine environmental traits recorded on the Holstein female population. Methane emission records, from the GreenFeed system and the Sniffer type systems, milk-spectral records, from mid-infrared spectroscopy of milk labs, ruminal content, and microbiota composition, collected from key individuals on the population, will feed into the central ANAFIBJ data flow system. This will allow in the near future to set-up a genetic evaluation for these innovative traits and build up stronger cooperation at the international level. The Life Cycle Assessment (LCA) has been applied to several dairy herds enrolled in the national herd book. Now, LCA predictions can be made for all national dairy herds using the routinely recorded data in the ANAFIBJ national database. More herds are expected to have LCA recorded scores in the future. Further, an innovative report, named the "green passport", was generated to summarize the methane emissions and feed and water intake records of each bull housed in the experimental farm managed by ANAFIBJ. Each tool plays a pivotal role in allowing farmers across the country to assess the environmental impact of their herd and inform decisions regarding herd management.

Keywords: greenhouse gas emissions, data-collection, GreenFeed, sniffer.
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Introduction

Enteric methane emissions from ruminants are a major contributor to atmospheric greenhouse gas accumulation. Accurate measurement of methane production in ruminants is crucial to not only develop reliable national greenhouse gas emission inventories, but also evaluate mitigation strategies for methane emissions. Measuring actual enteric emissions in livestock is complex, expensive and time consuming. Many different research and industry bodies globally are investigating the feasibility and accuracy of a range of different techniques for recording enteric methane emissions and to create an automatic data streamline to storage data.

Material and methods

Available on market there are two different ways to perform an environmental sustainability evaluation: direct methods and indirect one. In the first category there are many different instruments as respiration chamber, portable accumulation chamber, SF6, breath sampling during milking and feeding, Greenfeed® and laser systems. Indirect methods or proxies are predicted indexes, milk spectra records (MIR), ruminal microbiome data and LCA. Generally, these two methods are considered separated. In the Italian holistic approach these two methods need to be integrated because direct data are crucial to validate proxies, while proxies are necessary to extend an environmental sustainability evaluation on large scale.

- Objective of this project areCollect GHG emissions data using different methods:
  - Greenfeed®
  - Moologger®
- Collect innovative traits data:
  - Milk Spectra Records (MIR)
  - Ruminal Microbiome data
- Validate proxies;
- Developtools, certifications and services that meet community and farmers need of mitigation climate change;
- Set-up a genetic evaluation also including innovative traits.

The first step was in 2019 with the collection of methane, carbon dioxide emissions, feed intake and water intake data into ANAFIBJ Genetic Center on Italian Holstein young bulls candidates to Artificial Insemination in Italy.

Two years later, in 2021, a daily automatic data pipeline was created to incorporate new traits into the routine database maintained by ANAFIBJ.

In 2023 the Italian Sustainability Consortium (ISC) was founded including University, Experimental Farms, Research Centers and Private Companies. To be part of the project, key farms must have some features: they have already been equipped with an Automatic Milking System (AMS) or an Automatic Feeding System (AFS) and must be registered. ANAFIBJ into these key farms will install some additional equipments,as Greenfeed® or Moologger. This structure and organization allow to collect a large variety of data: CH_4 emissions from GreenFeed or Moologger, type traits, milk spectra records, ruminal microbiome data and weight.

In 2024 an automatic data streamline has been created to incorporate Consortium traits into the routine database maintained by ANAFIBJ.

Results

Up today, ANAFIBJ database in composed of the following data collected on 272 Italian Holstein young bulls:

- 36,653 CH_4 records from Greenfeed®.
- 559,800 feed intake records.
- 6,491 water intake records.
- 2,181 BCS records.
- 6,543 biometric measures records.
- 2,315 weight records.

In addition, some data are available also for Italian Holstein cows:

- 25,400 CH_4 records from Moologger®.
- 66,864 CH_4 records from Greenfeed®.
- 108,624 feed intake records.
- 2,997 weight records.
- 2,853 rumination records.

For each animal, male and female, as Italian Holstein Green Passport is produced. This passport can be considered as a animal functionality and environmental impact report. At the moment in the report are reported only phenotypic data. Phenotypic data are compared to average phenotypic data of the population. In the near future indexes will be includes.

For each farm enrolled in the project an environmental sustainability evaluation using LCA approach is performed. At the moment in the LCA evaluation, average predicted methane emission index is used, but in the near future it will be replaced by direct data.

Conclusion

Data collection on key-farms is crucial to create a national inventory about sustainability traits (direct and proxies) and to set up a genetic evaluation. Of course, data collection in commercial farms is going to be enhanced. LCA in a key-tool to perform high-quality technical assistance using an holistic approach.

Data collection and preparation for genetic analysis of methane emissions in Danish dairy cattle

T.M. Villumsen[1], P. Løvendahl[1], V. Milkevych[1] R. Krogh[1], H. Schneider[1], M. Bjerring[2] and G. Sahana[1]

[1]Center for Quantitative Genetics and Genomics, Faculty of Science and Technology, Aarhus University, C. F Møllers Allé 3, DK-8000 Aarhus C, Denmark
[2]Department of Animal and Veterinary Sciences at Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark
Corresponding Author: tmv@qgg.au.dk

Abstract

In Denmark, a carbon emission tax in the agricultural sector will be implemented to meet the 2030 greenhouse gas reduction target (Skm.dk, 2024). Enteric methane (CH_4) from cow digestion is a significant contributor, leading to interest in genetic selection for low CH_4 emitting dairy cows. Developing a genetic model for methane emission in dairy cattle requires a comprehensive database of individual CH_4 measures from many cows. This requires affordable, farm-installable equipment with high measurement capacity. Sniffers based on the Guardian NG CH_4 and CO_2 sensors, measuring gas concentrations in robotic milking systems (AMS), are used for this purpose in Denmark.

Currently, (September 2024) we have collected methane records from 15.000 dairy cows in 40 herds based on records from 38 sniffers with a two-channel multiplex setup. Installation and maintenance require ongoing technical support and daily data monitoring. Due to the high volume of daily data, an automated pipeline is needed to monitor, clean, and ensure high-quality data for CH_4 phenotypes. Equipment errors are detected based on data streams from AMS and sniffer, and measurements are matched to individual cows, using a CO_2 concentration-based filter which is also used to correct for potential time drift.

To filter environmental noise, data is split into baseline readings, based on empty periods in AMS and cow data where a cow is milked in the AMS. Both baseline readings and cow data are pruned. Reliable gas concentrations during milking are adjusted for baseline levels, and a head lifting criteria is added to discard records where a cow most likely does not have the head in the feed bin. Phenotypes such as CH_4 concentration and CH_4/CO_2 ratio are calculated. Additional information, such as milk yield, can be used to compute other methane traits. These phenotypes facilitate the development of genetic models for reducing methane emissions in dairy cattle.

Keywords: Methane emission, dairy cows, genetic selection, sniffer, data pipeline.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 11: Methane Emission-Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

Introduction

In Denmark a carbon emission tax for the agricultural sector increasing toward € 100 per ton CO2e by 2035 has been agreed on by the Danish Government and key stakeholders to meet the Denmark's 2030 climate goals. Methane (CH_4) released from dairy cows' enteric digestion constitute a substantial portion of greenhouse gas emissions (Beach *et al*., 2015, Charmley *et al*., 2016). There are many methane mitigation options such as production intensification, dietary manipulation and selection of low CH_4 producing animals (Beauchemin *et al*. 2022). There is a major focus on feed additives aimed at reducing enteric methane (e.g. Honan *et al*, 2022, Majgaard *et al*., 2024). Also, genetic selection of cows with low CH_4 emission pr produced unit of milk and meat has been investigated (for review see Lassen and Difford, 2020).

To facilitate the development of a genetic model and conduct genetic evaluations for methane emission in dairy cattle it is necessary to have access to large-scale recording of methane emissions, to establish a comprehensive database with individual CH_4 and CO_2 measures. Sniffers, installed in automatized milking systems (AMS) at private farms have a high capacity to measure CH_4 and CO_2 concentrations in the exhaled air continuously during milking. The sniffer is a relatively cost-efficient system to measure gas emissions, that can easily be upscaled. Although sniffer data are valuable for the development of genetic models, they often present challenges in its raw form and require substantial processing and filtering. Currently, sniffers are not integrated with AMS software synchronization with cow-ID from milking system and potential synchronization of time is required. In addition, early detection of equipment errors and filtering for environmental noise is essential. The following text will shortly describe how we measure methane with the sniffer in a Danish setup, our current data, and cleaning of data used to form methane phenotypes

The Danish sniffer

The sniffer units consist of a CH_4 sensor (Guardian NG, Edinburgh Instruments, UK) and a CO_2 sensor (Gascard, Edinburgh Instruments, UK). Air and cows' breath is led into the sensors from the feed-bins in the AMS through a de-humidifier tube (Nafion, https://www.permapure.com/environmental-scientific/products/gas-sample-dryers/md-gas-dryers/) using the pump in the Guardian CH4 unit. Concentrations of CH_4 and CO_2 are recorded in volume percent units. As dust may block the inlet pipe a "sneezer" system is retrofitted, so as to clean the inlet filter (Festo, Pneumatic silencer, Festo, UC-QS-6H, 6mm)) by back-flushing part of the inlet pipe with compressed air from the AMS. The sneezer valve (Pneumatic control valve, SMC, SYJA712-01F) is triggered by the "exit gate" pressure so that the filter is cleaned for a few seconds when the cow exits the AMS. The instrument runs continuously giving one record of CH_4 and CO_2 concentrations per second. The sniffers are equipped with a two-channel multiplex setup that makes it possible to switch between measurements in two AMS with a pre-defined intervall.

Tabe 1. Number of cows with individual methane records in the Danish methane database.

Breed	Cows
Holstein	~8,000
Jersen	~3,000
Red	~2,000
Crossbreds	~1,000

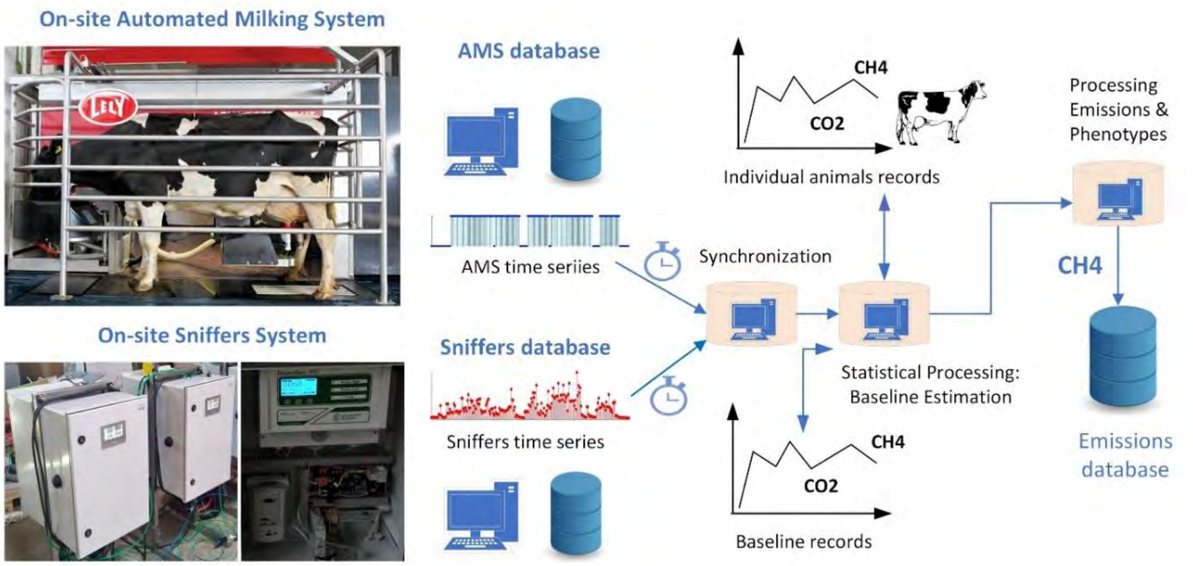

Figure 1. Fig. Diagram of experimental data acquisition system and processing pipeline. Top-left, AMS Milking unit where all measurements occur and milking data is acquired. Below, the sniffers where CH4 and CO2 is measured. Data processing follow the arrows, and the pipeline end-product is the phenotypes stored in the emissions database.

Animal data

By September 2024 the database with methane records consists of about 15.000 dairy cows from 40 herds with methane records. Models must be developed for all major dairy breeds in Denmark, therefore gas concentrations are measured in more breeds as summarized in table 1.

Data acquisition and processing pipeline for milking and emission phenotypes

The data system comprises two distinct pipelines for each milking unit, processing time series from both the AMS milking unit and the gas sniffer. The AMS pipeline collects cow identification data, milking details, and gate status to track the start and end of milking sessions. This data is downloaded weekly from the farmers' management system. The sniffer pipeline, operating on its own server, records CH_4 and CO_2 concentrations.

Data from the AMS milking unit and the sniffer are combined into a single processing pipeline, where key operations are executed. This integrated pipeline carries out critical tasks, including monitoring data flow, detecting and correcting equipment errors, cleaning data, estimating background gas concentrations, and analyzing cow data. These steps result in the calculation of condensed phenotypes, which are then stored as a single record for each milking event. This streamlined approach ensures that data from both the AMS system and gas sniffers is processed efficiently and consistently, as illustrated in Figure 1.

Monitoring of dataflow

Daily monitoring ensures smooth connection and reliable data transfer, with continuous tracking of cow visits in the AMS, maximum and mean gas values, as well as standard deviations for gas concentrations.

Detection of equipment error and cleaning of data

Several key issues arise with the sniffer techniques, including data association and synchronization, reliability detection, and significant embedded noise levels in measurements. To address these challenges, we employed the methods proposed by Milkewych *et al*. (2022), which utilize a matched filter approach based on milking times and CO2 gas concentrations to estimate potential time discrepancies and identify reliable data. These methods are grounded in the principles of linear filtering theory. The algorithmic implementation of this approach enables rapid and efficient automated data processing, resulting in an assessment of the proportion of reliable data. A high percentage of unreliable data may indicate equipment malfunction, necessitating a thorough check-up of the sniffer.

Analysis and estimation of background gas concentrations

The estimation of background gas concentrations is outlined in Løvendahl *et al*. (2024). Reliable data are categorized into baseline measurements taken when the AMS is unoccupied (idle), and emissions data recorded during cow usage. Idle periods of the AMS serve as the basis for calculating background gas concentrations. To mitigate potential carry-over effects from previous cows and address issues related to imperfect data synchronization, specific restrictions on the recording window are implemented to minimize edge effects. A baseline value is calculated for each restricted recording window, using data from 60 seconds after the start to 30 seconds before the end, with a minimum duration of 3 minutes. The diurnal effect of baseline is modelled using Fourier series as harmonics (Lassen and Løvendahl, 2016).)

Phenotypes

For cow visits, the recording window is limited to a range of 30 to 300 seconds. Concentration values that significantly exceed the baseline are used as indicators to ensure that the cows' heads are adequately positioned near the sniffer's air inlet.

The mean values of the selected gas records and their ratios form the basic response phenotypes. Additionally, other phenotypes can be generated when data such as milk yield (ECM) and dry matter intake (DMI) are available. These phenotypes include methane production (g/day), methane intensity (g/day/kg ECM), and methane yield (g/day/kg DMI), as described by Manzanilla-Pech *et al*. (2021).

Conclusions

Developing a genetic model for low-emission dairy cows requires a comprehensive database of individual CH_4 measures. Utilizing advanced sniffers integrated with automated milking systems (AMS), we have collected extensive methane emission data from 15,000 dairy cows across 40 herds.

By implementing automated pipelines for data processing, we ensure the maintenance of high-quality measurements through rigorous monitoring and error detection. The analysis of background gas concentrations, along with the establishment of key phenotypes for methane emissions, paves the way for effective genetic models and selection strategies aimed at reducing enteric methane emissions

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Validation of a previously developed enteric methane emission prediction model using individual cow milk mid-infrared spectra in Ireland

M. Frizzarin, B. Lahart and D.P. Berry

Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark,
Fermoy, Co. Cork, Ireland
Corresponding Author: maria.frizzarin@teagasc.ie

Abstract

The agricultural sector in Ireland contributes 38.4% of total greenhouse gas emissions and 71.2% of the agriculture greenhouse gases is generated from methane associated with livestock production. Mid-infrared (MIR) spectral data, which are routinely collected in a fast, cheap, and non-disruptive way, have been used to predict individual cow methane emissions in Canada, Belgium, France, Netherlands, and Ireland. The objective of this study was to validate, using data from the 2023 calendar year, predictions of enteric methane from milk MIR developed in Ireland based on data from the years 2020 to 2022.The Irish prediction model was developed using 93,888 individual spot measures of methane (i.e., individual samples of animal's breath when using a GreenFeed technology) from 277 cows. T

he enteric methane phenotype was based on the average of at least 20 individual spot measures taken over a 6-day period flanking each side of the milk sample with an associated milk spectral data. Predictions were based on a neural network algorithm populated with information on the MIR spectra, milk yield, and days in milk; the correlation between the actual and the predicted values in that 2020 to 2022 data varied from 0.68 to 0.75 in cross-validation, and from 0.55 to 0.71 in leave-one-experiment treatment-out validation. The validation dataset used in this study for the 2023 calendar year consisted of 45,196 individual cow spot methane measures from 157 cows which were collapsed into 1,715 methane records with associated milk MIR; none of the cows in the validation population were in the dataset used to develop the predictive model. The correlation between the real and the predicted values, the root mean square error (RMSE), and the ratio of performance to deviation were 0.38, 79.76 g/d, and 0.69, respectively.

The validation dataset was then stratified by estimated daily methane as the highest 10% emitting cows and the lowest 10% emitting cows. The mean (standard deviations) actual methane emitted by the cows predicted from the MIR to be the highest 10% emitter cows was 417.39 g/d (31.91 g/d), while that of those predicted to be the lowest 10% emitters was 220.56 g/d (26.69 g/d); the respective predicted mean methane of those two groups of animals was 402.59 g/d (27.73 g/d) and 358.26 g/d (27.00 g/d), respectively. Results from the present study indicated a relatively poor prediction accuracy in estimating individual cow methane emissions in a subsequent year. Nonetheless, differences in the actual mean methane between groups of cows predicted to be divergent in methane materialised. Hence, while individual animal predictions was poor, actual differences in enteric methane emissions differed between groups of animals stratified on predicted methane emissions.

Keywords: methane, mid-infrared spectroscopy, pasture based, validation.
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Introduction

Irish agriculture is responsible for 38.4% of national greenhouse gas emissions (GHG) and methane from enteric fermentation accounts for the 71.2% of these emissions (EPA, 2023). Nonetheless, the total amount of GHG emissions needs to be reduced by 25% by the year 2030. Strategies which will achieve this deliverable without impacting global are required. Environmental concerns from consumers necessitate future food production systems to demonstrate they are capable of accurately quantifying and mitigating their environmental footprint. Mitigating methane emissions from dairy cows presents a complex challenge due to the biological processes involved in rumen digestion. Different strategies have been proposed to reduce cow methane emissions such as improving feed management (e.g. by adjusting the composition and timing of feed; Hristov *et al*., 2013), inclusion of feed additives into the diet (Patra, 2012), genetic selection (Pinares-Patiño *et al*., 2013), and rumen manipulation (e.g. inoculating the rumen with specific microbes or introducing methanogen inhibitors; Eugène *et al*., 2015). Nonetheless, methods for estimating methane emissions are needed, ideally at an individual cow level, as they would allow for an assessment of methane output while also permitting to investigate methane-reducing strategies.

Mid-infrared (MIR) spectroscopy is a technology routinely applied to all bulk tank and individual animal milk samples to quantify the concentration of many milk components (e.g., fat, protein and lactose) in the milk sample. Moreover, it was successfully used to predict, with reasonable accuracy, the enteric methane emissions of individual animals (Vanlierde *et al*., 2015; Shadpour *et al*., 2022; Dehareng *et al*., 2012). Nonetheless, these previous studies were generally small in size (from 11 cows – Dehareng *et al*., 2012; to 202 cows - Shadpour *et al*., 2022), often limited to cows (likely) fed indoors (Shadpour *et al*., 2022; Wang and Bovenhuis, 2019; Dehareng *et al*., 2012; van Gastelen *et al*., 2018; Coppa *et al*., 2022) or measured over a relatively short period of time (Vanlierde at al., 2018; van Gastelen *et al*., 2018). In Ireland, a methane emissions prediction equation was developed in 2023 (McParland *et al*., 2024) using data collected between the years 2020 to 2022 from 277 grazing dairy cows. The aim of this study was to validate this Irish methane prediction equation using data collected during the 2023 calendar year.

Materials and methods

Calibration dataset

The calibration dataset included data from 93,888 individual spot measures of methane (i.e., individual samples of animal's breath when using a GreenFeed technology) from 277 dairy cows collected between the years 2020 and 2022. Different methane phenotypes were investigated and the one which produced the most accurate prediction results was based on the average of at least 20 individual spot measures taken over a 6 day period surrounding each side of the milk sample (McParland *et al*., 2024). The quantified phenotype was then merged with the same cow's daily average milk spectrum quantified as the milk yield weighted average of the milk spectrum originating from an evening milking and the milk spectrum originating from the following morning milking. A total of 531 wavelengths were used for the analyses (i.e., after discarding the water regions). The mean and standard deviation of the calibration dataset was 324.0 g/d and 94.0 g/d, respectively. The prediction equation was developed using a neural networks algorithm that was populated with data on the MIR spectra, milk yield, and days in milk. The R package brnn (Perez Rodrigez and Gianola, 2020) was used to develop the prediction equation, and the default tuning parameters were chosen, which included two hidden layers and a Bayesian regularization to the input layer to improve generalizability. Prediction results in the calibration dataset resulted in a correlation between the actual and the predicted values of 0.68 to 0.75 in cross-validation and from 0.55 to 0.71 in leave-one-experiment treatment-out validation.

Validation dataset

A further 45,196 individual spot methane measures from 157 cows were collected during the 2023 calendar year. These records were collapsed into 1,715 daily methane records with associated daily weighted average milk MIR; none of the cows in the validation population were in the calibration population. The mean and the standard deviation in the validation dataset was 313.20 g/d and 55.52 g/d, respectively.

Results

The correlation between the real and the predicted values, the root mean square error (RMSE), and the ratio of performance to deviation when validated in the 2023 data were 0.38, 79.76 g/d, and 0.69, respectively. The actual versus the predicted methane emissions values are in Figure 1.

The actual and predicted lactation profile for methane is in Figure 2. Actual daily emitted methane increased as the lactation progressed until week22 after which it declined; in contract, predicted daily methane reduced as the lactation progressed.

The correlation between the actual and the predicted methane emissions was then investigated within stage of lactation, where each stage was approximately 60 days in duration. The correlation between the actual and the predicted methane emissions was always 0.50 for the records collected between 5 to 59 DIM, between 60 and 119 DIM, and between 120 and 179 DIM, but weakened to 0.46 between 180 and 239 DIM and weakened further to 0.27 post 240 DIM.

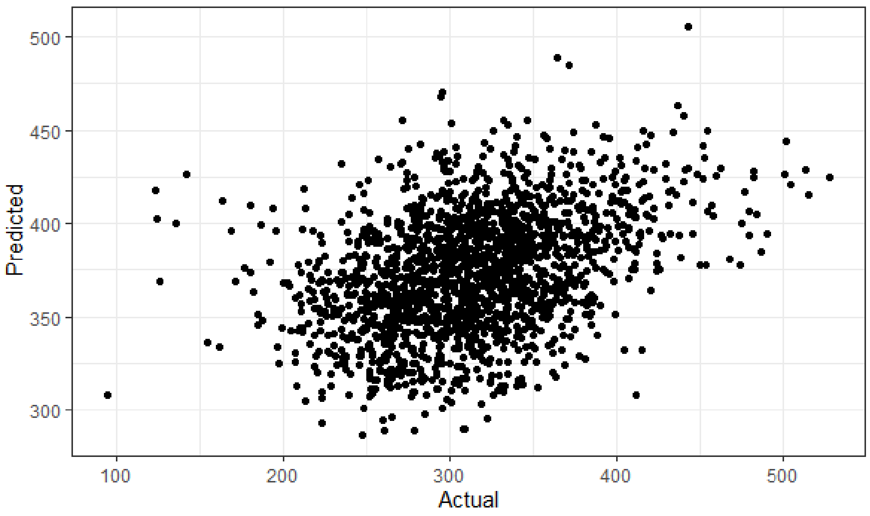

Figure 1. Actual (x-axis) versus predicted (y-axis) methane emissions (g/d).

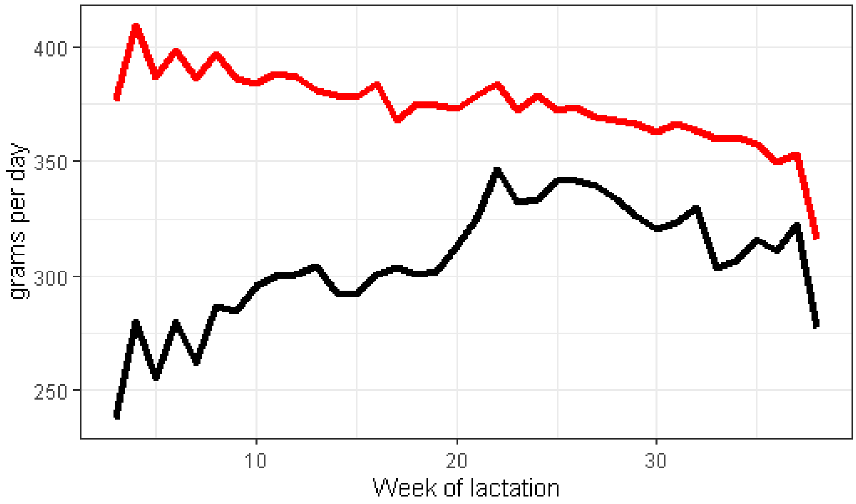

Figure 2. Actual (black) and predicted (red) methane emissions lactation profile.

The validation dataset was then stratified by predicted daily methane as the highest 10% emitting cows and the lowest 10% emitting cows. The mean actual methane emitted by the cows estimated to be the highest 10% emitter cows was 417.39 g/d, while that of those estimated to be the lowest 10% emitters was 220.56 g/d; the respective predicted mean methane of those two groups of animals was 402.59 g/d and 358.26 g/d, respectively.

Discussion

Quantification of methane emissions is essential to study the effect of different diets or the inclusion of feed additives on cow methane emissions, as well as to include methane emissions as a trait in national genetic evaluations. The reference method used for the quantification of methane emission are the respiratory chambers, which may not be a good reflection of the actual enteric methane emissions in grazing cows, since the cows are removed from their natural environment. Indeed, activities like walking, the grazing process itself (i.e., diet selection when grazing pasture), and the influences of weather conditions on grass quantity and quality are not detected with the respiratory chambers.

Therefore, alternative approaches to quantify methane emissions in the grazing system need to be explored. The utilization of milk MIR spectral data coupled with previously developed prediction equations generated reasonably accurate predictions. Nonetheless, the developed equations have to be properly validated before being used. Indeed, Wang and Bovenhius (2019) reported a coefficient of determination of 0.49 when methane emissions were quantified from MIR in dairy cows using random cross-validation, but a coefficient of determination of 0.01 when methane emissions were quantified using block cross-validation, with farm as blocks. As prediction equations are generally generated using data collected in a relatively small number of farms (often research farms), the developed equations need to be able to accurately quantify methane emissions for farms with no records in the calibration dataset. The validation dataset used in the present study included records collected from cows not included in the calibration dataset, collected in the successive year to the records in the calibration dataset, and majority of the data in the validation dataset were collected in a farm which was not included in the calibration dataset. While the accuracy metrics used to assess the model predictive ability were acceptable, the mean of the predicted methane emissions of the high 10% emitting cows was different ($P<0.05$) to the mean of the predicted methane of the low 10% emitting cows.

Conclusions

The results from the present study demonstrated that even if the actual methane emissions value for the different cows was not accurately quantified, groups of cows (i.e., high and low emitting cows) can be correctly identified. Therefore, the methane emission phenotypes quantified using the milk MIR spectra and the already developed equation could potentially be used for selection of lower emitting cows.

Acknowledgments

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Predicting methane emissions of Australian dairy cows using mid-infrared spectroscopy from milk samples

P.N. Ho[1], F. Almasi[1], L.C. Marret[2,3], S.R.O. Williams[2], J.L. Jacobs[2,3] and J.E. Pryce[1,4]

[1]Agriculture Victoria, AgriBio, Centre for AgriBioscience,
Bundoora, Victoria 3083, Australia
[2]Agriculture Victoria, Agriculture Victoria Research, Ellinbank, VIC 3821, Australia
[3]Centre for Agricultural Innovation, The University of Melbourne, VIC 3010, Australia
[4]School of Applied Systems Biology, La Trobe University,
Bundoora, Victoria 3083, Australia
Corresponding Author: phuong.ho@agriculture.vic.gov.au

Abstract

Genetic selection for reduced methane emissions from dairy cows has received increasing attention in the last decade, but this requires a large reference population. As such, a significant amount of research has been carried out to explore easy and cost-effective predictors of measure methane emissions from dairy cows that could become available on a large scale, including the performance of mid-infrared (MIR) spectra of milk. In this study, we investigated the relationship between MIR and methane emissions in Australian lactating dairy cows. Data on methane production (g/d), methane yield (g/kg of dry matter intake), methane intensity (g/kg of milk), and MIR spectra from 240 Holstein lactating cows that were part of two 32-day experiments conducted between October 2016 and December 2017 were used. Methane emissions were measured during a period of 5 consecutive days using the SF6 tracer technique, with corresponding morning milk samples were taken for milk composition analysis where MIR spectra were retained. Prediction models were developed using partial least-square regression and performance was evaluated through a leave-one (animal)-out cross-validation.

The prediction accuracy was measured by the coefficient of determination. Two modelling strategies were examined, that were predictions of methane emissions using MIR spectra collected on the same day and those collected on the following day. The effect of lactation stage was also explored. In the first scenario, the prediction accuracy of methane production, methane yield and methane intensity were 0.25, 0.20, and 0.24, respectively while these were 0.33, 0.53 and 0.38 when using MIR spectra collected a day after the methane measurements occurred. Further, incorporating the effect of lactation stage into the model greatly improved the prediction accuracy to 0.29, 0.24 and 0.33 versus 0.39, 0.55 and 0.42 when using MIR spectra collected on the same day versus the following day, respectively. In conclusion, our preliminary results indicate the potential of MIR spectra to predict methane emissions of Australian dairy cows. Additional data, especially that are measured on different feeding systems or breeds of cows, is essential to improve the prediction accuracy and the robustness of the models.

Keywords: methane emissions, dairy cows, mid-infrared spectroscopy.
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Introduction

In Australia, the latest report from the Australian Government Department of Climate Change, Energy, the Environment and Water shows that in 2022 agriculture produced 78.7 million tonnes of CO_2-e, which accounted for 17.4% of the total greenhouse gas emissions (NGER, 2022). The dairy industry contributes around 58% of the above figure, which is mainly due to the production of enteric methane (a by-product of the natural digestion in the rumen (Dairy Australia, 2023). As the result, various strategies to mitigate methane emissions (CH_4) have been evaluated, including dietary intervention, microbiome manipulation, feed additives and genetics (Króliczewska *et al*., 2023). Selective breeding offers additive and permanent benefits that can be used together with other strategies. However, genomic selection requires a large number of records to achieve acceptable prediction accuracy (van den Berg *et al*., 2019). Many current methane measurement methods such as respiration chamber, sulphur hexafluoride tracer (SF_6) and GreenFeed™ are accurate but they are still expensive and hard to implement on a large scale (Deighton *et al*., 2014, Patra, 2016). Lasers and sniffers have potential to increase the number of records provided they are highly correlated to gold-standard methods, such as calorimeters (Difford *et al*., 2018, Garnsworthy *et al*., 2019).

Mid-infrared (MIR) spectroscopy of milk samples has widely been reported to be able to predict methane emissions of individual cows, with accuracy (R^2) ranging between 0.04 to 0.79 depending on data and validation methods (i.e. random cross-validation or external validation) (Vanlierde *et al*., 2015, Shetty *et al*., 2017, Shadpour *et al*., 2022a). Given MIR has routinely been used by milk-recording organization worldwide to quantify fat, protein and lactose concentrations, genetic evaluations of methane emissions could be done for a large dairy population without any additional costs. This is especially powerful for genotyped populations, as it can be used to generate genomic predictions that can be validated with methane measurements on a subset of genotyped cows.

In April 2023, Lactanet in collaboration with Semex (Canada) published the first official Methane Efficiency genetic evaluations derived using phenotypes predicted from MIR spectra (Oliveira *et al*., 2024). Despite the extensive number of studies on MIR prediction of methane emissions, the transferability of such equations between countries is limited (Vanlierde *et al*., 2021). This is especially true when there are large differences in genetics, feeding and management which strongly affect MIR spectra and methane emissions.

The objective of this study was therefore to evaluate the performance of MIR data to predict methane emissions of Australian lactating dairy cows. Specifically, we examined the predictability of the MIR spectra collected either on the same day or the following day as well as the benefit of incorporating lactation stage to the model performance.

Materials and methods

Animal data

The data used in this study were collected from the research farm of the Agriculture Victoria Research (AVR) in Ellinbank, Victoria, Australia between October 2016 and December 2017. In total, there were 240 Australian Holstein lactating cows. Within each year, 120 cows were chosen and divided into three batches of similar stage of lactation, parity, and body weight. The experiment was run for a period of 32 days. Cows had *ad libitum* access to feed and water. The diet contained cubes that were ~74% alfalfa hay, 25% crushed barley grain, 1% minerals (calcium, phosphorus, and magnesium) on a dry-matter (DMI) basis and was provided by Multicube Ltd (Yarrawonga, Victoria, Australia). Cow dry matter intake (kg of DM/day) was recorded using feed bins mounted on load cells that were electronically monitored by linking the bin-weight data to the electronic identification of individual cows (Gallagher Animal Management Systems, Hamilton, New Zealand). Milk yield (L/day) was measured for each cow twice daily (morning and evening) using a DeLaval Alpro milking metering system (DeLaval International). Five days per week, morning milk samples were sent to TasHerd Pty Ltd (Hadspen, Tasmania, Australia) for analysis of fat, protein, and lactose concentrations by a mid-infrared spectrometer (Model 2000, Bentley Instruments, Chaska, MN, USA). The MIR spectra generated from the milk composition analysis were retained for this study. Methane production (MeP) were measured over a 5-d period using the SF6 tracer technique developed by Deighton *et al*. (2014). Using data of methane production, dry matter intake, and milk yield, methane yield (MeY) and methane intensity (MeI) were derived as gram of CH4/kg of DMI and gram of CH4/L of milk, respectively.

Data processing

Spectral records (899 wavenumbers) were first matched with methane phenotypes that were collected either on the same day (Day_0) or one day before (Day_1) which resulted in 937 and 530 records, respectively, available for future analyses. Several mathematical treatments were then applied to the spectra. Specifically, the spectral regions characterized by low signal: noise ratio ((2,998 - 3,998 cm^{-1}, 1,615 - 1,652 cm^{-1}, and 649 - 925 cm^{-1}), which is the consequence of high water absorption, were removed (Hewavitharana and van Brakel, 1997). There were 536 wavenumbers remained after this step. Then, a standardized Mahalanobis distance or also known as global H distance (Shenk and Westerhaus, 1995) between each spectrum and the population average was calculated. Spectra with a global distance larger than three were considered outliers and excluded as suggested by Williams (2004). Finally, first-order Saviztky–Golay derivative was applied to the reduced spectra (Savitzky and Golay, 1964). The final dataset included 930 and 525 for D_0 and D_1 scenarios, respectively. Further, the effect of lactation stage on prediction accuracy was accommodated using the method described by Vanlierde *et al*. (2015).

Model calibration and evaluation of performance

The prediction models were developed using partial least-square regressions (PLS) and implemented in R with the PLS package (Mevik and Wehrens, 2007). Because the current dataset contained repeated measurements, we chosen leave-one animal out approach to validate the model performance. In this validation, the data of a given animal were removed from the main dataset to be used as a validation against the model trained with the remaining records. The optimal number of latent variables for PLS model was determined based on first local minimum value in root mean-squared error of prediction. The prediction accuracy was measured using the coefficient of determination (R^2_{cv}) and root mean square error of prediction (RMSEP_{cv}).

Results and discussion

The means and standard deviations of MeP, MeY, and MeI were 515.4 ± 82.9, 22.6 ± 7.4, 21.5 ± 4.4 respectively. A high coefficient of variation (16 – 32%) were observed for methane emissions which is essential to have a robust prediction equation (Soyeurt *et al*., 2011). In general, Table 1 shows that the prediction accuracy obtained from this study (0.20 – 0.55) was within the range that were previously reported in Denmark (Shetty *et al*., 2017), Canada (Shadpour *et al*., 2022b), and (McParland *et al*., 2024), but slightly lower than that in Belgium (Vanlierde *et al*., 2015). When the lactation stage effect was incorporated, the accuracy was improved by 4 – 9%. Vanlierde *et al*. (2015) reported a similar prediction accuracy between the two models (0.75 – 0.77) on calibration, but when applying on an independent dataset, the model with lactation stage effect produced prediction accuracy of 0.48 compared to 0.09 in the simple model. A hypothesis behind such improved performance could be that the relationship between milk spectra and CH_4 changes during lactation which is resulted from the mobilization of body tissues, and this should be considered in the model. The same pattern was observed for the models developed using milk spectra collected a day after methane measurements (Table 2).

Interestingly, the models that used spectra from milk samples collected 1 day after methane measurement (D_1), resulted in a higher prediction accuracy compared to those collected on the same day (D_0): 0.33 vs. 0.39, 0.53 vs. 0.55 and 0.38 vs. 0.42, respectively (Table 2). Dehareng *et al*. (2012) also concluded that milk spectra collected at day 1.5 produced better results that that collected on the same day. Apparently, this might be explained by the delayed response of milk composition, that is captured by MIR spectra, to ruminal fermentation.

Conclusions

The results from this study indicate that it is feasible to predict methane emissions using the spectra of milk samples, with moderate accuracy. Further, the spectra collected one day after methane measurement produced better performance than that collected on the same day. It is also shown that incorporation of lactation stage effect improved prediction accuracy. Although the results are promising, the current dataset was rather small, and therefore more records need to be obtained especially on different breeds and feeding systems.

Table 1. Cross-validation accuracy of methane emissions predicted using MIR spectra collected on the same day, and with and without a correction for lactation stage.

	R^2_{cv}	**$RMSEP_{cv}$**
Independent of lactation stage		
Methane production (g/d)	0.25	72.0
Methane yield (g/kg of DMI)	0.20	4.5
Methane intensity (g/kg of MY)	0.24	6.4
Dependent on lactation stage		
Methane production (g/d)	0.29	70.2
Methane yield (g/kg of DMI)	0.24	6.4
Methane intensity (g/kg of MY)	0.33	3.6

Table 2. Cross-validation accuracy of methane emissions predicted using MIR spectra collected one day after, and with and without a correction for lactation stage.

	R^2_{cv}	$RMSEP_{cv}$
Independent of lactation stage		
Methane production (g/d)	0.33	68.5
Methane yield (g/kg of DMI)	0.53	5.1
Methane intensity (g/kg of MY)	0.38	3.5
Dependent of lactation stage		
Methane production (g/d)	0.39	65.1
Methane yield (g/kg of DMI)	0.55	4.9
Methane intensity (g/kg of MY)	0.42	3.3

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Reducing dairy cattle enteric methane emissions using rumen metagenome

B.J. Sepulveda[12], O. González-Recio[3], A.J. Chamberlain[1,2], B.G. Cocks[2], J. Wang[1], C.P. Prowse-Wilkins[1,4,5], M. Khansefid[1], R. Xiang[1,4], L.C. Marett[4,6], S.R.O. Williams[6], J.L. Jacobs[4,6], A. García-Rodríguez[7], J.A. Jiménez-Montero[8] and J.E. Pryce[1,2]

[1]Agriculture Victoria Research, AgriBio, Centre for AgriBioscience,
5 Ring Rd, Bundoora, VIC, 3083, Australia
[2]School of Applied Systems Biology, La Trobe University, Bundoora, VIC, 3083, Australia
[3]Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y
Tecnología Agraria y Alimentaria (CSIC), Madrid, 28040, Spain
[4]Faculty of Veterinary and Agricultural Science, The University of Melbourne,
Parkville, VIC, 3052, Australia
[5]Department of Animal Science, University of California, Davis, 95616, USA
[6]Agriculture Victoria Research, Ellinbank, VIC, 3821, Australia
[7]Department of Animal Production, NEIKER-BRTA, Granja Modelo de Arkaute,
Apdo. 46, 01080 Vitoria-Gasteiz, Spain
[8]Spanish Holstein Association (CONAFE). Ctra. Andalucía km 23600 Valdemoro,
28340 Madrid, Spain
Corresponding Author: boris.sepulveda@agriculture.vic.gov.au

Abstract

Ruminant digestion emits methane, a potent greenhouse gas contributing to global warming and reducing feed efficiency. Reducing enteric methane emissions (EME) via farming and breeding decisions is crucial, yet measuring these emissions on commercial farms is currently challenging and costly. It is common for EME to be measured using distinct technologies. However, different EME traits sometimes show weak correlations between countries, feeding systems or technologies, complicating the combination of reference populations. Here we show a methodology to predict and reduce EME with the use of the rumen microbiome. We identified a common *core* of 1,032 KEGG ontology identifiers (KO) from the rumen metagenome of 410 dairy cows located in Australia and 434 in Spain. This *core* explained 83% and 57% of EME (measured using SF6 in Australia and sniffers in Spain) with an accuracy of 0.38 and 0.19 respectively. This result suggest that the ruminal metagenome can be used to predict EME and make farming decisions to reduce these meissions. We also estimated reductions in EME of up to ~16% of the population mean per generation by selection on this *core*, being superior to direct selection on EME (~9 to 14%). A combination of direct selection on EME and indirect selection on the *core* would produce larger reductions (up to 19%). These results suggest that rumen metagenome features could be candidate for improvement with genomic selection in combination with EME traits. Combining reference populations through the ruminal metagenome can be used to predict EME irrespective of each population's EME trait. We propose a global effort to validate a common *core* of ruminal features associated with EME. If validated, our results could impact global ruminant emission reduction efforts.

Keywords: rumen microorganisms, metagenomics, methane production, phenotypic variation, genomic selection.
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Introduction

Ruminants have evolved in symbiosis with their rumen microbiota for over 50 million years, and for this reason can transform plant materials that humans cannot digest into vital nutrients and energy. This capability depends on a diverse microbial community that, unfortunately, produces methane—a potent greenhouse gas (GHG) that contributes to approximately 40% of global methane emissions (Moss *et al*., 2000) and makes up 40% of total GHG emissions from livestock (FAO, 2023). In addition, enteric methane emissions (EME) represent 2 to 12% of the energy loss in the ruminants' diet (González-Recio *et al*., 2023; Lassen and Difford, 2020).

Reducing enteric methane emissions (EME) through farm-management and breeding decisions is ideal. However, EME need to be measured for this purpose and recording these emissions in commercial farms is currently logistically challenging and expensive. Additionally, different EME traits are sometimes weakly correlated, complicating the combination of reference populations (Hristov *et al*., 2016). However, all EME traits have the same underlying biology – methane is mainly produced by the rumen microbiota (González-Recio *et al*., 2023).

The role of the host genetics and the rumen microbiome on EME remains unclear. The host genetics partially determines both EME (López-Paredes *et al*., 2020; Richardson *et al*., 2021) and ruminal microbial features associated with EME (Martínez-Álvaro *et al*., 2022; Saborío-Montero *et al*., 2021). For this reason, and according to the definitions of Pérez-Enciso *et al*. (2021), there could be two potential biological scenarios. Firstly, there is an indirect relationship where the host genome affects EME, but this is mediated by the microbiota. In the second scenario, a recursive model, both the host genetics and the microbiota exert influence on EME, and the host genetics also indirectly affects EME by modulating the microbiota (Saborío□Montero *et al*., 2020).

In the last decade one of the most widely employed approaches to study the effect of the rumen microbiome on EME is estimating the variance in EME explained by a microbial relationship matrix (MRM) (Ross and Hayes, 2022). Additionally, recent studies have estimated reductions in EME by implementing breeding programs selecting on ruminal microbial features, which are heritable and genetically correlated with EME (Martínez-Álvaro *et al*., 2022).

This study aimed to generate a methodology to: First, quantify the effects of an MRM constructed using a novel methodology on EME in two distinct dairy cattle populations of more than 400 animal each, one in Australia and one in Spain. Second, to estimate the response to selection on EME by indirectly selecting on ruminal metagenomic features. Third, to investigate whether these two dairy cattle populations with distinct EME traits could be connected through the rumen metagenome.

Material and methods

Ethical statement

The Australian experiments in this study were approved and undertaken in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Approval to proceed was granted by the Agricultural Research and Extension Animal Ethics Committee of the Department of Energy, Environment and Climate Action (application number 2013-14 was approved on August 22nd, 2013, and application number 2016-12 was approved on August 22nd, 2016). The Spanish experiments in this study were conducted in accordance with Spanish Royal Decree 53/2013 for the protection of animals used for experimental and other scientific purposes and were approved by the Basque Institute for Agricultural Research and Development Ethics Committee (Neiker-OEBA-2017-004) on March 28, 2017.

Data

The Australian population included 410 Holstein lactating cows located at the Agriculture Victoria's Ellinbank SmartFarm (Ellinbank, Victoria, Australia). These cows were measured for dry matter intake (DMI) and grams per day methane production (MeP) in 11 cohorts between 2013 and 2017. MeP was considered the EME trait in Australia. At the beginning of the study, cows averaged 110 ± 19.4 (mean ± standard deviation) days in milk, 2.5 ± 1.25 lactations, and weighed 539 ± 69.8 kg. Over a 32-day period in an experimental facility, they had continuous access to feed, water, and a bare paddock (loafing area) for rest. The cows were outside except for twice-daily milking. Cows were fed with the diet described by Moate *et al*. (2021) and DMI was measured using feed bins equipped with load cells and electronic monitoring linked to individual cow identification (Gallagher Animal Management Systems, Hamilton, New Zealand Daily DMI was recorded over the 32 days. Daily MeP was obtained with the sulphur hexafluoride (SF_6) tracer method described by Deighton *et al*. (2014). Further details of the environment of the Australian dairy cattle population are provided by Moate *et al*. (2021).

The Spanish population included 432 Holstein cows, either in their first or second lactation, from 14 commercial farms across four Northern Spanish regions (Cantabria, País Vasco, Navarra, and Gerona). Following the methodology of Rey *et al*. (2019), EME were measured using a non-dispersive infrared methane detector (The Guardian® NG) from Edinburgh Sensors (Livingston, Scotland, UK), also termed "sniffer", installed in the feed bin of an automatic milking system. Individual methane concentration (MeC) in Spain as parts per million (ppm) was recorded for each cow during milking over a period of two to three weeks. The recorded eructation peaks were averaged to obtain a single record per cow. The Spanish population was under commercial milk recording schemes consistent with ICAR accredited recording protocols.

Cows located in Australia were genotyped with SNP arrays including custom genotyping-by-sequencing (GBS) and selected SNP (XT) panels (approximately 8,800 SNP of which at least 6,900 overlapped with the BovineSNP50 BeadChip, Illumina, San Diego, California, USA) and imputed to the Bovine 50K SNP chip panel using FImpute (Sargolzaei *et al*., 2014) as described by Haile-Mariam *et al*. (2020). Cows located in Spain were genotyped with the EURO12K SNP chip (Illumina, San Diego, California, USA) and imputed to 54,609 SNPs using BEAGLE (Browning *et al*., 2018) as described in Jiménez-Montero *et al*. (2013) and the Spanish reference population provided by the Spanish Holstein Association (CONAFE, Madrid, Spain) containing more than 200,000 genotypes. A panel with 39,058 (40K) SNP shared by both populations and with a minor allele frequency greater than 0.05 was selected for analyses.

Ruminal metagenome processing

Ruminal fluid samples from all animals were collected via an oesophageal probe placed into the rumen via the mouth. In Australia a probe similar to the one described by Geishauser (2019), and a vacuum pump were used to collect samples (Moate *et al*., 2014). In Spain, samples were obtained as described by Saborío-Montero *et al*. (2021). Following collection, ruminal fluid samples were frozen using liquid nitrogen vapours. Microbial genomic DNA was extracted from the ruminal fluid using a ZymoBIOMICS DNA miniprep kit (Zymo Research, Irvine, California, USA) in Australia, and with DNeasy Power Soil Kit (QIAGEN, Valencia, California, USA) in Spain. After DNA concentration and purity assurance, long-read sequencing with Oxford Nanopore Technologies (ONT) and R9.4.1 flow cells was used for metagenome sequencing (Oxford Nanopore Technologies, Oxford, United Kingdom).

Microbiome sequence data analysis

Basecalling was conducted using Guppy (Oxford Nanopore Technologies, Oxford, United Kingdom) with high accuracy mode (HAC) with the versions 5.0.16 and 4.2.2 in Australia and Spain, respectively. Reads with a quality score greater than 7 and sequence length greater than 150 base pairs were retained for analysis. The long reads were aligned to the KEGG database (Kanehisa and Goto, 2000) for KEGG ontology identifiers (KO) identification using the script SQM_longreads.pl of SqueezeMeta pipeline (version 1.4) (Tamames and Puente-Sánchez, 2019).

Relative abundance matrix of KOs

KOs not present in all animals or that included genes of *Bos taurus* (cow) were removed, retaining 1,032 KOs for downstream analysis. These KOs were used to construct two absolute abundance matrices, one per population, with dimensions animals ×KOs and populated with the number of reads assigned to each KO in each animal. Subsequently, a relative abundance (RA) matrix was created from each population as the proportion of each variable's absolute abundance compared to the total abundances in the same animal. These RA matrices were CLR-transformed to account for their compositional nature (Gloor *et al*., 2017) using the unweighted option of the *CLR* function of the easyCODA R package (Greenacre, 2018).

Relationship matrices

A genomic relationship matrix (GRM) was created with genotypes of the SNP markers shared by both populations, utilising the *Gmatrix* function from the R package *AGHmatrix* (Amadeu *et al*., 2016) following the approach of Yang *et al*. (2010). Additionally, a MRM was constructed as:

$$\mathbf{M} = (1/p)\mathbf{XX}^{T} \qquad \mathbf{(1)}$$

Where M is the MRM, p is the number of KOs and X is the CLR-transformed matrix. A small constant value (1 x 10^{-8}) was added to the elements on the main diagonal of the MRM matrices to prevent singularity issues. Finally, the GRM and MRM were inverted with the function solve of R (R Core Team, 2022). We avoided the step of scaling and centring the KOs across animals as is widely used (Hess *et al*., 2023; Ross and Hayes, 2022; Ross *et al*., 2012) as this step decreases large effects of KOs on EME (López-García *et al*., 2022; Martínez-Álvaro *et al*., 2022; Roehe *et al*., 2016).

Variance in enteric methane emissions explained by the rumen microbiome

The variance in EME explained by the rumen microbiome was estimated with a microbiome BLUP (MBLUP) (Saborío-Montero *et al*., 2021) as:

$$y = 1'\mu + X\beta + Uh + Wm + e \qquad (2)$$

Where m is the EME population mean; 1 is a vector of ones with the same length of γ ; β is a vector of fixed effects; u is a vector of random additive genetic effects; and m is a vector of random microbiota effects. X and W are incidence matrices. The distribution of m is assumed N(0, MRMσm2); and e is a vector of random residuals distributed N(0,se2). EME of each country was included as the dependent variable (y) in this model. The fixed effects in Australia were cohort (11 levels), DMI, days in milk, energy corrected milk obtained with the methodology of Visscher *et al*. (1994), and daily body weight change during the experiment. In Spain, the fixed effects were lactation number (2 levels) and stage of lactation (3 levels). In Spain, the robots used to measure emissions nested within farms (24 levels) was used as a random effect and is represented by the effect h and the incidence matrix U. The models were conducted with the function asreml of the R package ASReml-R (version 3) (Butler *et al*., 2009). The proportion of EME variance explained by the rumen microbiome, microbiability (m2), was estimated as follow, where sp2 is the phenotypic variance on EME:

$$m^2=(\sigma_m^2)/(\sigma_p^2) \qquad (3)$$

The accuracy of prediction was estimated with a 10 repetition, 5-fold cross-validation, where the phenotypes of the validation group were removed and the prediction was developed with the phenotypes from the remaining four groups and the rumen microbiome KOs of all animals. The prediction accuracy was calculated as the correlation between the random coefficient regressors from the MRM of the validation group and their EME. Then, the mean and standard deviation of the accuracies across the groups were calculated. This process was repeated 10 times, and the mean and standard deviations were averaged between repetitions to obtain the final accuracy mean and standard deviation.

Phenotypic and genetic parameters

Univariate genomic BLUP (GBLUP) were conducted to EME and each KO as the response variable in:

$$y = 1'\mu + X\beta + Uh + Zg + e \qquad (4)$$

Where, g is a vector of random additive genetic effects with an assumed distribution N(0, GRM_g^2) and Z is an incidence matrix. The rest of Equation 4 are the same previously described for Equation 2.

The heritability (h^2) of the KOs was estimated as:

$$h^2=(\sigma_g^2)/(\sigma_p^2) \qquad (5)$$

The phenotypic correlation between KOs and EME was calculated as the Pearson correlation between them and the genetic correlations as the correlations between the genomic estimated breeding values obtained with Equation (4).

Selection response of enteric methane emissions

We calculated three different scenarios to select against EME: (1) by directly selecting against EME only, (2) by indirectly selecting on the ruminal KOs only, and (3) by using a combination of scenarios (1) and (2), selecting on both EME and the KOs. The KOs used as indicative traits of MeP in Australia were 87 KOs in the *core* that had a heritability ≥ 0.20 in Australia and a genetic correlation with EME in Australia ≥ 0.20 (*core breeding Australia*). Similarly, the KOs used as indicative traits of MeC in Spain were 159 KOs in the *core* that had a heritability ≥ 0.20 in Spain and a genetic correlation with EME in Spain ≥ 0.20 (*core breeding Spain*). Additionally, we also used 15 KOs reported by Martínez-Álvaro *et al*. (2022) as associated with EME in beef cattle that were present in our *core*. We calculated the response to selection in all scenarios with a selection index approach (Cameron, 1997) incorporating the estimated heritability, and genetic and phenotypic correlations previously described using an in-house R script (R Core Team, 2022). Further, we calculated the response to selection when 30% to 1% of the population with lowest methane emissions were selected.

Results

The MRM explained 83 ± 7% of the variance in EME in Australia and 57 ± 20 % in Spain, with prediction accuracies of 0.37 ± 0.08 and 0.19 ± 0.11, respectively. The heritability of EME was 0.28 ± 0.12 and 0.11 ± 0.10 in Australia and Spain, respectively. The maximum KOs' heritability was 0.56 in Australia and 0.47 in Spain, the genetic correlations between EME and KOs were up to |0.54| in Australia and |0.43| in Spain (Figure 1), and phenotypic correlations up to |0.49| and |0.22| in Australia and Spain. These results agree with that reported by (Martínez-Álvaro *et al*., 2022). The *core breeding Australia* had a heritability of 0.27 ± 0.06 and a genetic correlation of 0.30 ± 0.07. The *core breeding Spain* had a heritability of 0.28 ± 0.07 and a genetic correlation of 0.24 ± 0.03.

Larger reductions were estimated with indirect selection on the KO cores compared with direct selection on EME, agreeing with a previous study (Martínez-Álvaro *et al*., 2022). The mean MeP in Australia was 462 g/d, and the mean MeC in Spain was 1,310 ppm. We estimated that, by selecting the top 1% of the population, a reduction in MeP of 13.6% of the population mean in Australia per generation with direct selection (Figure 2), 15.8% with indirect selection on the *core breeding Australia*, and 19.4% by combining direct and indirect selection on the *core breeding Australia*. Similarly, by selecting the top 1% of the population in Spain, we estimated a reduction in MeC of 8.9% of the population mean per generation with direct selection, 12.6% with indirect selection on *core breeding Spain*, and 14.4% by combining direct and indirect selection on the *core breeding Spain*. Fifteen KOs were shared between our core and the KOs reported in beef cattle by Martínez-Álvaro *et al*. (2022). Reductions of 7.0% and 4.8% of the EME population mean per generation were estimated in Australia and Spain, respectively (Figure 2). These 15 KOs were also estimated to increase the reduction on EME when combined with direct selection, compared to use only direct selection.

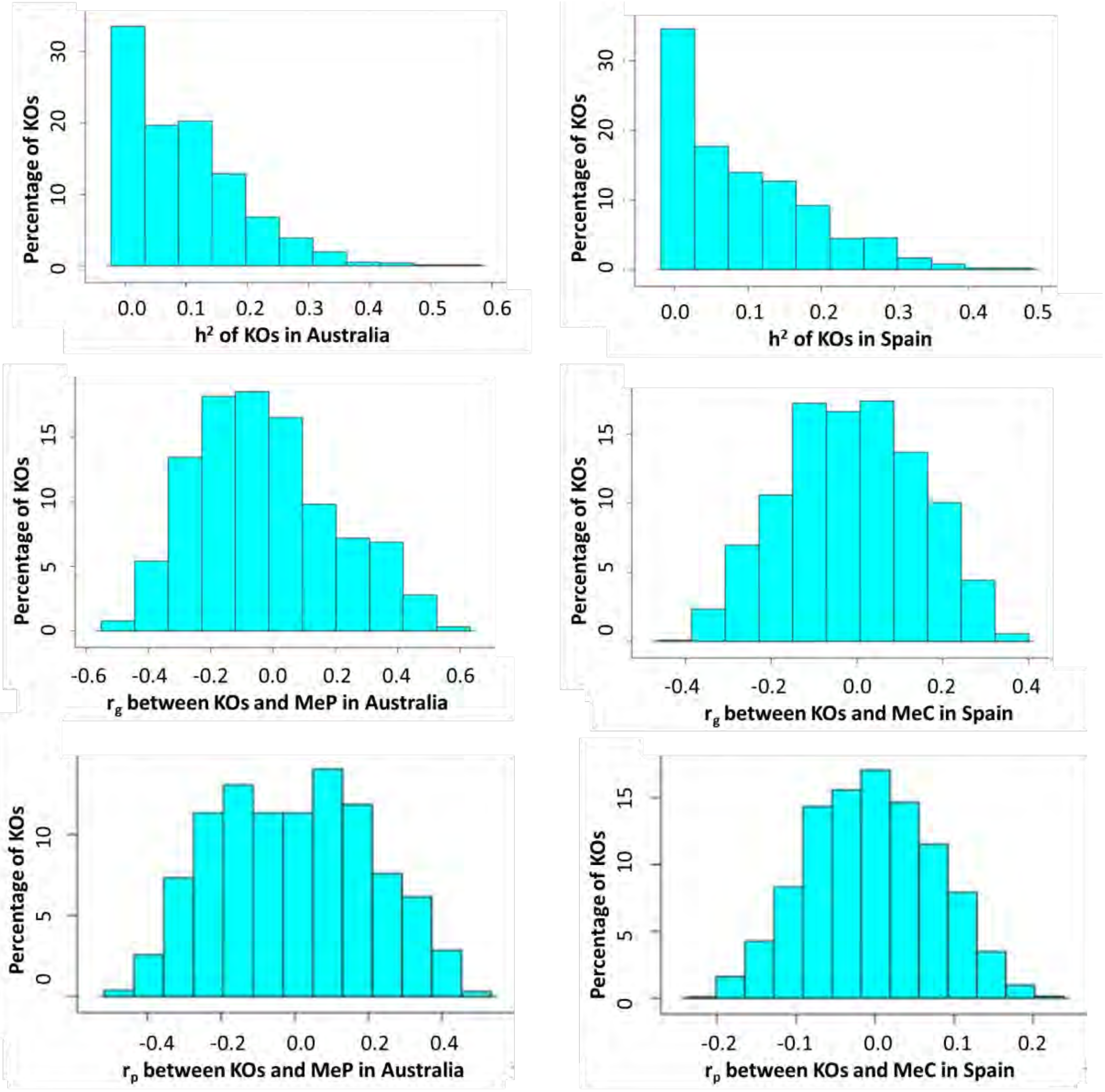

Figure 1. Heritability (h^2) of KEGG ontology identifiers (KOs) of a ruminal metagenome core shared between two dairy cattle populations located in Australia and Spain; and the genetic (r_g) and phenotypic correlation (r_p) between these KOs and methane production (MeP) in the Australian and methane concentration (MeC) in the Spanish populations.

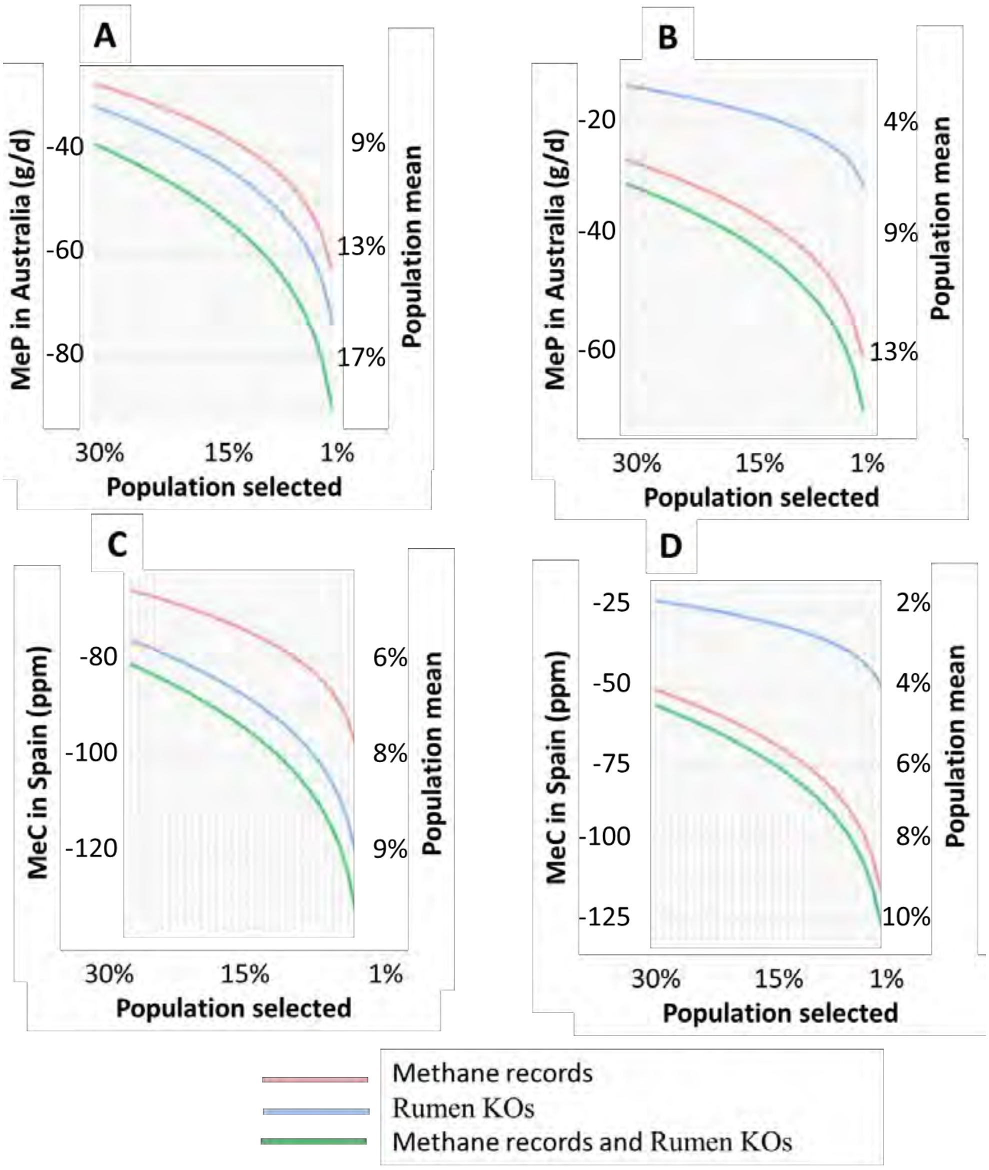

Figure 2. Estimated reduction by generation of enteric methane production (MeP) in Australian and enteric methane concentration (MeC) in Spanish dairy cattle populations. Red line: direct selection on enteric methane records. Blue: Indirect selection on ruminal microbial KEGG ontology identifiers (KOs). Green: Combination of direct selection on enteric methane records and indirect selection on KOs. In A and C, the KOs used are from a common core of 1,032 KOs shared between the populations located in Australia and Spain. A: Using 87 KOs with a heritability ≥ 0.20 in Australia and a genetic correlation ≥ with MeP. C: Using 42 KOs with a heritability ≥ 0.20 in Spain and a genetic correlation ≥ with MeC. B and D: Selection on 15 KOs shared between the Australian, Spanish dairy populations, and a beef cattle population (Martínez-Álvaro et al., 2022).

Discussion

The large variance in EME explained by our 1,032 KO *core* suggests that the ruminal metagenome could be used to reduce EME, for example, by identifying and removing high-emitter animals based on their ruminal microbiome profile or providing feed additives designed to reduce emissions exclusively to higher-emitting animals instead of the whole herd. Larger reductions on EME were estimated when using our *core* than when using direct EME and these reductions were even higher when combining the ruminal features and the EME records. These results are consistent with a previous study (Martínez-Álvaro *et al*., 2022). The large EME reductions by selecting on the KOs could be expected because EME is not an intrinsic animal trait, but a characteristic of the ruminal microbial community. This microbial community is heritable and genetically correlated with EME (Figure 1). The *core breeding Australia* and *core breeding Spain* used to estimate the selection response was heritable (~0.27) genetically correlation with EME (rg = 0.30 in Australia; rg = 0.24 in Spain). Based on our results, the *core breeding Australia* and *core breeding Spain* could be considered as target traits for improvement in emissions reduction genomic selection programs, in combination with EME records.

Genomic selection on a common ruminal metagenome *core* shared between Australia and Spain would lead to reductions in EME in both populations. These results indicate the potential for combining geographically diverse? reference populations in breeding programs through their ruminal metagenome, irrespective of each population's EME trait (Figure 3). Additionally, 15 out the 30 KOs reported as associated with EME in beef (Martínez-Álvaro *et al*., 2022) were used to estimate reductions of up to 7% of EME's population mean our dairy cattle populations (Figure 2). Further research could evaluate whether a common *core* between dairy and beef cattle, and other ruminants such as sheep, would reduce EME in all ruminant populations. Generating a reference population with EME measurements, ruminal metagenome and host genomics is costly and time consuming. Based on the results of this study, fostering international collaboration among the dairy, beef and other ruminant industries to combine diverse populations, EME traits, and environments through the rumen metagenomecould be beneficial for reducing global methane emissions. A common methodology is recommended for this purpose and based on our results, we present a methodology that (1) predicts most of the variance in EME, (2) potentially leads to significant EME

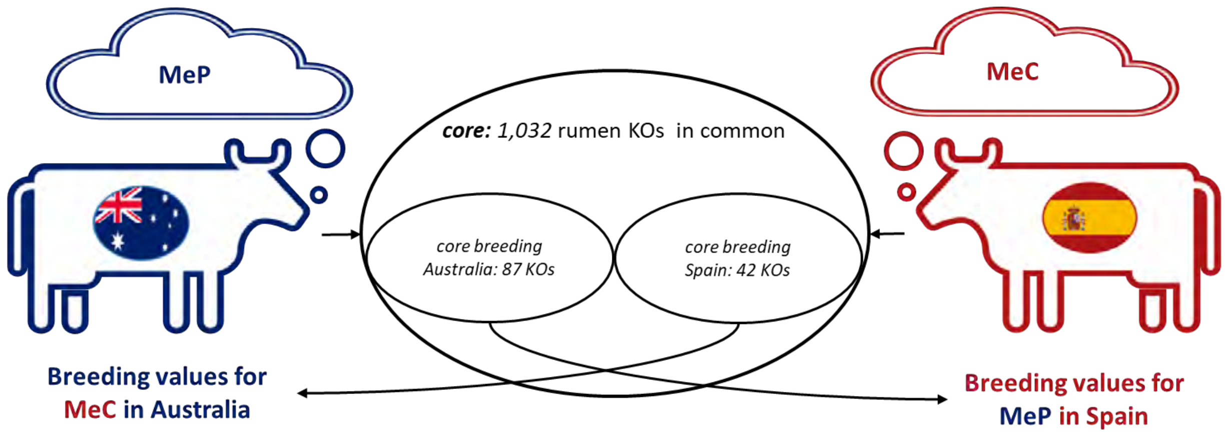

Figure 3. Sharing reference populations of the ruminal metagenome core facilitates prediction of enteric methane production (MeP) in Australia and enteric methane concentration (MeC) in Spain. Core breeding Australia: KOs with heritability ≥ 0.20 in Australia and genetic correlation with MeP ≥ 0.20. Core breeding Spain: KOs with heritability ≥ 0.20 in Spain and genetic correlation with MeC ≥ 0.20.

reductions through informed farming and breeding decisions, and (3) could potentially connect reference populations irrespective of their EME traits.

Conclusion

- We have developed a methodology to predict enteric methane emissions (EME) from ruminants. Using this methodology, we detected a common *core* of 1,032 KEGG ontology identifiers (KO) in the rumen metagenome of 834 dairy cows from Australia and Spain. This *core* explained up to 83% of the variation in EME with an accuracy of up to 0.38, which could potentially facilitate farming decisions aims to reduce methane emissions.
- Large reductions in EME, of up to ~16% of the population mean per generation, could be achieved by selection on this *core*, being superior to direct selection on EME. A combination of direct selection on EME and indirect selection on the *core* would produce larger reductions (up to 19% of the population mean). These results suggest that rumen metagenome features could be candidate traits to improved-genomic selection programs along with EME records.
- Sharing reference populations of the ruminal metagenome *core* facilitates prediction of EME irrespective of each population's EME trait. For this reason, we propose a global effort to validate a common *core* of ruminal features associated with EME.
- If validated, our results could impact global ruminant emission reduction efforts.

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The Swiss way of breeding dairy cattle for reduced methane: CH_4COW

B. Bapst[1], T.F.-O. Neuenschwander[2] and A. Butty[1]

[1]Qualitas AG, Chamerstrasse 56, CH-6300 Zug, Switzerland
[2]Holstein Switzerland, Route de Grangeneuve 37, CH-1725 Posieux, Switzerland
Corresponding Author: beat.bapst@qualitasag.ch

Abstract

In Switzerland, agriculture contributes 14.3% of greenhouse gas emissions, primarily methane from livestock, especially dairy cows. To meet emission reduction targets, a nationwide effort is underway to measure methane emissions and reduce the impact of dairy cows with genetic selection. The project named CH_4COW is funded by various entities and aims to install methane detection systems on 60 farms across Switzerland, focusing on different feeding systems and breeds. The project represents a significant step towards reducing methane emissions through breeding strategies tailored to Swiss dairy farming conditions. Long-term benefits include potential collaborations in areas like ruminant nutrition and life cycle assessment.

Keywords: methane mitigation, dairy cattle, phenotyping, sniffer, breeding.
Presented at the ICAR Anual Conference 2024 in Bled at the Session 11: Methane Emission-Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

Introduction

In Switzerland, 14.3% of greenhouse gas emissions come from agriculture. Although this is not much compared to transport, for example, agriculture is under societal and political pressure to reduce its emissions. Agriculture is responsible for 83.3% of methane emissions in our country (FOEN, 2024). Livestock, especially dairy cows, are a major contributor. Therefore, in order to meet the target of a 50% reduction in greenhouse gas emissions by 2030 compared to 1990 and CO_2 neutrality by 2050 (FOEN, 2023), action is needed at all levels. Besides farm and management level, the dairy industry has powerful tools at the cow level. Feeding measures can be very effective and have an immediate impact (Hristov et a., 2015). Breeding actions have a medium to long-term aspect (e.g. Pryce *et al*., 2014), but if implemented in the right selection strategy they are sustainable. For this reason, the umbrella organization of all Swiss cattle breeding organizations decided to launch a phenotyping offensive with the aim of implementing a routine genetic evaluation for reduced methane emission based at least in part on Swiss phenotypes.

The reasons for having our own phenotypes are the following:

1. Our feeding systems are often different from the rest of the world; we have distinctly different summer and winter diets and in general our diets are very emphasized on roughage (Zeitz, Soliva and Kreuzer, 2012).
2. Some farmers are not allowed to feed silage due to specific regulations for certain cheese manufacturing processes (FOAG, 2015).

3. Animal welfare regulations and programs require regular outdoor access or even grazing in some cases (TSchV, 2024).
4. Swiss milk production is not only based on Holstein (HOL) breeds: An important part comes from Brown Swiss (BSW) or certain local breeds such as Simmental or Original Braunvieh (Identitas, 2024).

The new genetic methane mitigation project

Description

The CH_4COW project started this year in January and will last for 4 years. The installation of 60 sniffers on farms across the country will take place this year. Thirty sniffers will be installed in HOL herds, and the other in BSW herds. The project is funded by the Swiss Federal Office of Agriculture (FOAG), some regional governments and the Association of Swiss Cattle Breeders (https://asr-ch.ch/en/). Although most sniffers will be placed in automatic milking systems (AMS), ten of the HOL farms are not allowed to have AMS due to regulations for certain cheese manufacturing processes, mainly Gruyère AOP (FOAG, 2015). There, the sniffers will be installed in automated feeding stations in parallel with the installation of an animal identification system.

Aims

The start of this project is a first step towards the reduction of methane emissions from dairy cattle in Switzerland through breeding. Several options for the next step will be available and need to be validated. In the short term, we will be able to contribute our methane phenotypes to an extension of existing methane phenotype predictions using mid-infrared spectroscopy milk data, or to develop our own prediction. After that, we should be able to perform genomic evaluation for routine purposes. In the medium or long term, based on the experience gained, we could extend the sniffer phenotyping process to perform a genomic evaluation with real measured phenotypes.

Outlook

The future pool of data and knowledge could form the basis for further collaborations. Not only in breeding, but also in related disciplines such as ruminant nutrition or life cycle assessment.

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breed4green: Recording of new phenotypes for methane emission and feed efficiency in Austrian dairy cattle

K. Linke[1], C. Schmid[2], A. Köck[1], F.J. Auer[2], F. Steininger[1], T. Guggenberger[3], B. Fuerst-Waltl[4], J. Sölkner[4], W. Zollitsch[4] and C. Egger-Danner[1]

[1]ZuchtData EDV-Dienstleistungen GmbH, Dresdner Str. 89, 1200 Vienna, Austria
[2]LKV-Austria, Dresdner Str. 89, 1200 Vienna, Austria
[3]HBLFA Raumberg-Gumpenstein, Altirdning 11, 8952 Irdning-Donnersbachtal
[4]BOKU University, Vienna, Department of Sustainable Agricultural Systems,
Gregor-Mendel-Str. 33, 1180 Vienna, Austria
Corresponding Author: linke@zuchtdata.at

Abstract

Climate change and the rising and fluctuating costs for energy and concentrated feed are major challenges for the livestock sector. The breed4green project focuses on researching strategies to reduce methane emissions and enhance feed efficiency within the Austrian cattle industry. Measurements of methane and CO_2 emissions are currently being conducted on both experimental and commercial farms using the GreenFeed system. The aim of the project is to collect methane and CO_2 measurements of approximately 1,000 Fleckvieh and 200 Brown Swiss cows. In addition, various phenotypes such as health, body weight, BCS, metabolism, energy intake and milk mid infrared (MIR) spectra are recorded. Data on feed intake from experimental farms are also available for validation. The genetic potential of direct traits like methane, CO_2 and feed efficiency, along with their correlations to health and other traits, will be analyzed. The project also includes the development and validation of MIR equations for emitted methane and energy balance. The focus will be on investigating the use of these indirect traits to reduce methane emissions and improve feed efficiency in breeding programs to pave the way for genomic selection. The results will also be used to optimize herd management. Furthermore, the environmental impact of relevant dairy and beef production systems in Austria will be investigated.

Keywords: GreenFeed, methane emission, feed efficiency, mid infrared, dairy cattle.
Presented at the ICAR Annual Conference 2024 in Bled at the Session 11: Methane Emission-Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

breed4green: direct and indirect traits for feed efficiency and greenhouse gas emissions for cattle breeding and herd management

The cattle sector is challenged by climate change and its implications on animal wellbeing and feed production but is also made responsible for methane emissions. breed4green aims to improve sustainability in Austrian cattle farming, focusing on individual animals. Genetic improvements in feed and energy efficiency, as well as reducing greenhouse gas emissions, are key leverage points to reduce environmental impacts and are focus of breed4green.

The goal of the project, which started in May 2023 and will run until December 2027, is to investigate breeding strategies focusing on feed efficiency and reduction of greenhouse gas emissions for the Austrian cattle industry. To achieve this, phenotypes to assess the genetic background of these traits will be generated. As they are expensive to record, proxies will be developed and validated. Data from research stations and commercial farms will be used to derive these novel traits for breeding in the areas of feed efficiency and greenhouse gas emission reduction.

Methane and CO_2 measurements on individual animals in experimental and commercial farms using the GreenFeed system, combined with extensive data collection on health and feed efficiency (health, weight, body condition score, metabolism, energy intake, mid-infrared (MIR) spectra) form the basis for research in this area. This will be complemented by existing data on feed intake at the station and additional data from station records as well as from commercial farms like performance data, data from the cattle data network, data from AMS systems and sensors. The goal is to conduct measurements on approximately 1,000 Fleckvieh and 200 Brown Swiss cows in commercial farms. At the moment, the data recording on the first farms is in progress.

The focus of the project is on the genetic improvement of feed efficiency and methane emissions. The genetic potential of the direct trait methane and CO_2 emission, and the genetic correlations to health and other traits in the total merit index, as well as the factors influencing the methane output of the animals, are analyzed. The new data are also used for the development and validation of MIR equations. The potential of the indirect trait MIR-methane is analyzed and indirect traits for feed efficiency are developed. The developed MIR estimators for energy balance and energy deficit are validated using station data, and their potential for improving feed efficiency is investigated in conjunction with other relevant traits. The genetic relationships between the traits feed efficiency and methane or CO_2 emissions are analyzed, and the potential of using indirect traits for feed efficiency and methane emissions for broad application in breeding is explored. The project aims to lay the foundation for genomic selection for feed efficiency and reduction of greenhouse gas emissions. Furthermore, the environmental impact of relevant milk and beef production systems in Austria is being researched.

Partners

In breed4green; the Cattle Breeders Austria; Higher Federal Teaching and Research Institute for Agriculture Raumberg Gumpenstein; LKV Austria; BOKU University; ZuchtData EDV Dienstleistungen GmbH; Fleckvieh Austria and Brown Swiss Austria work together in 10 work packages to achieve these goals; breed4green is supported by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management and dafne.at. The project has further cooperation and supporting partners.

Figure 1. Partners in breed4green.

Influence of housing system and season on methane and carbon dioxide concentrations in a dairy cattle barn

M. Bric, M. Podgornik Milosavljević, T. Perčič, S. Rogina and M. Klopčič

*University of Ljubljana, Biotechnical faculty, Department of Animal Science, Groblje 3,
1230 Domžale, Slovenia
Corresponding author: marija.klopcic@bf.uni-lj.si*

Abstract

Cattle barns are an important source of greenhouse gasses (GHG). In buildings for dairy cattle, the interaction of weather conditions and microclimatic parameters have an influence on the emission of GHG. The aim of the study was to determine the effects of the housing system and the seasons on the concentration of methane and carbon dioxide in dairy cattle barns. As part of the EIP-AGRI project "Innovative environmental and climate-based management systems of cattle farms to ensure feed production and optimal conditions for rearing cattle", we carried out monthly CH_4 and CO_2 concentration measurements at different points in the dairy cattle barn of ten farms with different housing systems (tied-in housing system, loose housing with cubicles and slatted floor or with concrete floor, compost bedded pack barn, deep straw housing and innovative housing system with permeable floor). The measurements were carried out from July 2022 to October 2023 at a height of 1.5 m. Each measurement lasted 5 minutes. For the measurements we used the portable gas analyser GASMET GT5000 Terra. In addition to greenhouse gas concentrations, microclimate parameters (temperature, relative humidity and air flow) were also measured using a TESTO 435 multimeter. Based on the 4,633 measurements, we find that there are differences in the measured CH_4 and CO_2 concentrations between farms with different housing systems, different methods of removal and storage animal secretions and in terms of measurement time. We find that, on average, the lowest CH_4 concentrations (11.46 ± 8.83 ppm) were measured in compost bedded pack barns and the lowest CO_2 concentrations (517.67 ± 85.13 ppm) in deep straw barn. The highest concentration of CH_4 (33.24 ± 23.40 ppm) and CO_2 (787.49 ± 254.12 ppm) was measured in barns with tied-in housing. The lowest concentration of CH_4 (17.70 ± 11.21 ppm) was measured in June 2023 and of CO_2 (558.04 ± 126.55 ppm) in August 2022. The concentrations of CH_4 and CO_2 measured in the winter months were on average higher than the concentrations measured in the summer months. Higher CH_4 and CO_2 concentrations were found in closed barns where air flow was poorer. The differences between the CH_4 and CO_2 concentrations measured in the summer and winter months were smaller in more open barns. A correlation coefficient of 0.755 indicates a relatively strong linear relationship between CH_4 and CO_2 concentrations. This means that changes in CH_4 concentrations are closely associated with corresponding changes in CO_2 concentrations across the measured data points and vice versa.

Keywords: cattle, dairy cows, housing system, methane, carbon dioxide, season, Slovenia. Presented at the ICAR Anual Conference 2024 in Bled at the Session 11: Methane Emission-Free Communications: Genetics, Environmental, and Life Cycle Assessment Studies

Introduction

Methane (CH_4) is a greenhouse gas (GHG) whose global warming potential is 23 times higher than that of carbon dioxide (CO_2) (IPCC, 2001). Enteric fermentation and manure management account for 35 to 40 % of total anthropogenic CH_4 emissions and 80 % of CH_4 released from agriculture (FAO, 2006). With the intensification of milk production, dairy cattle barns have been identified as an important source of GHG emissions (Qu *et al.,* 2021). Quantifying GHG emission rates in dairy cattle barns with natural ventilation is a challenging task, as many different factors influence the release of these emissions (Samer *et al.,* 2011), but if done properly, it could contribute to the development of accurate emission inventories and effective mitigation strategies (Qu *et al.,* 2021). Therefore, it is necessary to carry out this type of research on farms under realistic conditions if we want to obtain representative and reliable measurement results. Conducting the trial on several farms at the same time (multi-farm trial) contributes to more reliable results. In addition, attention must be paid to representative sampling, which requires an appropriate spatial distribution of the measurement locations within the barns. In order to take into account, the influence of climatic factors, measurements must be carried out throughout the year (Schrade *et al.,* 2012). Indeed, the climatic conditions surrounding livestock buildings are considered to be an extremely important factor for GHG emissions, as these conditions are likely to be essential for naturally ventilated buildings, as they have a direct influence on the ventilation rate and most likely also on the emission rate (Ngwabie *et al.,* 2009). Numerous studies have shown that heat stress, which is a function of relative humidity and air temperature, affects both the behaviour and performance of dairy cows (Joo *et al.,* 2015; West, 2003). Therefore, further research into the effects of environmental factors on GHG emissions from dairy cattle barns is important (Joo *et al.,* 2015).

The aim of the study was to determine the influence of the housing system and the season on the CH_4 and CO_2 concentrations in ten different dairy cattle barns with different housing systems.

Material and methods

As part of the EIP - AGRI project “Innovative environmental and climate-based management systems of cattle farms to ensure feed production and optimal conditions for rearing cattle”, we carried out monthly measurements of CH_4 and CO_2 concentrations on ten milk production-oriented farms with different housing systems. The study therefore included three dairy cattle barns with cubicles and slatted floors (farm 2, farm 3 and farm 6), one dairy cattle barn with cubicles and concrete floor (farm 7), two compost bedded pack barns (farm 8 and farm 9), two barns with tied-in housing system (farm 4 and farm 10), one barn with deep straw housing (farm 5) and one farm with an innovative housing system with a permeable floor (farm 1). The measurements were carried out from July 2022 to October 2023 at a height of 1.5 m above the floor at various locations within the barns: in the feeding alley, on the cow traffic routes, in the lying alley and in the young stock housing area (Figure 1). Each measurement at each selected location inside and outside the barn lasted 5 minutes. The GHG concentrations were measured with a portable gas analyser Gasmet GT5000 Terra. It works on the principle of FTIR technology (Fourier transform infrared spectroscopy), which enables fast, accurate and reliable measurements of up to 300 different gas components simultaneously based on the absorption of IR light (Gasmet, 2022). In addition to the GHG concentrations, microclimate parameters (temperature, relative humidity and air flow) were also measured at the same locations as the GHG measurements. These measurements were carried out using a Testo 435 Multi-Metre.

The data analysis of the measurements of greenhouse gas concentrations and microclimate parameters was carried out using the SAS Stat statistical package. We were interested in the effects of different housing systems and the influence of season on the concentrations of CH_4 and CO_2 in different housing systems for dairy cows. For the statistical analysis we used two different statistical models. The systematic part of both models was developed using the least square means method with the GLM procedure in the SAS statistical package, and the differences within each influence were tested using analysis of variance (ANOVA) (F-test).

In the first model, CO_2 was used as a variable, and in the systematic part of the model, M was used as the month, F as the farm where we took the measurements, and T(M) as the housing system nested within the month. The influence of CH_4 concentrations was included in the model in the form of a linear regression (see equation 1). The systematic influence of the month of measurement had a statistically significant influence on CO_2 concentrations (p<0.0001), as did the systematic influence of the farm (P <0.0001) and the influence of the housing system within the month (P <0.0001). In addition to the systematic influences on the CO_2 concentrations, the CH_4 concentrations in the barn also had a statistically significant influence (p<0.0001). With this model, we were able to explain 78.99 % of the variance (R^2 = 78.99 %).

$$y_{ijkl} = \mu + M_i + F_j + T_{ki} + b_1 (x_{ijk} - \bar{x}) + e_{ijkl} \qquad (1)$$

In the second model, we used CH_4 concentration as a variable, and in the systematic part of the model, we used M as the month, F as the farm where the measurements were taken, and T(M) as the housing system nested within the month of the measurements. A linear regression coefficient was used for the influence of CO_2 concentration (see equation 2). The influence of the month and the influence of the farm had a statistically significant influence on the measured CH_4 concentrations in the barn (P<0.0001), the same applies to the influence of the housing system, which was nested within the month and the influence of the linear regression (P <0.0001). With the second model, we were able to explain 72.29 % of the variance (R^2 = 72.29 %).

$$y_{ijkl} = \mu + M_i + F_j + T_{ki} + b_2 (x_{ijk} - \bar{x}) + e_{ijkl} \qquad (2)$$

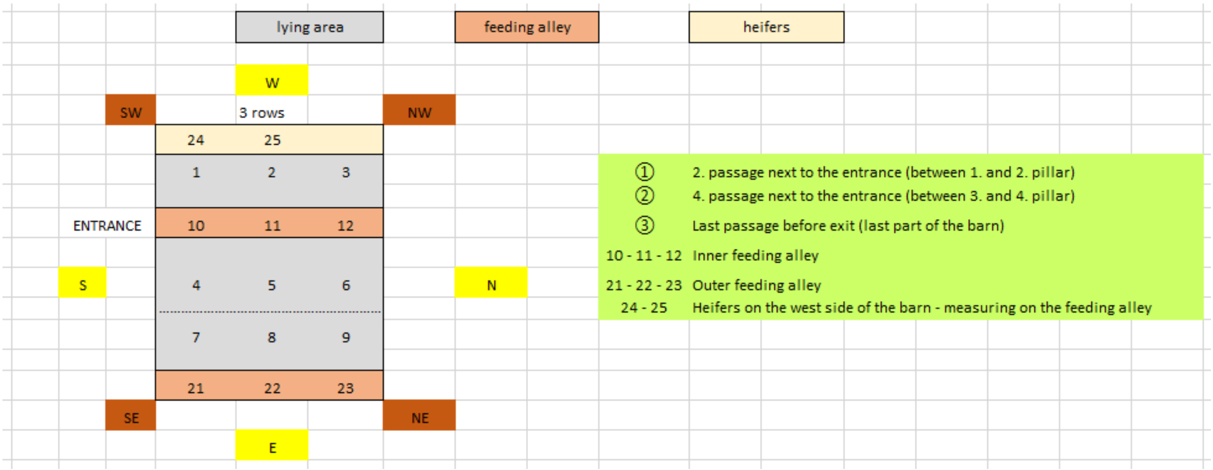

Figure 1. Schematic representation of the measuring points in the barn.

Results and discussion

Based on the 4,633 measurement results, we find that there are differences in the measured CH_4 and CO_2 concentrations between the individual farms with different housing systems, different methods of removal and storage of animal secretions and with regard to the measurement time. On average, the lowest CO_2 concentrations (529.09 ± 51.71 ppm) were measured in deep straw barn and the highest (833.54 ± 204.90 ppm) in barns with tied-in housing system. In August 2022, when the average air temperature was 23.64°C ± 3.05°C and the average relative humidity was 63.98 % ± 9.05 %, CO_2 concentrations (558.04 ± 126.55 ppm) were the lowest on average, and in March 2023, when the average air temperature was 11.26°C ± 4.14°C and the relative humidity was 57.86 % ± 13.77 %, the measured CO_2 concentrations were the highest on average (789.77 ± 240.88) (Figure 2, Table 1).

On average, the lowest CH_4 concentrations (10.23 ± 3.00 ppm) were recorded in compost bedded pack barns and the highest similar to the CO_2 concentrations, in barns with tied-in housing (36.38 ± 14.54 ppm) (Figure 3). The CH_4 concentrations measured in the winter months were on average higher than the concentrations measured in the summer months. The lowest CH_4 concentrations (17.69 ± 11.21 ppm) were measured in June 2023, when the average air temperature was 21.15°C ± 2.59°C, and the average relative humidity was 63.28 % ± 10.86 %. The highest average CH_4 concentrations (28.51 ± 21.32 ppm) were measured in January 2023, when the average air temperature was 5.74°C ± 3.82°C, and the average relative humidity was 72.92 % ± 6.16 % (Table 1).

The concentrations of the two gases investigated, CH_4 and CO_2, were on average higher in the winter months than the concentrations measured in the summer months. The

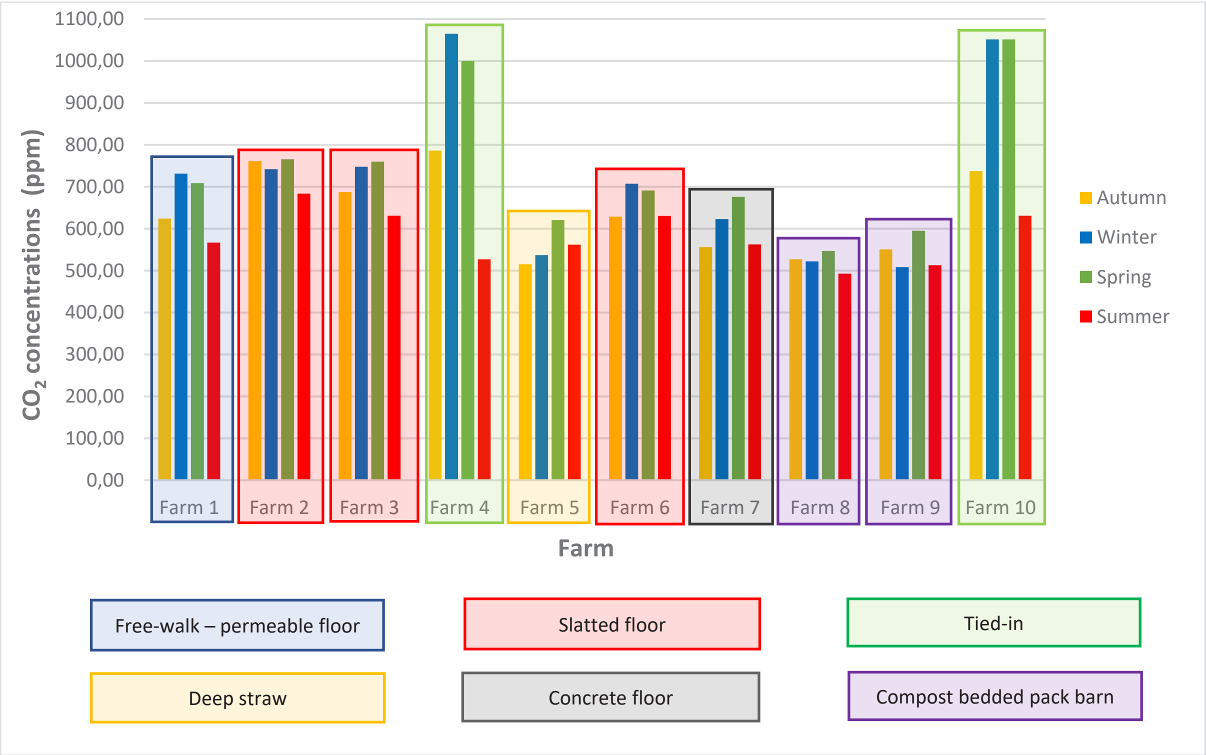

Figure 2. Differences in average CO2 concentrations between farms and seasons.

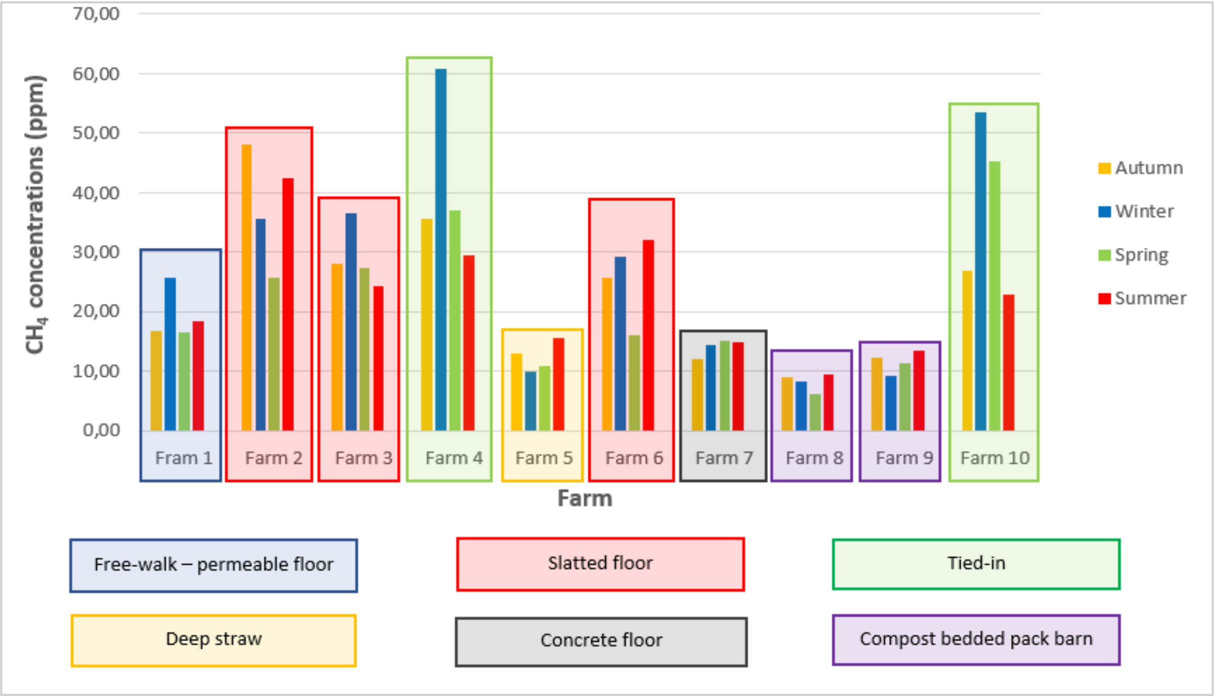

Figure 3. Differences in average CH4 concentrations between farms and seasons.

Table 1. Descriptive statistics (mean and STD) for CO2 and CH4 concentrations in the different measurement months.

Month	N	CO_2 (ppm)	CH_4 (ppm)	Temperature (°C)	Humidity (%)
July 2022	294	594.53 ± 136.93	21.92 ± 25.40	25.59 ± 2.72	54.63 ± 11.82
August 2022	277	558.04 ± 126.55	25.11 ± 27.32	23.64 ± 3.05	63.98 ± 9.05
September 2022	329	582.01 ± 121.13	21.83 ± 17.25	16.33 ± 2.65	71.66 ± 8.71
October 2022	311	631.67 ± 142.09	21.53 ± 15.54	15.78 ± 2.02	71.64 ± 8.71
November 2022	311	673.82 ± 192.92	20.45 ± 18.00	7.87 ± 3.46	75.17 ± 5.52
December 2022	277	662.97 ± 179.67	21.72 ± 16.08	4.64 ± 3.04	73.83 ± 5.49
January 2023	295	726.58 ± 237.17	28.51 ± 21.32	5.74 ± 3.82	72.92 ± 6.16
February 2023	277	695.35 ± 255.34	26.55 ± 24.43	5.35 ± 3.74	62.05 ± 8.38
March 2023	311	789.77 ± 240.88	24.17 ± 21.52	11.26 ± 4.14	57.86 ± 13.77
April 2023	277	752.00 ± 209.69	19.47 ± 16.81	12.60 ± 2.86	51.21 ± 13.27
May 2023	311	596.52 ± 144.07	17.76 ± 11.55	16.47 ± 2.27	64.83 ± 9.29
June 2023	277	570.12 ± 131.80	17.69 ± 11.21	21.15 ± 2.59	63.28 ± 10.86
July 2023	275	609.24 ± 152.27	23.64 ± 20.05	23.38 ± 3.05	70.16 ± 9.07
August 2023	277	570.96 ± 112.32	21.07 ± 20.36	20.82 ± 2.88	68.73 ± 9.08
September 2023	259	614.34 ±143.10	26.05 ± 25.28	20.91 ± 3.11	65.77 ± 17.35
October 2023	277	575.33 ± 133.58	24.37 ± 26.06	14.73 ± 3.49	72.70 ± 8.03

differences between the CH_4 and CO_2 concentrations measured in the summer and winter months were smaller in more open barns. We also found that higher CH_4 and CO_2 concentrations were detected in more closed barns where airflow was poorer. Qu *et al.* (2021) indicate that CH_4 emission rates tend to increase with increasing temperature. Poteko *et al.* (2019) also find similar findings. In their report, Joo *et al.* (2015) investigated the influence of environmental factors on various GHG concentrations. They found that air temperature had the greatest influence on the increased CO_2 concentrations in the dairy cattle barn, while the contribution of relative humidity had the least influence. Similar to CO_2 concentrations, elevated CH_4 concentrations in the barn were significantly influenced by air temperature and air velocity, while the contribution of by relative air humidity was the smallest (Joo *et al.,* 2015). The air temperature between 5°C and 25°C is referred to as the thermoneutral range for lactating dairy cows. Outside this comfort zone, animal activity can be negatively affected, resulting in low metabolism, reduced appetite, low CO_2 levels in the bloodstream and lower respiration, which in turn leads to lower CO_2 emissions (West, 2003). High temperatures, which reduce the time cows devote to eating and rumination, also lead to a reduction in the amount of CH_4 produced (Ngwabie *et al.,* 2011). However, Qu *et al.* (2021) note that data synthesis shows large differences between CH_4 emission rates in dairy cow barns in different publications.

A correlation coefficient of 0.755 indicates a relatively strong linear relationship between CH_4 and CO_2 concentrations, which is in line with the results of Joo *et al.* (2015) ($R^2 = 0.67 – 0.74$). This implies that changes in CH_4 concentrations are closely associated with corresponding changes in CO_2 concentrations and vice versa, across the measured data points, due to the common origin (enteric fermentation and respiration) in ruminants (Joo *et al.,* 2015).

Conclusions

In the future, it is expected that major changes will be required from agriculture in terms of reducing greenhouse gas emissions (Pathak *et al.,* 2013). Quantifying gas emission rates in dairy cow barns could help to develop accurate emission inventories and effective mitigation strategies (Qu *et al.,* 2021). Estimates of gas emissions in dairy barns are highly dependent on the measurement of ventilation rates and gas concentration (Qu *et al.,* 2021). Changes in some husbandry practices with the aim of reducing GHG emissions, such as feed production strategies and feeding practices, animal housing facilities, animal excreta handling practices, etc., will be a major challenge for agriculture in the future (Pathak *et al.,* 2013), but at the same time could help to tackle climate change and improve air quality on a large scale (Hassouna *et al.,* 2016).

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Phenotyping sheep in a portable accumulation chamber and using devices with different accuracies measured the same methane trait

M.N. Aldridge[1], D.J. Brown[1], P.T. Fitzgerald[2], E.H. Clayton[3], A. Donaldson[3], V.H. Oddy[4] and J.H. van der Werf[2]

[1]Animal Genetics and Breeding Unit, A Joint Venture of NSW Department of Primary Industries and University of New England, Armidale, NSW 2351, Australia
[2]School of Environmental and Rural Science,
University of New England, Armidale, NSW 2351, Australia
[3]NSW Department of Primary Industries, Armidale, NSW, 2351 Australia
[4]Beef Centre, NSW DPI, Armidale, NSW, 2351, Australia
Corresponding Author: michael.aldridge.@une.edu.au

Abstract

Across countries and ruminant species, animal selection has been identified as a desirable method of reducing methane (CH_4) emissions. A possible strategy is to develop a reference population to enable genomic selection for emission traits. However, the high cost and slow throughput of phenotyping make it challenging to rapidly collect sufficient information for publishing CH_4 breeding values in sheep. This project intends to measure methane emissions from 10,000 animals.

The most common method of measuring CH_4 emissions from sheep is with a portable accumulation chamber (PAC) where CH_4, carbon dioxide (CO_2) and oxygen (O_2) are measured at a mid-point (20 or 25 minutes) and end point (40 or 50 minutes) in the PAC. Two measurement devices can be used, referred to as FID (for CH_4) and FoxBox (for CO_2 and O_2); a third device known as an 'Eagle' can measure CH_4, CO_2, and O_2. The Eagle device is both cheaper and simpler to use. However, there are concerns about the lower sensitivity and precision of the Eagle compared to FID and FoxBox. The aim of this study was to compare both the duration of measurement, and the devices used to measure methane traits in sheep.

Data from 3,729 lambs and ewes were fitted with a bivariate animal model for methane rate (mL/min) from different measurement durations or measured with different devices. The following significant fixed effects were fitted for Site.Day.Run, birth and rearing type, age, age of dam, sire breed, and sex. Estimates of heritability of CH_4 ranged from 0.15 to 0.19 and were not significantly different between CH_4 measurement device or measurement duration. The genetic correlation for CH_4 measured using FID or Eagle was 0.96 for the short duration and equal to one for the long duration, and the phenotypic correlation between the two devices was 0.94 for the short duration and 0.97 for the long duration. The genetic correlation for CH_4 measured at 20-min and 40-min was equal to one for both measurement devices, with a phenotypic correlation of 0.80 when CH_4 was measured with the with the Eagle and 0.82 when measured with the FID.

Among other factors, the accuracy of genomic prediction depends on the heritability of the trait and the number of animals measured. We used the heritability of CH_4 according to different measurement methods to predict accuracy of genomic prediction. Assuming a heritability of 0.17 from the Eagle long measurement and 10,000 animals measured, gave an accuracy of genomic prediction of 0.42. We assumed that shortening the measurement time from 40 mins to 20 mins would allow 40% more animals measured

(14,000 total), which resulted in a accuracy of genomic prediction of 0.48. We found that the heritability was not significantly different between measurement durations, however if it were lower for the shorter measuring period (0.15) the accuracy of genomic prediction would be 0.45.

By reducing the measurement duration with the PAC methodology, there is limited or no loss of precision indicated by heritabilities that are not significantly different. The time saved with shorter measuring periods can be used to phenotype more animals or reduce labour costs. The overall benefit is a lower cost per animal with potentially more animals measured and an overall increase in accuracy of genomic prediction. However more clarity is needed regarding how many additional animals can be recorded with shorter measurement durations.

Keywords: methane, sheep, small ruminant, phenotyping, protocol.
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Introduction

Globally, genetic selection for lower methane has been identified as a promising method of reducing the contribution ruminant species have on greenhouse gas output. Over the past two decades, several Australian studies measured methane (CH_4) on over 7,000 sheep and reported estimates of heritability (0.11 to 0.18) for various methane traits (Robinson *et al*., 2014, Goopy *et al*., 2016, Paganoni *et al*., 2017, Wahinya *et al*., 2022, Sepulveda *et al*., 2022). Additionally, selection line experiments in New Zealand have demonstrated genetic selection does lower methane production (Rowe *et al*., 2019). One of the main challenges to an industry-wide implementation of methane selection is the publication of reliable breeding values for a methane trait as the trait is currently not measured by breeders. A possible strategy is to develop a reference population to enable genomic selection for emission traits, but rapid collection of sufficient phenotypes for this purpose is not easy.

The majority of sheep production is based on pasture systems. Measuring many animals for methane output in pasture production systems is a challenge. One method used in sheep to measure methane production is the use of portable accumulation chambers (PACs). The use of PACs has improved the feasibility of measuring large numbers of sheep. Each PAC chamber is an airtight box, a sheep is placed inside the box for a period of time (less than one hour), the methane (CH_4), carbon dioxide (CO_2), and oxygen (O_2) concentrations are measured at multiple time points during the PAC occupation. As the volume of the box is known, the gas concentration accumulated over the measurement duration can be converted to methane rate (ml/min). Across studies and between protocol methods, different gas measuring devices have been used, it is important to determine if the different devices are measuring the same trait, if the various datasets are to be used in the same genetic evaluation. The typical protocol requires a large amount of experienced and technical labour, and throughput is limited by the number of chambers and the occupation duration.

Simplifying the protocol by using cheaper and easier to use devices, could reduce the cost of measurements. However, cheaper and easier to use devices tend to have lower precision and can lower the accuracy of measurements. Additionally, the amount of time within a PAC chamber could potentially be reduced. While this would lower the accuracy of the measurement, it would allow for additional animals to be measured and/or reduce labour costs per animal measured. Reducing the accuracy either with less accurate devices or shorter measurement durations, will also lower the heritability for the same trait. It is important that the sheep industry is provided with accurate breeding values for methane, both heritability and number of animals phenotyped affect the accuracy of genomic prediction. It is therefore important to investigate how potential changes to protocol will change the accuracy of genomic prediction.

This work aimed to demonstrate that reducing measurement duration, may decrease the accuracy of measurements, but the reliability of genomic prediction would increase, as more animals could be phenotyped. An additional objective of this analysis, was to determine if different measurement devices are measuring the same methane trait. This would help determine the feasibility of including all 17,000 records from the current and historic projects in a single genetic evaluation, especially as the historic data did not have access to the recent developments in measurement technology. This could allow the current protocols to be simplified by reducing the number of gas measuring devices and thereby reducing labour intensity.

Material and methods

Data collection

Methane phenotyping

Between March 2022 and February 2024, CH_4, CO_2, and O_2, was measured on 3,769 sheep across seven sites (Four research sites and three industry breeder flocks) in New South Wales (NSW), Australia. At one of the research sites, 501 lambs were measured in 2022, and another 504 lambs in 2023, all other sites measured mixed aged ewes and were only visited once. At each site, up to a maximum of 84 animals were measured each day, with the aim of phenotyping 500 sheep over consecutive days. Animals were placed in a holding paddock near to the site of PAC measurements, with access to feed and water. Animals were measured in up to seven batches (six batches per day is the current standard practice) across twelve PACs. The 12 chambers were occupied in a staggered order with animals taken off feed one hour earlier. The measurement of 12 sheep constituted one run, and after allowing air circulation the protocol was repeated with a new run of 12 sheep. The gases were measured within seconds of the set times, at a mid-point 20 minutes (25 minutes for lambs) and again at the end point 40 minutes (50 minutes for lambs). After the end point measure, the animal was released from the PAC. For each chamber, the three gases were measured using both the Eagle-2 device (Eagle) and a combination of FID (CH_4) and FoxBox (CO_2 and O_2) devices, hereafter this combination will be referred to as FID. The historic data (not used in this study) only measured with FID.

Genetic parameter estimation

Univariate animal models with restricted maximum likelihood (REML) were used to estimate all variance components, using WOMBAT (version 2022). The model can be summarised with matrix notation:

$$y = Xb + Za + e \qquad (1)$$

Where y is a vector of trait observations, for methane (CH4). Four traits were considered, being different measures of methane output: mid-point (20min for ewes, 25min for lambs) and end-point measure (40min for ewes, 50min for lambs) and measured with either the Eagle or the FID. Only animals with both Eagle and FID measurements at both time points were included. Due to differences in means and variation between sites, each methane rate was centred to the site mean and standardised by site standard deviation. The matrices X and Z are incidence matrices associated with the fixed effects vector b (Site.Day.Run, birth and rearing type, age, age of dam, sire breed, and sex), and the vector of random additive genetic effects $a \sim (0,Hs^2_a)$, respectively. Heritability and genetic and phenotypic correlations between related pairs of methane traits, i.e. between the two measurement devices and between the two time points, were estimated in bivariate analyses.

Power calculations

Different measurement protocols may result in different trait heritabilities and numbers of animals measured and these parameters were used to predict the accuracy of genomic prediction. The first equation of Daetwyler *et al*. (2008), was used for this purpose

$$r_{g\hat{g}} = \sqrt{\frac{\lambda h_o^2}{\lambda h_o^2+1}} \quad (2)$$

Where, λ is the ratio (Me/T) of number of observed phenotypes (T) to the number of effective chromosome segments and h^2 is the heritability. Assuming M = 50,000 SNP markers, Ne is effective population size = 150, L is average chromosome length of 1 Morgan and k is the number of chromosomes = 27, such that the effective number of chromosome segments was 8,100, calculated as Me = 2NeLk. The heritabilities used were from the genetic parameters estimates from the univariate analyses. The number of animals tested was based on measurements to be made during this project (10,000 animals), if historic data can be included (17,000 animals), if at least 2,500 animals are measured every year after the conclusion of this project (29,500, total animals after 5 years), and the previous scenarios repeated if an additional 40% more animals could be recorded with time saved with short measurement durations (14,000 with current project, 21,000 with historic added, 38,500 with future measurements).

Results and discussion

Phenotypic comparison

The relationship between short measurements (20, and 25 minutes) and long measurements (40 and 50 minutes) was very strong for both Eagle and FID devices. (Figure 1). This is an indication that the methane production during the time in the PAC is relatively constant. It also suggests the duration of measurement in the PAC could potentially be decreased. Historic projects used a range of measurement durations, these results suggest that the current and historic datasets could be combined regardless of the measurement duration used.

The relationship between measurement devices (Eagle and FID) was also very strong for both the short measurement and long measurement durations (Figure 2). The reason for measuring with both devices in this project, was to ensure that the Eagle

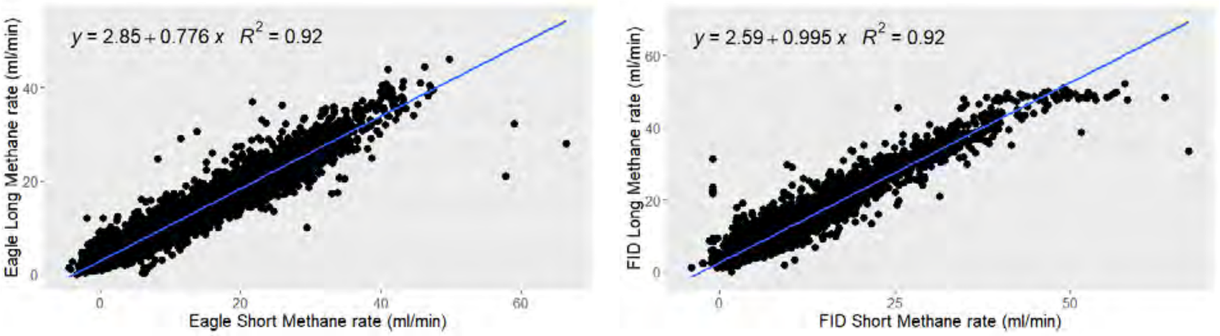

Figure 1. Relationship between methane rate (ml/min) measured with a short duration (mid-point 20 or 25 minutes) and long duration (end-point 40 or 50 minutes) with either the Eagle (Left) or FID (Right).

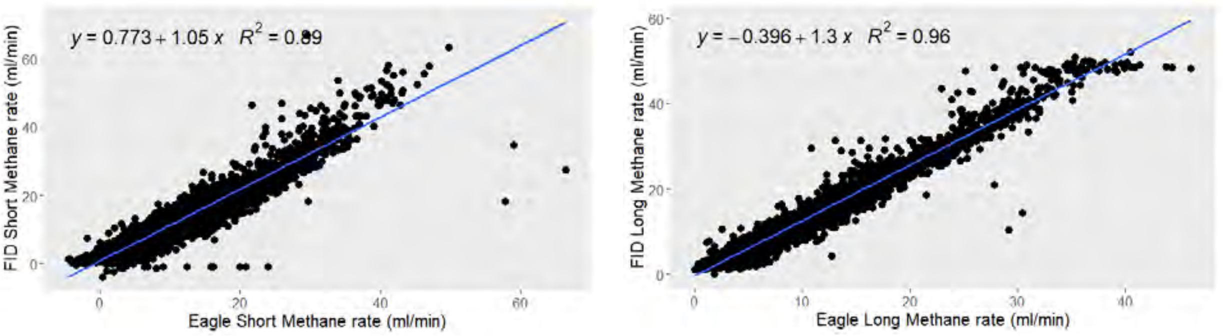

Figure 2. Relationship between methane rate (ml/min) measured with either Eagle or FID for a short duration (20 or 25 minutes) (Left) and long duration (40 or 50 minutes) (Right).

device was measuring the same trait as the historic data which used the FID. These results support that both devices are measuring the same trait. Only measuring with one of the devices for the remainder of the project could simplify the protocol, reduce labour, and consumable costs.

Genetic parameters

The variance components and estimates of heritability (0.15 to 0.19) were not significantly different regardless of measurement duration or measurement device (Table 1). The Eagle device tended to have lower estimates of heritability, the shorter measurement durations also tended to have lower estimates of heritability. This could be due to the lower sensitivity of the Eagle device, and the higher precision of the FID capturing more variation between animals, similarly the longer measurement duration allows for more variation to be captured. The genetic correlation between measurement durations was not different from one, with phenotypic correlations of 0.80 ± 0.01 (Eagle) and 0.82 ± 0.01 (FID). This indicates that the measurement durations are genetically the same trait. Furthermore, the genetic correlation between Eagle and FID was 0.96 ± 0.02 for the short duration and not different to one for the longer durations, indicating that the two devices are also measuring the same trait. This suggests that the protocol could be simplified by only measuring with the Eagle. This also implies that datasets with measurements with different devices or with different measurement durations could be combined in a single genetic evaluation. This provides the confidence that the historic data only measured with the FID is measuring the same trait as more recent projects that use the Eagle, and that future projects only need to use the Eagle. Further investigation which includes both recent and historic datasets is needed.

Table 1. Parameter estimates (σ^2_e, residual variance; σ^2_a, , additive variance; h^2, heritability) for methane traits recorded with the Eagle and FID.

Trait (ml/min)	N	$\bar{x}$ ± SD[1]	σ^2_e	σ^2_a	h^2
Eagle Short	3,729	0.00 ± 1.00	0.43	0.08	0.15 ± 0.04
FID Short	3,729	0.00 ± 1.00	0.44	0.09	0.18 ± 0.04
Eagle Long	3,729	0.00 ± 1.00	0.39	0.08	0.17 ± 0.04
FID Long	3,729	0.00 ± 1.00	0.40	0.09	0.19 ± 0.04

[1] Each site was centred to the mean and standardised by standard deviation.

Power calculations

If higher heritabilities with more accurate devices or longer recording periods are realised, higher accuracies of genomic prediction would be achieved by measuring more animals but with shorter measurement durations (Table 2). Assuming the protocol continues to measure with FID and the heritability is higher (0.19), an accuracy of genomic prediction of 0.44 would be achieved at the completion of this project (10,000 animals measured). However, if only the Eagle is used and a lower heritability of 0.17 is realised, the accuracy of genomic prediction will also be slightly lower at 0.42, and with the shorter measurement duration the accuracy would be 0.40 due to the lower heritability of 0.15. If the extra time from measuring for only 20 minutes was used to measure 40% more animals (14,000) the accuracy of prediction would be higher at 0.45. The current estimates of heritability are not significantly different, if we assumed the Eagle long and Eagle short both had a heritability of 0.17, the accuracy of genomic prediction is further increased for Eagle short to 0.48. This trend continues if historic data is added or with expected measurement goals in the future.

While the shorter protocols allow for more animals to be measured and to increase the accuracy of genomic prediction, it does not consider the logistical issues that come with phenotyping more animals. As it is not possible to retrospectively measure for longer durations it is recommended that the current protocol not be changed. However, labour is a key limiting factor and the shorter measurement duration would significantly reduce these costs per animal, if the extra time is not used to measure additional animals.

Table 2. Accuracy of genomic prediction using different protocols of recording.

Device	h^2	Duration	Number of animals	Accuracy of prediction
FID	0.19	40min	10,000	0.44
FID	0.19	40min	17,000	0.53
FID	0.18	20min	10,000	0.43
FID	0.18	20min	17,000	0.52
Eagle	0.17	40min	10,000	0.42
Eagle	0.17	40min	17,000	0.51
Eagle	0.17	40min	29,500	0.62
Eagle	0.15	20min	10,000	0.40
Eagle	0.15	20min	14,000	0.45
Eagle	0.15	20min	21,000	0.53
Eagle	0.15	20min	38,500	0.65
Eagle	0.17	20min	14,000	0.48
Eagle	0.17	20min	21,000	0.55
Eagle	0.17	20min	38,500	0.67

Conclusion

The largest challenge for prediction of breeding values for methane based on genomic testing is the phenotyping of enough animals to form a reference population. We demonstrated that measurement with the Eagle is sufficiently accurate to replace the FID and FoxBox devices. The amount of time each animal is in the portable accumulation chamber can be shortened to about 20 min without losing measurement accuracy and could be considered to allow phenotyping of additional animals.

Acknowledgments

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Greenhouse gas emission intensity of milk production in three Slovenian sheep breeds

M. Bizjak and A. Cividini

University of Ljubljana, Biotechnical faculty, Department of animal science,
Groblje 3, 1230 Domžale, Slovenia
Corresponding Author: Marko.bizjak@bf.uni-lj.si

Abstract

The aim of the study was to determine the intensity of greenhouse gas (GHG) emissions from the milk production of three sheep breeds in Slovenia, to identify the trends and to determine the main impacts on greenhouse gas emissions. Based on information on milk yield, protein, fat and lactose content, average body mass of each breed, litter size, and lambing interval, we estimated methane and nitrous oxide emissions for the period 2010-2022. Emissions were estimated for 21,655 lactations. GHG emissions were expressed in carbon dioxide equivalents. Emission intensity was expressed as emissions per kg of milk produced. Enteric methane contributed to almost 92% of the total GHG emissions. Methane from manure stores contributed about 2% to total GHG while the total contribution of nitrous oxide was about 6%.

The differences in the intensity of GHG emissions among sheep were fivefold, ranging from about 0.7 to more than 3.6 kg of CO_2 equivalent per kg of milk. On average, the emission intensity expressed in kg CO2 equivalent per kg milk was 1.555 for Bovec sheep, 1.379 for Improved Bovec sheep, and 2.026 for Istrian Pramenka. The intensity of GHG emissions decreased between the first parity (1.682 kg CO_2 equivalent per kg milk) and the fourth parity (1.534 kg CO_2 equivalent per kg milk) and then gradually increased until the tenth parity (1.714 kg CO_2 equivalent per kg milk). The emission intensity decreased with increasing litter size. The average emission intensity, expressed in kg CO_2 equivalent per kg milk, was 1.639 for sheep delivering single lambs, 1.442 for sheep delivering twins, and 1.241 for sheep delivering triplets.

The emission intensity increased with increasing lambing interval from 1.430 kg CO_2 equivalent per kg milk for sheep with a lambing interval between 280 to 314 days to 1.769 kg CO_2 equivalent per kg milk for sheep with lambing interval between 416 to 450 days. This means that the total milk yield of sheep with a longer lambing interval, was not high enough to compensate for the higher maintenance requirements of these sheep. The intensity of GHG emissions from milk production in flocks with controlled sheep varied over the years (ranging from 1.522 kg CO_2 equivalent per kg of milk in year 2021 to 1.657 kg of CO_2 equivalent per kg of milk in years 2013 and 2014). Overall, the intensity of GHG emissions decreased by around 6% during the study period. To summarise, some fertility traits are correlated with milk production and consequently also with the intensity of GHG emissions. In particular, a relatively short lambing interval could reduce the intensity of GHG emissions by increasing daily milk production.

Keywords: milk yield, litter size, parity, lambing interval, trends.
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Introduction

Livestock production contributes to anthropogenic (human) greenhouse gas (GHG) emissions. Globally, small ruminant production contributes 6.5% of all emissions in livestock production (Opio *et al*., 2013). Major greenhouse gases produced by small ruminants are methane (CH_4) and nitrous oxide (N_2O). While methane is produced both by fermentation in the digestive tract (enteric methane) and by manure management, the main source of emissions of nitrous oxide in small ruminant production is the manure management (Opio *et al*., 2013). In 2023, a study about GHG emissions from milk production of three Slovenian goat breeds was made. Still, no study about GHG emissions in sheep production has been prepared in Slovenia. The present study was conducted to determine the intensity of GHG emissions from milk production of three sheep breeds in Slovenia (Bovec sheep, Improved Bovec sheep and Istrian Pramenka), to identify the trends, and to determine the main impacts on the GHG emissions.

Material and methods

Records were provided by the Slovenian breeding programs for dairy sheep collected from the year 2010 to 2022. Data about animal breed, flock, lambing date, litter size, parity, date of the end of lactation and records of milk recording were acquired from the Central Database for Small Ruminants in Slovenia. GHG emissions were estimated on an annual basis. Methane emissions from enteric fermentation were calculated based on the net energy requirements (IPCC, 2019). The sum of net energy for maintenance, activity, lactation, pregnancy and wool growth was considered for the estimation of gross energy intake. Methane emissions from manure management were estimated based on the amount of daily volatile solid excreted (VS), maximum methane producing capacity for manure produced (B_o) and methane conversion factors for each manure management system (MCF). The estimation of direct and indirect nitrous oxide emissions based on the assumption that each sheep excretes 15.5 kg of nitrogen per year (EMEP, 2019) and was used only to estimate full GHG emissions in dairy sheep. Greenhouse effect of methane and nitrous oxide emissions were expressed in carbon dioxide equivalents (CO_2 eq). To calculate GHG emissions expressed in CO_2 eq, methane emissions were multiplied by GWP_{100} factor 28 while nitrous oxide emissions were multiplied by GWP_{100} factor 265. GHG emission intensity was calculated as the ratio of the quantity of GHG emissions and total milk yield.

Results and discussion

The intensity of GHG emissions by sources is presented in table 1. Enteric methane contributed almost 92% to the total GHG emissions. Total contribution of nitrous oxide was around 6%, while the methane from manure stores contributed around 2% to total GHG. These findings are confirmed by Gerber *et al*. (2013) who reported that emissions from manure were very low because excretes of small ruminants are mainly deposited

Table 1. Intensity of greenhouse gas (GHG) emissions by sources

		Mean	S.D.	Minimum	Maximum
GHG emission intensity (kg CO_2 eq/kg milk)	Enteric methane	1.457	0.383	0.655	3.302
	Methane from manure stores	0.027	0.007	0.012	0.062
	Nitrous oxide from manure stores	0.090	0.032	0.025	0.253
	Indirect nitrous oxide	0.013	0.004	0.003	0.036
	Total	1.587	0.426	0.695	3.636

on the pasture. The differences in the intensity of GHG emissions among sheep were fivefold, ranging from about 0.7 to more than 3.6 kg of CO_2 equivalent per kg of milk.

The intensity of GHG emissions by sheep breed is presented in table 2. The intensity of GHG emissions was the highest in the Istrian Pramenka (2.026 kg CO_2 eq/kg milk) and the lowest in Improved Bovec sheep (1.379 kg CO_2 eq/kg milk) while the intensity of GHG emissions was slightly higher in Bovec sheep (1.555 kg CO_2 eq/kg milk). The relatively high intensity of GHG emissions in Istrian Pramenka is due to low total milk yield as well as high dry matter content of the milk compared to the other two breeds.

Table 3 shows the intensity of GHG emissions by litter size. The emission intensity was the lowest in sheep delivering triplets (1.241 kg CO_2 eq/kg milk) and the highest in sheep delivering single lambs (1.639 kg CO_2 eq/kg milk) which is a consequence of increasing total milk yield with increased litter size.

The intensity of GHG emissions by parity is shown in figure 1. The emission intensity decreased between the first parity (1.682 kg CO_2 eq/kg milk) and the fourth parity

Table 2. Intensity of greenhouse gas (GHG) emissions by the sheep breed.

	Breed		
	Bovec sheep	**Improved Bovec sheep**	**Istrian Pramenka**
GHG emission intensity (kg CO_2 eq/kg milk)	1.555	1.379	2.026

Table 3. Intensity of greenhouse gas (GHG) emissions by the litter size.

	Litter size		
	Single lambs	**Twins**	**Triplets**
GHG emission intensity (kg CO_2 eq/kg milk)	1.639	1.442	1.241

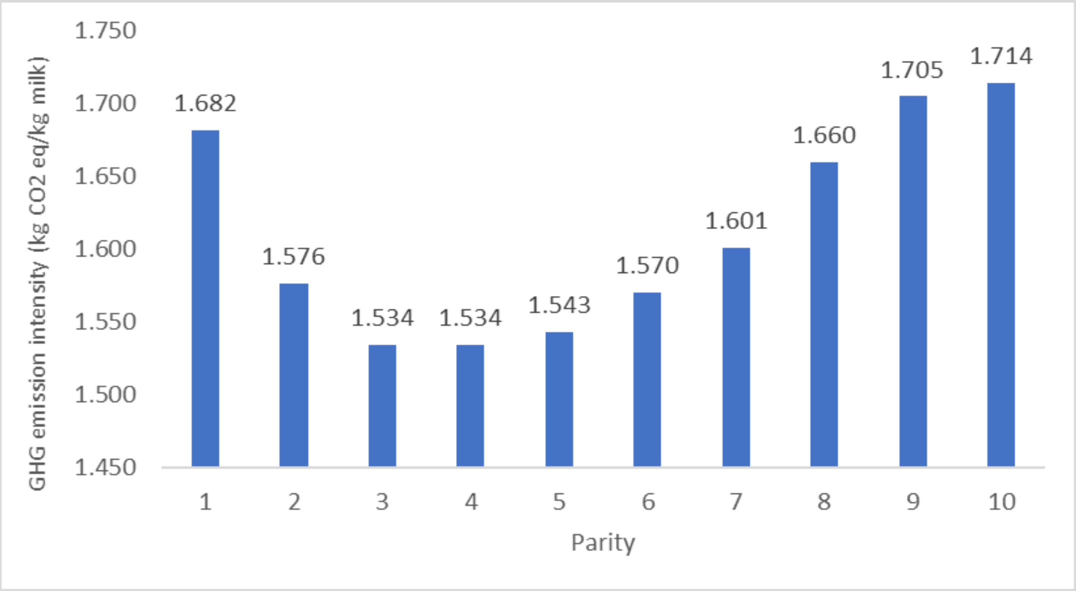

Figure 1. Intensity of greenhouse gas emissions by parity.

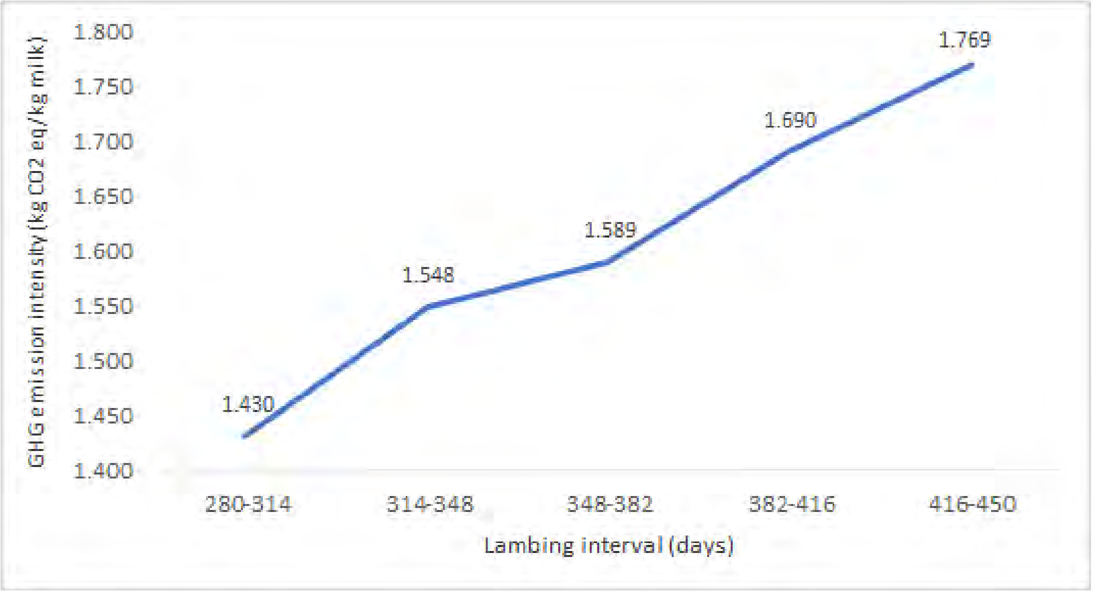

Figure 2. Intensity of greenhouse gas emissions related to lambing interval.

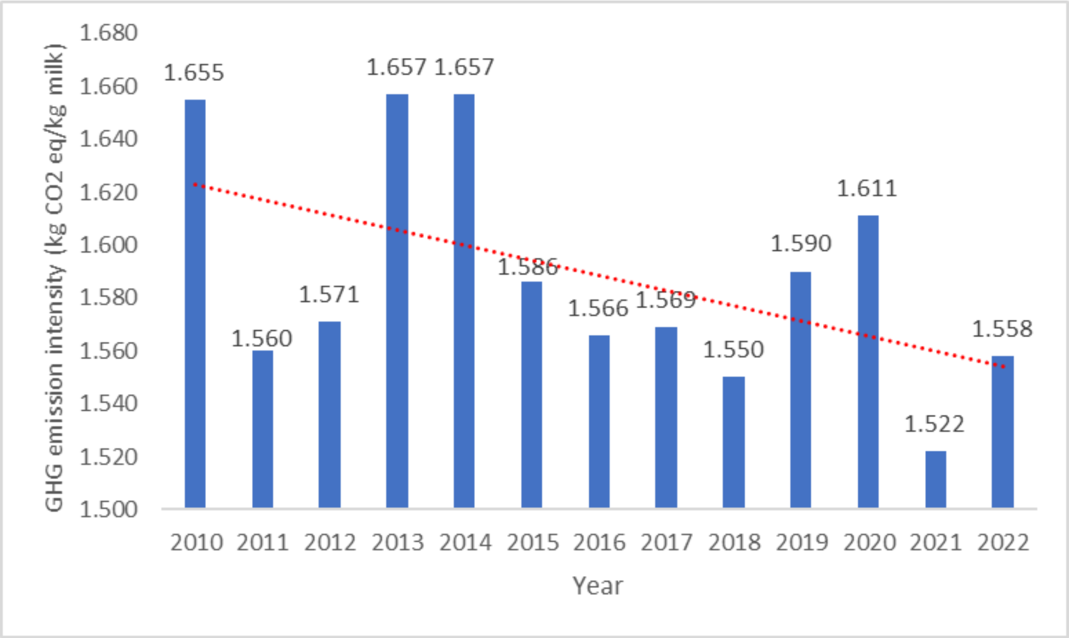

Figure 3. Intensity of greenhouse gas emissions by the year.

(1.534 kg CO_2 eq/kg milk) and then gradually increased until the tenth parity (1.714 kg CO_2 eq/kg milk).

Figure 2 shows the intensity of GHG emissions related to lambing interval of sheep. It could be noticed that the intensity of GHG emissions increased with increasing lambing interval, from 1,430 CO_2 eq/kg milk in ewes with lambing interval of 280-314 days to 1,769 CO_2 eq/kg milk in ewes with lambing interval of 416-450 days. These results indicate that total milk yield of sheep with extended lambing interval was not high enough to compensate the higher maintenance requirements of these sheep.

GHG emission intensity trends in the period 2010-2022 are presented in figure 3. The intensity of GHG emissions from milk production in flocks with sheep in breeding programs varied over the years (ranging from 1.657 CO_2 eq per kg of milk in years 2013 and 2014 to 1.522 kg CO_2 eq per kg of milk in the year 2021), but decreased overall by 5.9 % during the study period.

Conclusions

We conclude that the selection for high milk production of dairy sheep could be a useful tool to reduce the intensity of GHG emissions. Furthermore, some fertility traits such as lambing interval, parity, and litter size are correlated with milk production and consequently with the intensity of GHG emissions as well. In particular, relatively short lambing interval could significantly reduce the intensity of GHG emissions.

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Methane phenotyping using different techniques and estimates of parameters for the Nordic Red cattle in Finland

E. Negussie, A.R. Bayat, T. Stefanski, A. Chegini, and M. H. Lidauer

Natural Resources Institute (Luke), 31600 Jokioinen, Finland
Corresponding Author: enyew.negussie@luke.fi

Abstract

The aim of this paper is to compare the performances of different methane (CH_4) measurement techniques, to estimate some genetic parameters and highlight CH_4 phenotyping methods used in the Nordic Red cattle (RDC) in Finland. Data were from CH_4 measurements of RDC cows in Jokioinen dairy research farm of the Natural Resources Institute Finland (Luke). Three CH_4 measurement techniques were used and in total 32, 137 and 310 cows had daily average CH_4 measurements from cattle respiration chamber (RC), GreenFeed (GF) and F10 multi gas analyser (F10 sniffer, Gasera Ltd, Turku, Finland), respectively.

For comparison among techniques, data from simultaneous CH4 measurements by any two techniques and Lin's concordance analyses were used for comparative assessment. Estimates of genetic correlations from repeatability animal models were used to assess the association of CH_4 phenotypes with some production and functional traits included in the dairy cattle breeding goals. Methane phenotypes: CH_4 production (MeP=CH_4 g/day), CH_4 yield (MeY= g CH_4/kg DMI), CH_4 intensity (MeI=g CH_4/kg ECM) and residual CH_4 production (RMP) were considered. Production traits: energy corrected milk (ECM), metabolic body weight (mBW), residual feed intake (RFI) and dry matter intake (DMI) were included in the analyses.

The mean MeP, MeY and MeI from RC were 453.0±55, 21.3±1.4, 17.1±1.6 whilst from GF were 467.1±61, 21.6±1.5 and 14.8±1.8, respectively. Corresponding means from the F10 technique were 400.1±32.7, 20.6±4.3 and 13.9±3.5. The Lin's concordance correlation coefficient for MeP between the RC and GF techniques were 0.70. Whereas the 95% confidence interval of the Lin's concordance correlation coefficient for MeP between the RC and F10 techniques ranged from 0.40 to 0.85. Heritabilities using data from F10 measurement for traits: MeP, MeY, MeI and RMP were 0.04, 0.04, 0.08, and 0.16, respectively. Genetic correlations between MeP and production traits: ECM, mBW, RFI and DMI were moderate to high positive with 0.42, 0.67, 0.48 and 0.49, respectively.

Some disparities in the estimates of CH_4 phenotypes from different techniques were observed. In view of the scarcity of individual animal CH_4 data, to make effective use of every available CH_4 measurements in livestock, methods and tools should be developed for integrating records from different techniques into standardised and harmonised set.

Keywords: methane, dairy cattle, measurement methods, heritability, correlations, concordance analyses.
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Introduction

Attempts to lower the environmental footprint of milk production needs a sound understanding of the basis of CH_4 emissions from the dairy production systems. Accurate and reliable CH_4 measurements are therefore important not only for understanding of the basis of livestock system CH_4 emissions, but also for national inventory and to identify potential mitigation strategies. However, the accurate measurement of CH_4 emissions particularly from individual animals is difficult and expensive (Pickering *et al*., 2015, Negussie *et al*., 2017). As a result, so far, routine measurements CH_4 and large-scale recordings are rare. One of the main reasons for this has been the lack of accurate, low-cost, portable, and non-invasive methods that are also suitable for application on commercial farms. Lately, with the advances in digital and sensor technologies there has been a gradual rise in new and advanced applications for CH_4 measurement. There is, therefore, a need to understand the comparative performances of the different techniques and the associated challenges and opportunities to develop suitable CH_4 phenotyping strategies for management, targeted nutritional studies or genetic selection. The aim of this paper is to compare the performances of few of the widely used CH_4 measurement techniques and to estimate some genetic parameters for CH_4 output traits in the Nordic Red cattle (RDC) in Finland.

Material and methods

To evaluate the performances of GF in measuring CH_4 emission compared to respiration chambers, thirty-two lactating Nordic Red cows (RDC) were used. Measurements of CH_4 emission were done using two GF units and cattle respiration chambers (n = 4) in a complete block design (8 blocks). The experimental period for every block lasted for 5 weeks; in the first 2 weeks the cows were measured in GF units, on the 3rd week cows were measured in the chambers and on the 4th and 5th weeks they were measured again in the GF units.

The cows were fed a grass silage-based diets with 55:45 forage to concentrate ratio. The data collected from the study was edited in that GF records above or below 2.5 × SD of all measurements for every cow over 4 weeks period were considered as outliers and were deleted resulting in removal of 217. In a similar but separate study, F10 multigas analyser (F10, sniffer) was compared to respiration chambers (n = 4) using twenty-one first lactation RDC cows to assess the agreement between the two techniques. Here CH_4 measurements on cows were taken in a three-weeks sequence, where a one-week F10 measurements was followed by another one-week CH_4 measurements in the chamber which was then followed by a one-week F10 measurements.

Data on CH4 measurements of twenty-one cows from the two techniques were made available for analysis. In both comparative assessments of CH_4 measurement techniques: the GF *versus* respiration chamber and F10 sniffer *versus* respiration chambers, the agreement between the techniques was assessed using the Lin's concordance correlation analyses (Lin, 1989). Data on CH_4 measured from a relatively large number of RDC cows using the F10 technique was then used to estimate genetic parameters for the different CH_4 output traits and to assess their genetic and phenotypic associations with some production traits.

Data was from 309 RDC cows including 13,573 weekly average records. Production traits were energy corrected milk (ECM), metabolic body weight (mBW), residual feed intake (RFI) and dry matter intake (DMI). Methane phenotypes included were: CH_4 production (MeP=CH_4 g/day), CH_4 yield (MeY = g CH_4/kg DMI), CH_4 intensity (MeI= g CH_4/kg ECM) and residual CH_4 production (RMP). Univariate and bivariate repeatability animal models were used for the estimation of heritabilities and genetic correlations between traits, respectively. The models included fixed effects of age, feeding kiosk number, lactation week, measurement year-month and random permanent environment and animal effect.

Results and discussion

Comparison of CH_4 measurement techniques

Respiration chambers are calibrated to be accurate and precise, and are the gold standard for benchmarking new methods (Garnsworthy *et al.*, 2019). Where an alternative method may be cheaper, less invasive, easier to implement, or have a wider scope of applications, it is of value to assess their relative accuracy and agreement with the gold standard. In actual comparison between methods simultaneous repeated measures per cow with two or more techniques are required in order to establish agreement between the techniques. In such comparisons, it is also important to have short time interval between repeated measures per subject to ensure that the underlying biology of the cow has not changed (Garnsworthy *et al.*, 2019).

The main difficulty faced in most comparisons involving CH_4 measurement techniques is that not all techniques can be recorded simultaneously on the same individual and the methane emission of cows changes both throughout the day and over the lactation period. In such cases, either cross-over designs are needed, or else matched-pair repeated measures designs. In both cases, the Lin's concordance correlation analysis (Lin, 1989) is useful to validate and establish agreement between any two methods.

Lin's concordance analysis computes agreement on a continuous measure obtained by two methods. It is widely used in validation studies because of its ability to combine measures of both precision and accuracy to determine how far the observed data deviate from the line of perfect concordance. In this study, the overall average daily CH_4 emission using GF units was 467 ± 61.4, g/d and was 453 ± 55 g/d for respiratory chambers. For CH_4 production, the Lin's concordance correlation coefficients between the GF and chamber was 0.68.

On the other hand, in the F10 sniffer *versus* chamber comparison, the concordance correlation coefficient for the first week before chamber F10 measurements was 0.70 with 95% lower and upper confidence limits of 0.41 and 0.85, respectively. Whereas the concordance correlation coefficient for the third week after chamber F10 CH_4 measurements was 0.69 with corresponding lower and upper confidence limits of 0.37 and 0.86, respectively.

The concordance correlation coefficient for combined before and after chamber F10 measurements was 0.84 with the 95% lower and upper confidence limits of 0.65 and 0.93, respectively. Here the indications are that when the week before and week after chamber F10 CH_4 measurements were combined, the agreement between the methods has markedly improved. The result shows that a combined weekly mean F10 measurements taken in a week interval can provide a much closer prediction of the respiration chamber measurements.

Comparing different CH_4 measurement methods, Garnsworthy *et al.*, (2019) reported that for the methods with repeated measures per cow, the mass flux-based methods had the highest repeated measures correlations which outperformed the concentration-based methods. They have reported a concordance correlation coefficient of 0.87 and 0.81 for comparison between SF6 *versus* chamber and GF *versus* chamber which is close to the results obtained in this study.

Estimates of genetic parameters

Genetic selection provides a reliable route towards permanent and cumulative reductions in quantitative traits such as enteric CH_4 emissions. This requires estimation the amount of available genetic variations for the CH_4 traits and their genetic associations with other dairy breeding goal traits. Unfortunately, such estimates particularly for dairy cattle are rare in literature. In this study, the mean MeP, MeY and MeI from the F10 technique CH_4 measurements were 400.1±32.7 g/d, 20.6±4.3 g/kg, 13.9±3.5 g/kg. The estimates of heritability for the CH_4 output traits: MeP, MeY, MeI and RMP were 0.04, 0.04, 0.08, and 0.16, respectively.

The genetic correlations between MeP and production traits: ECM, mBW, RFI and DMI were moderate to high positive with 0.52, 0.67, 0.48 and 0.49, respectively. The estimated genetic associations among the traits ranged from moderate to high and are in line with literature estimates. Analysing a combined dairy cows data from four countries Manzanilla-Pech *et al*. (2021) reported heritability (SE) for MeP of 0.21 (0.04), and heritabilities of 0.30 and 0.38, respectively for the MeY and MeI, respectively. Difford *et al*. (2020) on the other hand reported 0.26 for CH4 concentration (in ppm) for Danish Holstein, whereas Breider *et al*. (2018) reported 0.33 for MeP using SF6 in Australian Holstein.

Compared to these literature estimates, our estimates of heritability for MeP is slightly lower than the reported estimates for Holstein cattle. However, our estimate is in line with an earlier estimate obtained on part of the same data. Any disparity between the present estimates and above cited literature reports could be in part due to the methods of CH_4 measurement, data size, the population under consideration and the model used for the evaluation.

Conclusion

In general, results from comparing the performances of different methane measurement methods have shown some differences. The main question is if we measure CH_4 by sniffer or by GF or respiration chamber are they the same phenotype. The answer for this is clear and particularly in analyses involving animal evaluations, efforts should be made, and methods should be developed to standardize and harmonize CH_4 measurements coming from the different methods. This enables us to make effective use of the small, scattered, rare and unique CH_4 data sets for accurate estimation of the genetic merit of animals and planning mitigation strategies.

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Selection of cows at risk of subclinical ketosis as part of the milk recording of dairy cattle in Poland in 2014-2022

J. Kwaśniewska

Polish Federation of Cattle Breeders and Dairy Farmers

Abstract

In 2013, in Polish Federation of Cattle Breeders and Dairy Farmers, the new service was introduced - selecting cows which are in a state of subclinical ketosis (SK). This method is based on checking beta-hydroxybutyrate and acetone levels in milk sample, taken during test day. Those results constitute components for a logistic regression model, which indicates the probability of ketosis incidence. Above specific threshold, cow is marked on reports delivered to farmer. During period of 10 years, the incidence of SK in cows decreased with each subsequent year. The incidence of SK in cows is influenced, among other things, by milk yield in the herd and the size of the herd, as well as the housing system. The lower the herd efficiency and the smaller the herd size, the higher the incidence of SK. The results indicate a constant improvement in the health situation of recorded cows in Poland, in the context of the frequency of subclinical ketosis. This is due to breeders expanding their knowledge about nutrition, prevention and good practices related to the keeping of dairy cows, as well as greater awareness of the negative effects of metabolic diseases.

Materials and methods

The data for analysis was downloaded from the Fedinfo database, belonging to the PFCBDF, in which the results of test milkings carried out as a part of milk recording are collected and processed. Set 1 contained data on milk samples taken from cows of various breeds, mainly the Holstein-Friesian breed, from 2014-2022. Set 2 contained herd data (average number of cows, average yield and location). Cows marked with K! index in Fedinfo were probably in a state of ketosis on test day. Sensitivity and specificity of the method are 0.7 and 0.9, respectively (7).

Based on the K! index prevalence in the herd, as well as the herd size, the probable prevalence of SCK in the herd is calculated. If it exceeds 10%, herd is at risk of SCK. If this indicator exceeds 20%, the herd is considered to be at high risk of SCK. Data analysis and graphs were performed using the R language (https://www.r-project.org/) and the RStudio program. To check the relationship between the health status of a cow and the size and average milk yield of the herd, the chi-square test and measures based on this test were used, including the V-Cramer and the contingency coefficients.

Results

The share of primiparous cows was 33%, cows in the second lactation 26%, and in the third and beyond - 41%. The animals were on average in 31.7 DIM, with a daily milk yield of 31.97 kg (Table 1).

Table 1. The share of primiparous cows

Variable	n	Average	Median	Minimum	Maximum	SD	CV
Number of samples							
All	10 631 322						
Primiparous cows	3 554 722						
2nd lactation cows	2 761 429						
From cows in 3rd and further lactation	4 315 171						
Parity		2,54	2	1	22	1,7	65,9
Days in milk		31,7	32	5	60	16,3	51,6
Daily milk yield, kg		31,97	31,0	1	107	9,6	29,9
Number of records containing farm data	1 651 657						
Number of cows in herd		42,53	30	1	1546	56,6	133,1
Average milk yield on test day/per whole herd, kg		20,33	20,3	0,2	61,3	5,4	26,5
Average milk yield on test day/milked cows, kg		24,30	23,4	2,3	61,9	5,7	23,4

The median for the parity was 2. The average number of animals per herd was 43 (from 1 to 1546), and the average milk yield of cows on test day was 24.3 kg. Since the introduction of SCK monitoring in 2013, the prevalence (cows with the K! index) in the recorded population has decreased. However, there is a slight increase in this frequency in 2022. In all parity groups, there has been a continued tendency to reduce the share of the K! cows. Within the ≥ 3rd lactation cows, the share of such cows was higher than in younger cows (Figure 1).

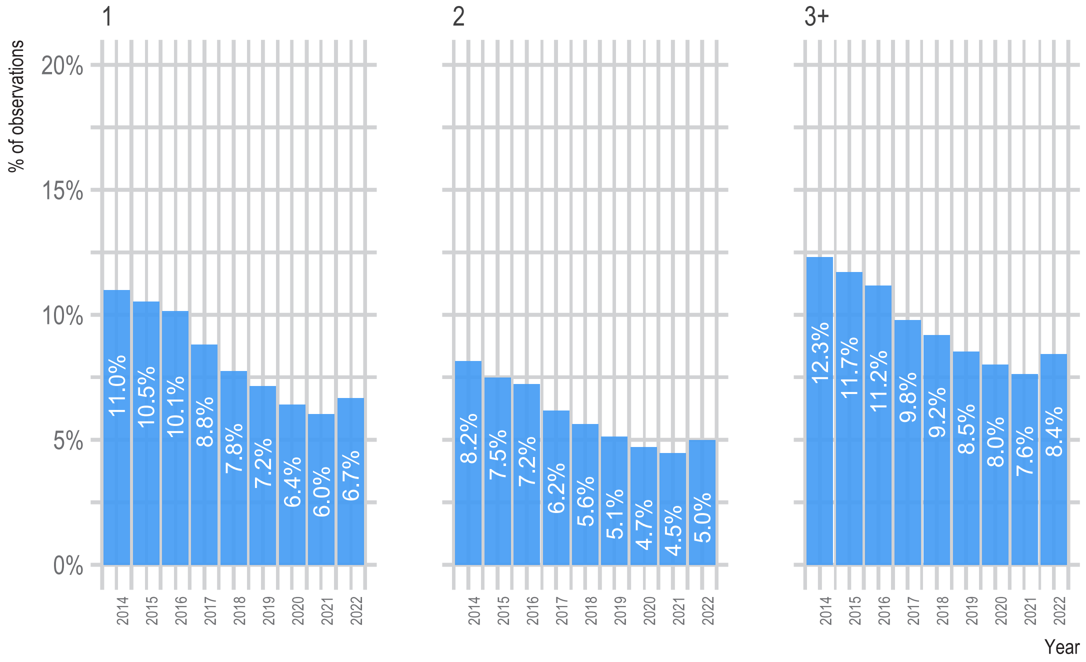

Figure 1.Tthe share of 3rd lactation cows compared to younger cows.

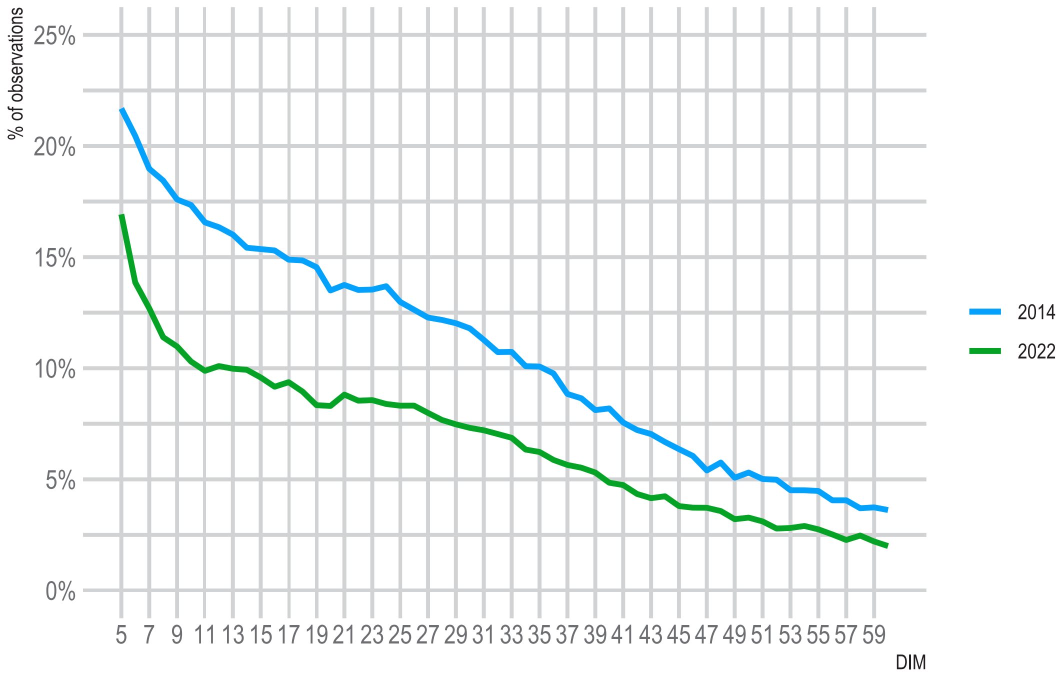

Figure 2. Percentage of cows suspected of being in the SCK state.

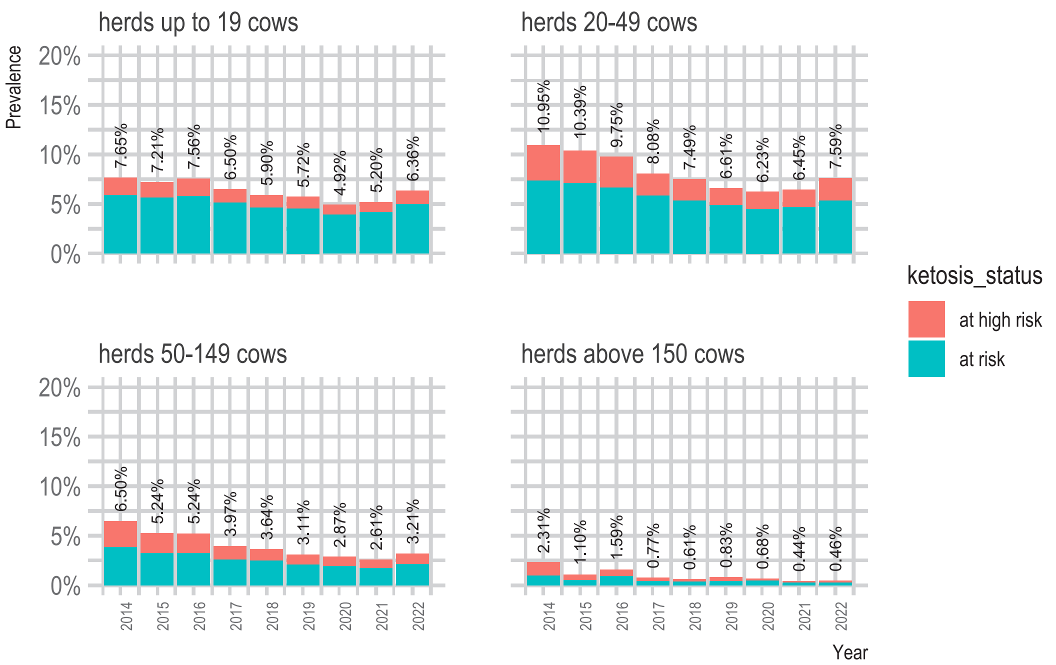

Figure 3. Risk of SCK by herd-size classes.

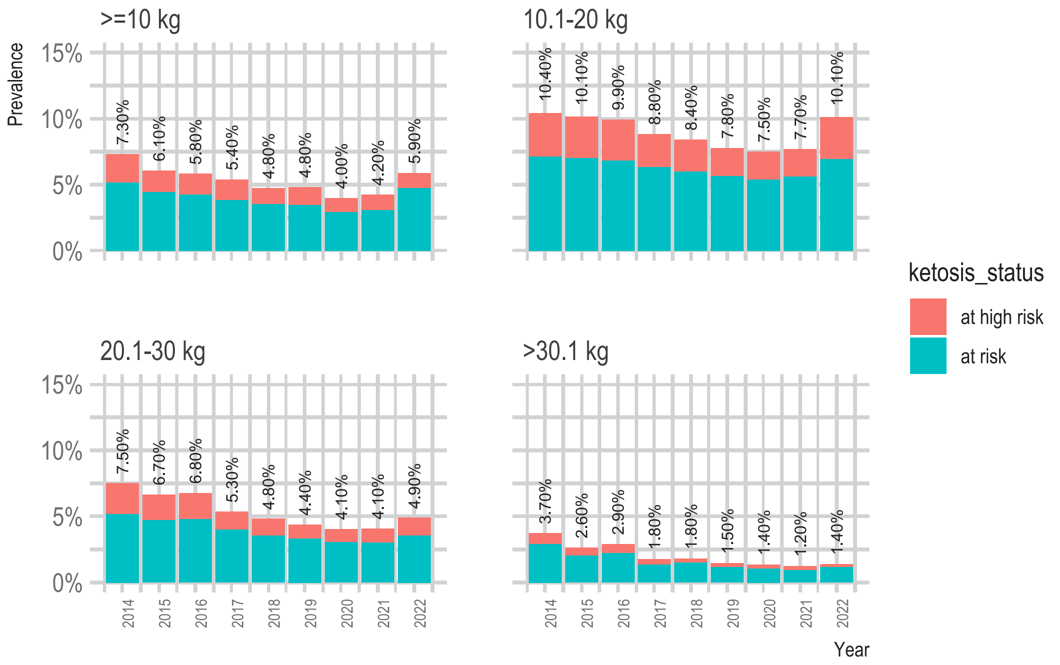

Figure 4. Risk of SCK by milk production classes.

In 2022, the number of the K! cows between 5 and 7 DIM decreased faster than in the corresponding group in 2014. In 2014 19.6% of cows between 5 and 7 DIM were suspected of being in the SCK state (in 2022 13.6% cows; Figure 2). The K! cows were the most frequent among the lowest producing cows, i.e. ≤ 10 kg/d. As the daily milk yield increased, the prevalence of SCK decreased and the relationship was significant, although very weak (p-value = 0.0005, Cramer's V coefficient = 0.143, contingency coefficient = 0.142). The share of herds at risk or high risk of ketosis has decreased from year to year. Herds with 20 to 49 cows were at the highest risk of SCK, and the relationship was significant, although very weak (p-value = 0.0004998, V-Cramer coefficient = 0.045, contingency coefficient = 0.078). SCK occurred least frequently in herds with over 150 animals (Figure 3).

SCK most often occurred in herds with an average daily milk yield 10.1-20 kg and 20.1-30 kg, while the most productive herds were characterized by a low share of the K! cows (Figure 4). The ketosis status of herd and yield level were significantly, although very weakly, correlated (p-value = 0.0005, V-Cramer coefficient = 0.05, contingency coefficient = 0.088).

Discussion and conclusions

Compared to the average prevalence of SCK recorded in dairy cow populations in 10 European countries, which was 11.2-36.6% (9), the prevalence of SCK in the Polish population was much lower. The increase in SCK prevalence in 2022 can probably be associated with rising inflation and prices of products and services, which could translate into lower standards of veterinary care, the quality of cow nutrition or the use of consultancy. Lower prevalence, than in the other studies (1,8,9) may result from different diagnosis models. Our model is very conservative and therefore selects significantly fewer cows in the SCK status. SCK most often occurred in older cows, which is consistent with the results of previous studies (3,5). The fact that SCK was much more common in herds with a daily milk yield of 10.1-20 and 20.1-30 kg is surprising, since other studies have shown that the occurrence of ketosis increases with increasing milk yield (2,4). The presented population data, based on a large amount of data, indicate that SCK is not a metabolic disorder associated with a high cow performance, but a disorder associated with poor cow welfare, including poor nutritional standards (6). In the herds with the largest number of animals, SCK occurred less frequently, which could be related to the fact that large farms more often use the services of nutritional consultants, had the opportunity to divide animals into production groups, and consequently adjust feed rations to the needs of cows in particular stages of lactation. The above results indicate a constant improvement in the health situation of recorded cows in Poland. This is due to breeders expanding their knowledge of nutrition, prevention and good practices related to the maintenance of dairy cows, as well as greater awareness of the negative effects of metabolic diseases. Introduction of this type of service contributed to the gradual reduction of the prevalence of ketosis.

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Carcass traits of Holstein, Simmental and Brown Swiss calves, bulls and heifers and their crossbreeds with Charolais, Limousin, and Belgian Blue in Slovenia

M. Voljč, M. Klopčič and S. Žgur

*University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3,
1230 Domžale, Slovenia*

Abstract

The effects of crossing Holstein (HOL), Simmental (SIM) and Brown Swiss (BS) cows with Charolais (CHA), Limousin (LIM) and Belgian Blue (BB) sires on growth and carcass traits were evaluated. The crossbred animals were compared with purebred HOL, SIM and BS animals. A total of 174,365 calves, 397,962 bulls younger than 24 months and 160,731 heifers slaughtered in Slovenian abattoirs between 2009 and 2022 were included in the comparison. Carcass weight was determined within 45 minutes of slaughter. Net daily gain was calculated on the basis of carcass weight and age at slaughter. The conformation and fatness of the carcass were evaluated according to the EUROP classification system and divided into 15 subclasses. The most popular breed for crossbreeding was LIM, as their crossbreeds in all three categories of slaughtered cattle represented 72 %, crossbreeds with BBP 18 % and with CHA 10 % of all crossbreeds. The CHA breed increased carcass weight the most (by an average 10 % in all three categories of slaughtered cattle, followed by BBP with 8 % and LIM with 6 %). The CHA breed increased carcass weight by 10 kg for calves, 40 kg for young bulls and 17 kg for heifers, while the BBP breed increased carcass weight by 8, 40 and 16 kg and the LIM breed by 9, 19 and 4 kg in the three categories of slaughtered cattle. Net daily gain was also improved by crossbreeding, with the highest value again achieved by the CHA breed, which was 15 % or 71 g/day higher than the average of all three categories in purebred cattle. The most significant improvement in carcass conformation for all three categories was achieved by the BBP breed (42 % or 2.4 subclasses), followed by the CHA and LIM breeds (31 % and 1.8 subclasses). The BBP breed achieved slightly lower values (-4 % and -0.3 subclasses), while the crosses with the CHA (+3 % and +0.1 subclasses) and LIM (+5 % and +0.3 subclasses) breeds showed a higher carcass fatness than the purebred animals. The improvement of all carcass traits through crossbreeding was more pronounced in Holstein dairy cattle than in Brown Swiss and Simmental cattle.

Keywords: crossbreeding, Holstein, Simmental, Brown Swiss, beef breeds, carcass traits.
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Introduction

In the last ten years, the number of cows and cattle as whole in Slovenia has been relatively stable, but the proportion of different breeds has changed considerably. The number of dual-purpose cows of the Simmental (SIM) and Brown Swiss (BS) breeds decreased, while the number of dairy cows of the Holstein (HOL) breed and beef cows of the Charolais (CHA), Limousin (LIM) and other beef breeds increased (Sadar *et al*., 2024). Crossing of dairy and dual-purpose breeds with beef breeds can significantly improve both growth and carcass traits of fattening animals as well as beef production. The positive effects of crossbreeding differ depending on the dam and sire breeds used (Keane and Drennan, 2008; McGee *et al*., 2008, Huuskonen *et al*., 2014, Eriksson *et al*., 2020, Bittante *et al*., 2021). In Slovenia, the CHA, LIM and Belgian Blue (BBP) breeds have recently been used to cross SIM, BS and HOL. Therefore, the aim of our study was to investigate the effects of crossing our dual-purpose and dairy breeds (SIM, BS, HOL) with beef breeds (CHA, LIM, BBP) on growth rate and carcass traits in the entire Slovenian cattle population.

Material and methods

The study included records of animals of three different breeds, Holstein (HOL), Simmental (SIM), Brown Swiss (BS) and their crosses with Charolais (CHA), Limousin (LIM) and Belgian Blue (BBP), slaughtered in Slovenian abattoirs from 2009 to 2022. Three different categories of slaughtered animals were considered: young bulls aged 12 to 24 months (A), heifers aged more than 12 months without calving (E) and calves aged less than 8 months (V). A total of 174,365 calves, 397,962 young bulls and 160,731 heifers were included in the comparison (Table 1).

The data included hot carcass weight (HCW), net daily gain (NDG), carcass conformation and fat content. Hot carcass weight was measured within 45 minutes after slaughter. Net daily gain was calculated on the basis of carcass weight and age at slaughter. The carcass conformation and fatness of the carcass was assessed according to the EUROP classification system (The Commission of the European Communities, 2008) and divided into 15 subclasses.

Table 1. Number of animals of different breeds or crossbreeds and different categories included in the study.

Breed	A	E	V
SIM	258 269	101 583	42 430
SIM/BBP	5 228	3 614	1 369
SIM/CHA	4 144	2 544	897
SIM/LIM	23 108	16 022	7 711
BS	28 589	8 758	11 383
BS/BBP	2 431	1 628	1 028
BS/CHA	1 212	672	377
BS/LIM	10 628	7 500	5 182
HOL	57 458	14 116	98 061
HOL/BBP	2 139	1 297	1 572
HOL/CHA	607	322	392
HOL/LIM	4 149	2 675	3 963
Total	397 962	160 731	174 965

A - Young bulls, aged 12 to 24 months
E - Heifers, aged more than 12 months with no calving recorded
V - Calves, animals aged less than 8 months

Data were analyzed separately for each category (A, E, Z) of slaughtered animals using the GLM procedure of SAS (2002), where breed was included as a fixed effect and differences between breeds were tested using the PDIFF option.

Results and discussion

Young bulls of the SIM breed and its crosses had the highest HCW and NDG compared to young bulls of the HOL and BS breeds. The greatest improvement in HCW and NDG was achieved in all three breeds by crossing animals with CHA breed. The improvement

Table 2: Carcass traits (LSM±SE) of Holstein (HOL), Simmental (SIM) and Brown Swiss (BS) young bulls (A) and their crosses with Charolais (CHA), Limousin (LIM) and Belgian Blue (BBP).*

Breed	Hot carcass weight (kg)	Net daily gain (g/d)	EUROP conformation (1 – 15)	EUROP fatness (1 – 15)
SIM	367.1±0.1 ^{a}	566.1±0.2 ^{a}	8.63±0.00 ^{a}	6.34±0.00 ^{a}
SIM/BBP	379.9±1.0 ^{b}	589.7±1.4 ^{b}	10.26±0.03 ^{b}	5.58±0.03 ^{b}
SIM/CHA	382.0±1.1 ^{b}	598.0±1.5 ^{c}	9.57±0.03 ^{c}	6.26±0.03 ^{c}
SIM/LIM	361.9±0.5 ^{c}	564.6±0.7 ^{d}	9.33±0.01 ^{d}	6.23±0.01 ^{c}
BS	319.5±0.4 ^{a}	488.5±0.6 ^{a}	6.43±0.01 ^{a}	6.13±0.01 ^{a}
BS/BBP	359.7±1.4 ^{b}	551.1±2.0 ^{b}	8.97±0.04 ^{b}	5.71±0.04 ^{b}
BS/CHA	368.6±2.0 ^{c}	565.5±2.9 ^{c}	8.53±0.06 ^{c}	6.23±0.06 ^{ac}
BS/LIM	343.6±0.7 ^{d}	529.8±1.0 ^{d}	8.35±0.02 ^{d}	6.34±0.02 ^{c}
HOL	314.3±0.3 ^{a}	491.1±0.4 ^{a}	5.07±0.01 ^{a}	6.16±0.01 ^{a}
HOL/BBP	359.6±1.5 ^{b}	549.2±2.1 ^{b}	8.51±0.04 ^{b}	5.79±0.04 ^{b}
HOL/CHA	370.2±2.8 ^{c}	568.8±4.0 ^{c}	7.89±0.08 ^{c}	6.47±0.08 ^{c}
HOL/LIM	351.0±1.1 ^{d}	534.5±1.5 ^{d}	7.82±0.03 ^{c}	6.44±0.03 ^{c}

*A - young bulls, aged 12 to 24 months
LSM - Least square means; SE - standard error; ^{ab} LSM within breed of cows without the same superscript differ significantly (P<0.05)

Table 3. Carcass traits (LSM±SE) of Holstein (HOL), Simmental (SIM) and Brown Swiss (BS) heifers (E) and their crosses with Charolais (CHA), Limousin (LIM) and Belgian Blue (BBP).*

Breed	Hot carcass weight (kg)	Net daily gain (g/d)	EUROP conformation (1 – 15)	EUROP fatness (1 – 15)
SIM	278.5±0.2 ^{a}	391.6±0.3 ^{a}	7.68±0.01 ^{a}	7.73±0.01 ^{a}
SIM/BBP	294.2±1.0 ^{b}	439.8±1.5 ^{b}	9.43±0.03 ^{b}	7.24±0.04 ^{b}
SIM/CHA	292.4±1.2 ^{c}	433.2±1.7 ^{c}	8.65±0.4 ^{c}	7.92±0.05 ^{c}
SIM/LIM	275.2±0.5 ^{c}	404.9±0.7 ^{d}	8.42±0.02 ^{d}	7.78±0.02 ^{d}
BS	249.8±0.7 ^{a}	326.1±0.9 ^{a}	5.89±0.02 ^{a}	7.65±0.03 ^{a}
BS/BBP	269.3±1.6 ^{b}	403.4±2.2 ^{b}	7.93±0.05 ^{b}	7.21±0.07 ^{b}
BS/CHA	270.1±2.4 ^{b}	406.7±3.4 ^{b}	7.35±0.08 ^{c}	7.59±0.11 ^{a}
BS/LIM	258.6±0.7 ^{c}	380.1±1.0 ^{c}	7.52±0.03 ^{d}	7.91±0.04 ^{c}
HOL	262.4±0.5 ^{a}	347.3±0.7 ^{a}	4.69±0.02 ^{a}	7.45±0.02 ^{a}
HOL/BBP	276.7±1.7 ^{b}	409.2±2.4 ^{b}	8.00±0.06 ^{b}	7.11±0.08 ^{b}
HOL/CHA	278.3±3.5 ^{b}	415.0±4.9 ^{b}	7.35±0.11 ^{c}	7.73±0.15 ^{a}
HOL/LIM	268.6±1.2 ^{c}	395.3±1.7 ^{c}	7.43±0.04 ^{c}	8.08±0.05 ^{c}

* E - Heifers, aged more than 12 months with no calving recorded
LSM - Least square means; SE - standard error; ^{ab} LSM within breed of cows without the same superscript differ significantly (P<0.05)

in HCW was 4 % and NDG 6 % for the SIM breed, 15 % and 16 % for the BS breed, 18 % and 16 % for the HOL breed. Crossbreeding SIM cows with LIM sires resulted in a lower carcass weight and a slightly lower net daily gain. The best conformation was achieved in animals crossed with the BBP breed. The most significant improvement in carcass conformation for all three breeds was achieved with the BBP breed, followed by the CHA and LIM breeds. Young bulls derived from a cross with the BBP breed had the lowest fatness scores.

Crossing SIM, BS and HOL cows with CHA, LIM and BBP sires resulted in higher HCW, NDG and EUROP conformation in heifers, with the exception of crossing SIM cows with LIM sires, which resulted in lower carcass weight of SIM/LIM crosses compared to purebred SIM heifers. The most significant improvement in HCW and NDG in all three breeds was achieved with the CHA and BBP breeds. For carcass conformation, the most significant improvement was achieved with the BBP breed. Heifers from the SIM and BBP crosses had the highest conformation with, 9.43 on the scale from 1 to 15 EUROP. The heifers from the cross with the BBP breed had the lowest fatness scores.

Crossbreeding was also found to improve the calves. Crossbreeding SIM, BS and HOL cows with the CHA, LIM and BBP breeds resulted in better HCW, NDG and EUROP conformation of the calves. In the SIM breed, the greatest improvement in HCW and NDG was achieved with the CHA breed, while in the BS and HOL breeds the improvement was the same for all crosses. The most significant improvement in carcass conformation for all three breeds was achieved with the BBP breed.

The CHA breed increased carcass weight the most. The improvement was 10 % for all categories of slaughtered cattle, followed by BBP with 8 % and LIM with 6 %. The CHA breed increased carcass weight by 10 kg in calves, by 40 kg in young bulls and by 17 kg in heifers, while the BBP breed increased carcass weight by 8, 40 and 16 kg and the LIM breed by for 9, 19 and 4 kg in all three categories of slaughtered cattle. Crossbreeding also improved NDG, the highest was achieved with CHA breed and it was 15 % higher than the average of all three categories in purebred animals. The greatest improvement in carcass conformation was achieved in all three categories with the BBP breed, followed by the CHA and LIM breeds. The cross with the BBP breed resulted in a slightly lower carcass fatness (-4 % and -0.3 subclass), while the crosses with CHA (+3 % and +0.1 subclass) and LIM (+5 % and +0.3 subclass) breeds had a higher carcass fatness than the purebred animals.

Table 4. Carcass traits (LSM±SE) of Holstein (HOL), Simmental (SIM) and Brown Swiss (BS) calves (V) and their crosses with Charolais (CHA), Limousin (LIM) and Belgian Blue (BBP).*

Breed	Hot carcass weight (kg)	Net daily gain (g/d)	EUROP conformation (1 – 15)	EUROP fatness (1 – 15)
SIM	101.6±0.1 ^{a}	714.3±0.8 ^{a}	7.54±0.01 ^{a}	4.43±0.01 ^{a}
SIM/BBP	110.9±0.6 ^{b}	805.8±4.5 ^{b}	9.36±0.05 ^{b}	4.36±0.04 ^{a}
SIM/CHA	116.2±0.8 ^{c}	779.7±5.6 ^{c}	8.72±0.06 ^{c}	4.45±0.05 ^{a}
SIM/LIM	113.9±0.3 ^{d}	763.5±1.9 ^{d}	8.81±0.02 ^{c}	4.58±0.02 ^{b}
BS	94.3±0.2 ^{a}	690.8±1.6 ^{a}	6.24±0.02 ^{a}	4.08±0.01 ^{a}
BS/BBP	105.7±0.7 ^{b}	799.1±5.2 ^{b}	8.63±0.06 ^{b}	4.21±0.05 ^{b}
BS/CHA	104.9±1.2 ^{b}	797.6±8.6 ^{b}	8.10±0.10 ^{c}	4.25±0.08 ^{b}
BS/LIM	104.6±0.3 ^{b}	772.4±2.3 ^{c}	8.16±0.03 ^{c}	4.48±0.02 ^{c}
HOL	96.6±0.1 ^{a}	618.0±0.5 ^{a}	5.14±0.01 ^{a}	3.91±0.00 ^{a}
HOL/BBP	100.9±0.6 ^{b}	705.6±4.2 ^{b}	8.11±0.04 ^{b}	4.11±0.03 ^{b}
HOL/CHA	101.4±0.6 ^{b}	712.9±8.4 ^{b}	7.27±0.09 ^{c}	4.27±0.07 ^{c}
HOL/LIM	101.0±0.6 ^{b}	672.3±2.6 ^{c}	7.46±0.03 ^{c}	4.40±0.02 ^{c}

* V - Calves, animals less than 8 months old
LSM - Least square means; SE - standard error; ^{ab} LSM within breed of cows without the same superscript letter differ significantly (P<0.05)

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