Occurrence of contaminated/carryover samples in automated milking systems during milk recording

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Abstract

During a genetic study about milk protein variants in different breeds, contaminated milk samples on one farm were observed, where an automated milking system (AMS) was used. In a following study about 1800 milk samples from 11 different farms using 5 different automated milking systems (AMS) within official milk recording were included. The samples were analysed using isoelectric focusing (IEF) and the milk protein variants were used for quality control. The occurrence of contaminated milk samples was up to 52% with variations between farms and AMS. The additional alleles/different concentrations as reason for the contaminated samples could in all cases traced back to the previously milked cow. Depending on the milk protein genotypes a minimum of about 3% - 10% contamination could be identified. In addition, in several cases the milk protein genotypes were not in agreement to the genotypes obtained during the other milkings of the same animal. One source of those misidentifications might be the management of the samples on farm during milk recording due to the limits of the autosampler.

Therefore we conclude that milk samples from routine milk recording using an AMS are a source of incorrect data used for management and estimation of breeding values and are not suitable as DNA source for genomic evaluation and identification of health status. Therefore, it is necessary to improve milk recording using AMS.

Keywords: milk recording, automated milking system, sample quality

Introduction

More than 95% of the proteins contained in bovine milk are coded by the four casein (CSN1S1, CSN2, CSN1S2, CSN3) and the two whey protein (LAA, LGB) genes encoding - α_{S1}-CN, β-CN, α_{S2}-CN, κ-CN, α-LA and β-LG and genetic variation has been identified and characterized. The importance of the genetic variation in animal breeding and human nutrition is reviewed by Caroli et al. (2009). Genetic variation can be detected at the phenotypic level by different identification techniques at which isoelectric focusing (IEF) in polyacrylamide gels with carrier ampholytes is the most effective screening method (Erhardt and Giambra, 2012).

Milk protein polymorphisms provide also useful information for identity control within official milk recording systems by analysing individual milk samples by IEF (Erhardt and Senft, 1991). During routine screening for milk protein variants within diversity studies using residual samples within official milk recording contaminated milk samples were observed in a frequency never observed before. It came out that these samples were collected using automated milking system (AMS).

Therefore it was the aim of the studies to analyse milk samples collected during official milk recording within different AMS on the basis of milk protein variants separated by IEF.

Material and Methods

a) 1767 residue milk samples taken within official milk recording from 11 farms with 5 different AMS (DeLaval VMS, GEA MIone, Lely A3 and A2, Lemmer Fullwood Merlin) were analysed using IEF in ultra thin layer polyacrylamide gels according to Erhardt (1989) after routine analyses within official accredited routine milk testing laboratory (HVL, Alsfeld, Germany). Analysis and identification of the genetic variants was done using cow milk samples with known variants as reference samples. The data were combined with the AMS dataset.
b) Confirmation/verification of the sample identity by combining AMS data and numbering the sampling tubes before official milk recording took part in 4 farms of a).
c) Dilution (1 to 50 %) of milk samples from Research Station Oberer Hardthof of 2 cows each with different milk protein genotypes and variation within fat-, protein- and cell content and analysing the samples within official milk testing laboratory under a).

Results and Discussion

Isoelectric focusing of the milk samples allowed simultaneous phenotyping of the milk protein variants and identification of contaminated samples.

As shown in Figure 1, in sample 1 beside a major band (β-CN A2) a faint band of β-CN A1 is visible. As the relation of β-CN A2 to β-CN A1 is far away from the 50:50 relation in a heterozygous sample, this indicates that the sample is contaminated by β-CN A1 as β-CN milk protein variants are inherited in a codominant autosomal manner without quantitative differences. Composite phenotypes with 3 (A2, B, A1) or 4 (A1, A2, B, C) pattern belonging to the β-CN fraction are clearly visible in sample 2, respectively 3. In addition sample 5 (β-CN BC) is contaminated by β-CN A2.

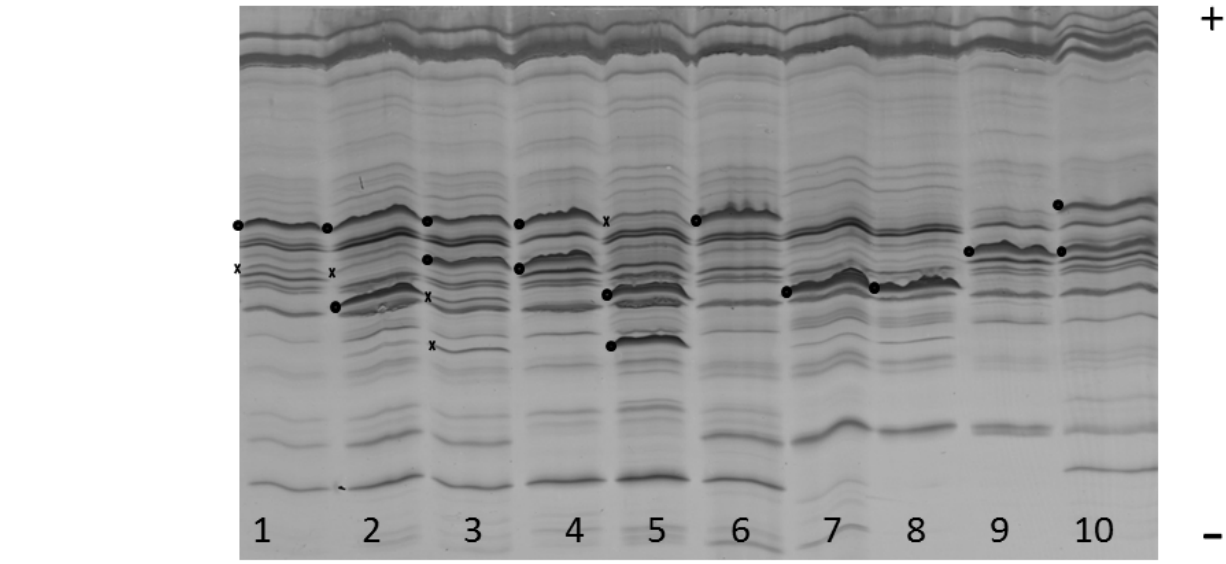

Figure 1: Detail of an IEF gel with contaminated samples in the β-*CN fraction. The major band of the homozygous and heterozygous forms of* β-*CN are marked by a dot, while contaminated pattern with* β-*CN are denoted with a cross.*

In Table 1 the phenotypes in the milk samples within consecutively sampling (S. No. 14-24) are shown. Within this screening contaminated samples occurred within β-CN in samples 18, 21 and 22 which could be related in each case to the milk sample from the previously milked cow (17, 20, 21). It is evident that a possible contamination of a milk sample by the previously milked cow is not visible if within the consecutively sampling the genotypes of the cows in the milk proteins are not different. As demonstrated in Table 1, this would be the case in S. No. 23 and 24.

Table 1. Identification of contaminated samples (S. No. 18, 21 and 22) using IEF within AMS – consecutively sampling.

Milk samples: Cattle
Origin: HVL
Time period: 24.10.2011 to .2012

Year	LabNr.	S.No.	K-CN	cont. K-CN	β-CN	cont. β-CN	α_{S1}-CN	cont. α_{S1}-CN	β-LG	cont. β-LG	α_{S2}-CN	α-LA	Farm	AMS	Date
2011	4869	14	A		A1		B		B		A	B	3	3	31.10.11
2011	4870	15	AB		A1B		B		B		A	B	3	3	31.10.11
2011	4871	16	A		A1A2		B		AB		A	B	3	3	31.10.11
2011	4872	17	A		A2		B		AB		A	B	3	3	31.10.11
2011	4873	18	A		A1	A2	B		AB		A	B	3	3	31.10.11
2011	4874	19	AB		A2B		B		B		A	B	3	3	31.10.11
2011	4875	20	AB		A2B		B		B		A	B	3	3	31.10.11
2011	4876	21	A		A1A2	B	B		B		A	B	3	3	31.10.11
2011	4877	22	A		A1	A2	B		AB		A	B	3	3	31.10.11
2011	4878	23	A		A1A2		B		AB		A	B	3	3	31.10.11
2011	4879	24	A		A1A2		B		AB		A	B	3	3	31.10.11

The results of trial a) with the screening of milk samples from 11 farms with 5 different AMS including different sampler are shown in Table 2. The % of contaminated milk samples varied between farms (0% - 52.3%). Contaminated samples were identified in each of the 5 AMS.

Table 2. Trial a: Percentage of detected contaminated milk samples within eleven farms (1 – 11) within milk recordings using different AMS (1 – 5).

Farm	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
System											
1	1.5%					6.4%					
2		0.0%			20.6%						
3							29.3%	31.9%			26.2%
4			52.3%						15.9%		
5				25.5%						35.6%	

Within the screening of the milk samples from the eleven farms it became evident, that beside the occurrence of contaminated milk samples the phenotypes of the milk proteins in the samples collected from each cow within the 24 hours of sampling did not correspond in each case. The result of repeated analyses of cow no. 148 within two milk recordings is demonstrated in Table 3. As shown in Table 3, analyses of the milk protein variants of cow no. 148 during a) with finally 3 samplings let to three different genotypes of which one (S. No. 93) was in addition contaminated. The results of repeated phenotyping of 3 further samplings (S. No. 37, 84, 143) within another milk recording b) demonstrates identical

genotypes within the recording of this cow of which two (S. No. 37, 84) were contaminated. The sampling by hand milking (S. No. 12) confirmed the genotype κ-CN AA, β-CN A1A1, αS1-CN BB and β-LG AB of cow no. 148. The number of invalid cow genotypes, on the basis that at least one of the cow's multiple samples collected within one milk recording period over 24 hours showed a different genotype, was very high in trial a. One reason for the high number of invalid genotypes could be the additional handling within milk recording at AMS with a transfer of the tubes from the sampler to the transportation box which could result in a modified order.

Table 3. Comparison of the milk protein variants in cow no. 148 within 2 milk recordings with finally 6 samples.

Farm	Cow no.	DE-no.	Date	Time	Tube	Shuttle no.	S. No.	κ-CN	Cont. κ-CN	β-CN	Cont. β-CN	α_{S1}-CN	Cont. α_{S1}-CN	β-LG
H.	148	DE..148215	2011-12-03	10:59	28	1	28	*AE*		*A1A2*		BB		AB
H.	148	DE..148215	2011-12-03	19:45	93	1	93	*AB*		*A2A2*	A1	BB		AB
H.	148	DE..148215	2011-12-04	03:51	12	2	152	AA		A1A1		BB		AB
H.	148	DE..148215	2012-08-26	16:22	37	1	37	AA		A1A1	A2	BB		AB
H.	148	DE..148215	2012-08-27	00:14	84	1	84	AA		A1A1	A2	BB		AB
H.	148	DE..148215	2012-08-27	10:17	3	2	143	AA		A1A1		BB		AB
H.	148	DE..148215	2013-02-27				12	AA		A1A1		BB		AB

The repeated analysis of milk samples on 4 farms (trial b) within a further official milk recording with the numbering of sampling tubes before official milk recording lead to different results (Table 4).

Table 4. Trial b: Percentage of contaminated milk samples in 4 farms already included in trial a).

Farm System	1	4	5	7
1	0.0%			
2			2.3%	
3				29.7%
5		10.5%		

Farm 1 with a very low level (1.5%) of contaminated samples already in trial a showed a reduction to 0 in trial b. This reduction was significant in farm 4 and 5, while on farm 7 the level was still close to 30% of composite/contaminated samples.

The numbering of the sampling tubes lead to a reduction of the number of invalid cow genotypes close to 0. Therefore, invalid cow genotypes can be overcome in a first step by numbering the test tubes. In addition technical solutions are necessary in a second step.

Based on the experimental dilution of milk samples within c), we could identify a minimum of about 3% -10% contamination depending on the milk protein genotypes. This would result in a carryover of about 1-4 ml milk from the previous milked cow/s into the regular tube of about 40 ml milk volume. As in several cases 20% contamination occurred as in the already documented tracer studies of Løvendal et al. (2010) it is evident that sample collection on farm especially in the final step (sampler) has to be improved. In addition, the demonstrated invalid cow genotypes which can be detected using genetic markers as demonstrated by using milk protein variants need an improved solution.

Therefore within the approval of sampler by ICAR not only milk yield has to be considered but also the origin of the sample. Under these circumstances IEF is a cost saving high throughput method which allows an effective screening of milk samples on a genetic basis and a first evaluation of new developments to improve the milk sample quality collected within milk recording using an automated milking system.

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