

# Estimating the Effect of the Kappa Casein Genotype on Milk Coagulation Properties in Israeli Holstein Cows

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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 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., 1999).



# Introduction, cont.

Cheese-making traits can be affected by environmental factors such as feeding, udder health, season, parity, lactation stage..... Furthermore, they are also genetically influenced (Cecchinato et al., 2011).

Milk coagulation properties are heritable and can therefore be improved by selective breeding (Ikonen et al., 2004).

In the Holstein population, the gene encoding  $\kappa$ -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  $\kappa$ -CN has faster and firmer gelling ability and is more suitable for cheese production than other variants (Jõudu et al., 2009).



# Aims of Study

Our study hypothesis was that cows carrying B allele will show superior milk coagulation properties (MCP) over cows carrying A & E allele.

To test this hypothesis, our objectives were to analyze milk coagulation properties as a function of the cow's  $\kappa$ -CN genotype, including time in minutes until the beginning of coagulation (RCT); and curd firmness after 60 minutes, in volts, as measured in an Optigraph device.



# Description of the analyzed data sets

Two data sets were analyzed:

Dataset 1: analyzed the allele distribution.

- Data set was used to estimate the  $\kappa$ -CN allele frequency in Holstein cow populations in Israel.
- A total of 1447 bulls and 4430 cows that were born between 2011 and 2021, were genotyped.



# Results – $\kappa$ -CN frequency in the Israeli Holstein population

## Genotype frequency

Genotype <sup>1</sup>	n	Frequency (%)
AA	1908	32.47
AB	2723	46.33
AE	167	2.84
BB	943	16.05
BE	134	2.28
EE	2	0.03
Total	5877	100

## Alleles frequency

Allele	n	Frequency (%)
A	6706	57.11
B	4743	40.28
E	305	2.61
Total	11754	100

B allele frequency in the early 90's where 17%.



# Description of the analyzed data sets

Dataset 2: analyzed the MCP by  $\kappa$ -CN genotype.

- We analyze the MCP in a group of cows ( $n = 359$ ).
- Cows were selected from the Israeli herdbook according to their sire's and grandsire's allele for  $\kappa$ -CN, to reach a balanced sample that includes all genotypes.
- We didn't find EE genotype cows due to low frequency of the allele E.



# Sampling protocol

- Cows were sampled according to the following protocol:
- Cows in mid-lactation (average of 148 d)
- Cows with SCS (Somatic Cell Score) lower than 4 (average of 2.03)
- Cows were checked for clinical or subclinical mastitis using the California mastitis test (CMT) on the quarter level. Quarter with positive CMT was not sampled.

The selected cows were tested for their  $\kappa$ -CN genotypes from a hair sample (Neogen).

In total, 359 cows from 15 dairy farms were included in the study.





# Sampling protocol, cont.

A milk sample was collected (30–45 mL of a mixture of whole udder yield) and divided 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) or COMBI FTS (Bentley instruments, Chaska, Minnesota, USA).

The second sample was tested for curd firmness after 60 min (CF-60) and RCT with an optigraph (Ysebaert, Frepillon, France).



# Statistical analysis

The MCP were analyzed by the GLM procedure of SAS (2009, SAS Institute Inc., Cary, NC).

Multiple comparisons for significance among the genotype effects were tested by Bonferroni procedure.

Results for a level of a specific variable included in the model was based on least square (LS) mean values.



# $\kappa$ -CN frequency found in cows included in dataset 2

## Genotype frequency

Genotype <sup>1</sup> n <sup>2</sup>	Sample (%)	Population (%)
AA	64	17.83
AB	142	39.55
AE	41	11.42
BB	65	18.11
BE	47	13.09
EE	0	0.03
Total	359	100

## Alleles frequency

Allele	N	Sample (%)	Population (%)
A	311	43.31	57.11
B	319	44.43	40.28
E	88	12.26	2.61
	718	100	

<sup>1</sup>Cow genotype was tested in a hair sample taken from each cow.

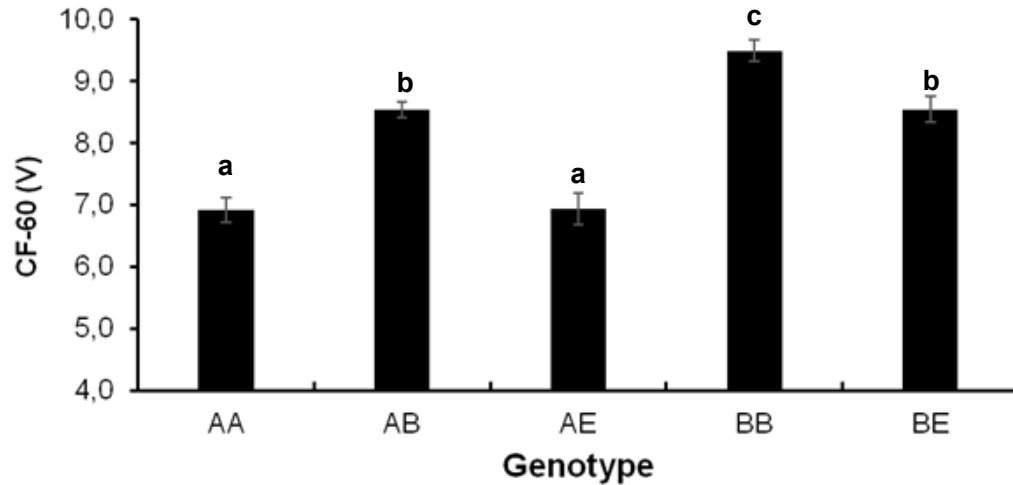
<sup>2</sup>Data of 359 cows from 15 different dairy farms.



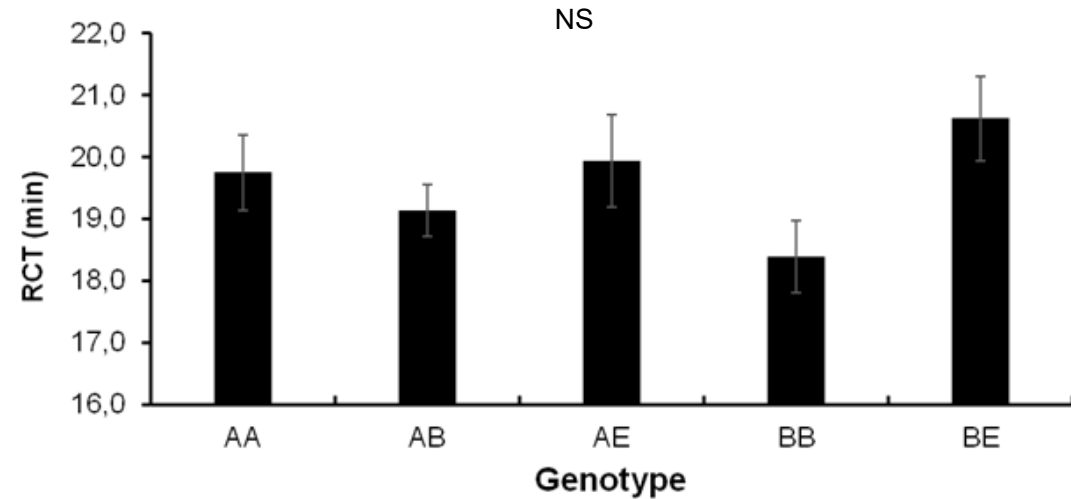
# Results

Genotype effect on:

(A) curd firmness after 60 min (CF-60)



(B) rennet coagulation time (RCT).



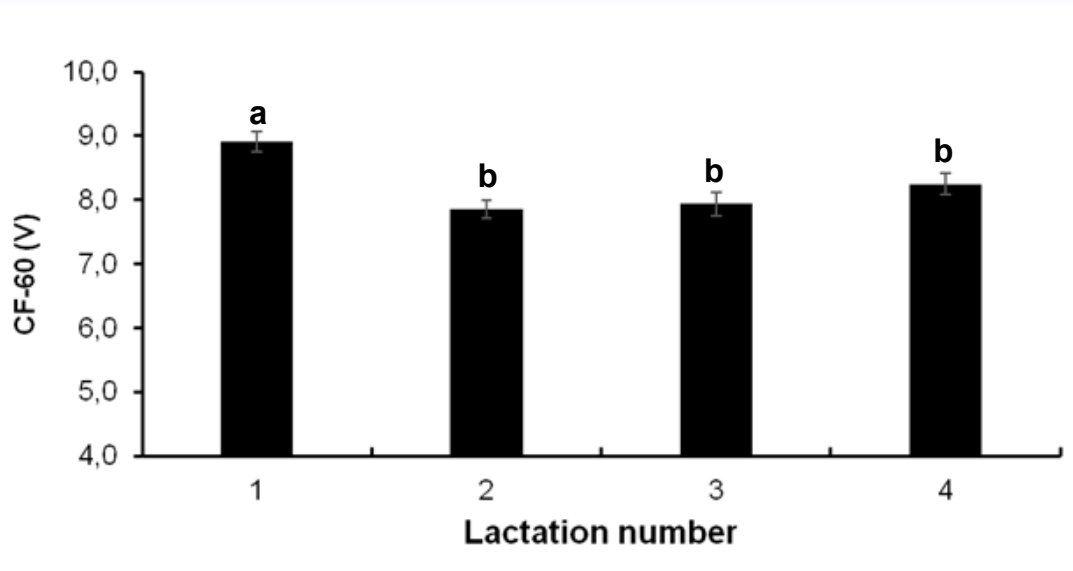
There was no difference in the milk yield and milk components.



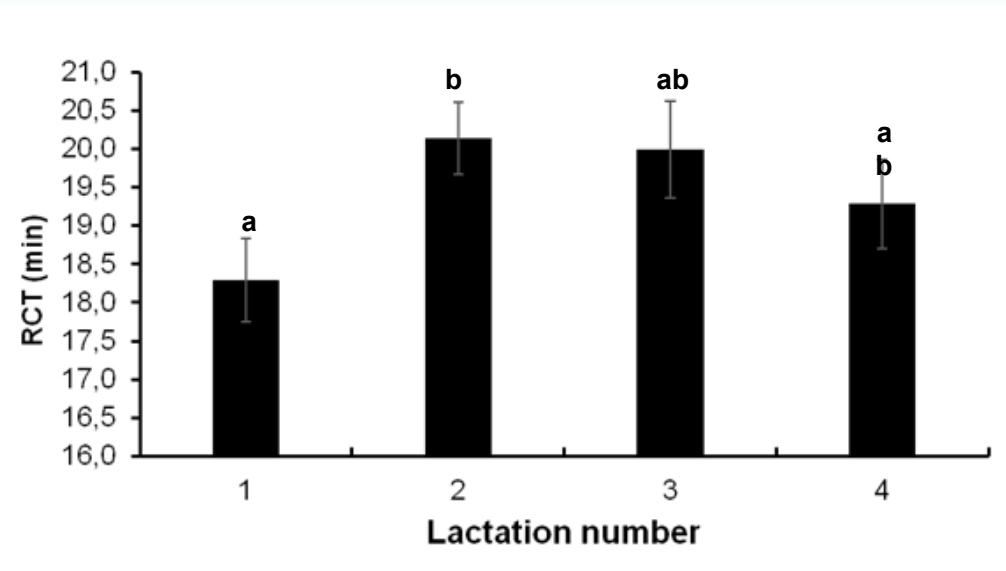
# Results

Effect of lactation number on:

(A) curd firmness after 60 min (CF-60)



(B) rennet coagulation time (RCT).



## Effect of $\kappa$ -CN genotype on milk coagulation parameters.

### 10 cows with the lowest CF-60 scores

Cow (#)	Genotype	CF-60	RCT
527	AE	4.2	14.3
4651	AA	4.2	21.9
9801	AE	4.1	19.1
4455	AE	4.0	17.3
1201	AE	3.8	18.6
1623	AB	3.4	42.4
9982	AE	3.4	42.1
10824	AE	3.3	12.7
10988	AA	2.8	13.7
216	AA	0.3	31.6

### 10 cows with the highest CF-60 scores

Cow (#)	Genotype	CF-60	RCT
7346	BB	14.6	15.8
7946	BE	14.4	19.5
4529	AA	14.1	18.5
1277	AB	13.4	16.1
7617	BB	13.2	22.8
1231	BB	13.0	16.4
5678	AB	12.8	21.1
9744	BB	12.8	14.9
2802	BB	12.3	20.8
513	BB	12.3	18.9



# Discussion

This study presents the current distribution for  $\kappa$ -CN genotypes and alleles in the Israeli Holstein population.

The prevalence of allele B in Israeli Holstein cattle in the early 1990s was about 17%.

In the current study, prevalence of the B allele has more than doubled, to around 40% in the entire population.

Elevation of the B allele distribution was in direct and related to breeding for high protein.



# Discussion

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.





# Discussion

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  $\kappa$ -CN in the selection of bulls for general service.

Inclusion of the  $\kappa$ -CN genotype in the Israeli selection index could further raise the frequency of the B allele in the population as a direct effect, leading to a faster rise in the B allele and improved MCP for the milk industry in Israel.



# A few questions for discussion not necessarily with answers

Should we change the breeding index and give higher weight to bulls with the B allele

Is there a place to put this parameter in the breeding index

Are the dairies interested in this issue for the purpose of increasing cheese production yields? Are they willing to pay for it?



# Acknowledgment

Thanks to Afimilk for performing the optigraph tests

Thanks to the Sion company for sending the hair samples for the characterization of the genotypes

Thanks to the Israeli Dairy Council for funding the research

Thanksto the ICAR conference organization committee



# Thank you for listening

