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Genetic Variants of Milk Proteins - *Relevance to Milk Composition and Cheese Production*

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The Science of Farming and Food

Genetic Variants of Milk Proteins and their Association with Milk Production and Processing Properties

(Genetic Variants of Milk Proteins - Relevance to Milk Composition and Cheese Production)

ARMIS No. 4245

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Summary and Conclusions

The genetic variants of protein in milk affect both cheesemaking characteristics and cheese composition. The effect of the κ-casein genotype in particular has generated considerable interest as it has been shown to affect rennet coagulation properties of milk as well as cheese composition and yield in cheese varieties such as Cheddar, Parmigiano-Reggiano, Svecia, Edam and Gouda. It is also evident from the literature that the κ-casein BB variant is associated with higher cheese yields when compared with κ-casein AA or AB variants. However, all information to date has been obtained from small-scale (laboratory) cheesemaking studies. Little or no information is available on the impacts of different κ-casein variant milks on the characteristics of Mozzarella cheese (pasta filata), the production and consumption of which has grown rapidly in recent years due to the increase in popularity of pizza pie, particularly in the United States, where annual Mozzarella production is now close to 1 million tonnes.

However, before milk protein genotypes can be introduced as a selection criteria in a breeding programme, their associations with all economically and biologically important characteristics in dairy cattle must be carefully evaluated.

The objectives of this project were:

(i) to develop rapid screening procedures for the determination of milk protein polymorphism (genetic variants)

(ii) to determine the frequency distribution of milk protein genetic variants in a large population of Irish Holstein-Friesians and to determine if there was an association between κ-casein variant and milk yield and composition in this group of animals, and

(iii) to make Cheddar and low-moisture part-skim Mozzarella cheese from different κ-casein genetic variant milks and to assess any effect on cheese yield, composition and functional characteristics.

The main conclusions were as follows:

Analysis of 6,007 individual Irish Holstein-Friesian milks showed that the phenotype distribution of the κ-casein BB variant was very low at 1.98% compared to 53.07% for κ-casein AA and 44.95% for κ-casein AB (Fig.1).

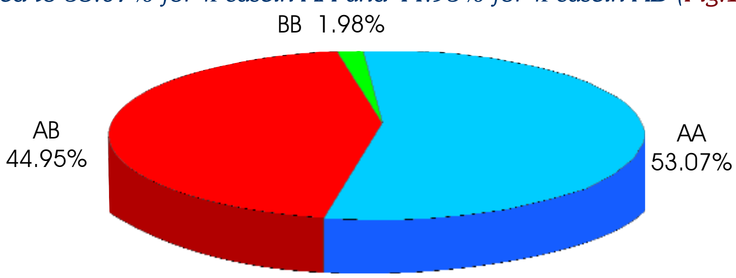


Fig. 1. Frequency distribution of κ-casein genotype for Holstein-Friesians in Ireland.

While no statistically significant associations were observed between κ-casein variant and milk yield and composition, κ-casein BB variant milks had superior rennet coagulation properties to that of the AA or AB variants.

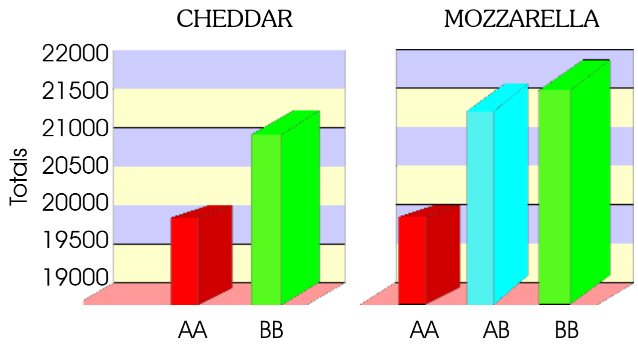
Generally, κ-casein variant had little effect on compositional attributes of cheese apart from FDM (fat in dry matter) which was significantly higher in cheeses from κ-casein BB milk than in those from κ-casein AA milk.

Generally, κ-casein variant had no significant effects on either primary or secondary proteolysis, or on the sensory and/or textural characteristics of Cheddar or Mozzarella cheese throughout ripening; or on the functional characteristics (e.g. flow and stretch) of baked Mozzarella on storage for 90 days at 4°C.

However, κ-casein BB variant milk gave significantly higher actual, and moisture adjusted yields of Cheddar and Mozzarella cheese than either κ-casein AB or AA variant milks. For example, the moisture adjusted Cheddar yield from κ-casein BB milk was 8.2% higher than from κ-casein AA milk. In the case of Mozzarella, the moisture adjusted yield was 12% higher.

Based on the results, it is estimated that the actual yield of cheese in a plant producing 20,000 tonnes per year from κ-casein AA milk would increase to approximately 21,180 tonnes of Cheddar, or 21,780 tonnes of Mozzarella if made from κ-casein BB milk. Where κ-casein AB milk is used instead of κ-casein BB milk, the estimated yield of Mozzarella would increase to 21,580 tonnes (*Fig. 2*).

Fig. 2. Estimated yield differences using κ-casein AA, AB and BB variant milks.



Research and Results

Screening procedures for genetic variants

An isoelectric focusing method was adapted and modified to give greater resolution of the A and B variants of κ-casein in individual bovine milk samples. A DNA based (allele specific primer) protocol was developed for the rapid screening of blood and semen for κ-casein genotypes. The technique was used to screen blood and semen from sires standing in a commercial AI station.

Individual milk samples from 6,007 Irish Holstein-Friesian cows in herds supplying milk to a large local dairy company, were typed for κ-casein phenotype by isoelectric focusing. The frequency distribution of the κ-casein variants were as follows: κ-casein AA, 53.07%; κ-casein AB, 44.95% and κ-casein BB, 1.98% (*Fig.1, Table 1*). Individual cow phenotypes were merged with milk recording records. The resulting data were analysed using the GENSTAT statistical package to determine if there were associations between κ-casein variant, milk yield and milk composition.

While no statistically significant associations (p>0.05) were found, trends in the data indicated that the κ-casein BB variant cows had higher milk yield and protein yields than κ-casein AA or AB variant cows.

Table 1. Percentage distribution of κ-casein variants in Holstein-Friesian and other breeds.

Breed	**No. of cows sampled**	**AA**	**AB**	**BB**
Irish Holstein-Friesian	*6007 (typical local herds)*	*53.1*	*44.9*	*2.0*
Irish Holstein-Friesian	*696 (5 Moorepark herds)*	*54.2*	*42.1*	*3.7*
Jersey	*116 (single herd)*	*4.5*	*47.7*	*47.7*
Montebeliarde	*35 (single herd)*	*20.0*	*37.1*	*42.9*
Normande	*30 (single herd)*	*10.0*	*33.3*	*56.7*
Kerry	*41 (single herd)*	*80.5*	*19.5*	*0.0*

In addition to the 6007 Holstein-Friesian cows, 696 Holstein-Friesians in the five Moorepark herds, as well as Jersey, Montebeliarde, Normande and Kerry cows were typed for κ-casein variants (*Table 1*.) By contrast with the Holstein-Friesians, the frequency of variant AA in the Jersey herd was very low at 4.5%, with 47.7 each for AB and BB. The Montebeliarde and Normande breeds also had relative high frequencies of κ-casein BB (42.9% and 56.7% respectively), while no κ-casein BB was found in the Kerry herd. With the exception of the Holstein-Friesian cows, the numbers typed in the other breeds were relatively small and hence the data should be taken as indicative only.

Rennet coagulation properties of different κ-casein variant milks

Milks of similar casein levels were collected from two Spring-calving Holstein-Friesian herds (one genotype AA and one genotype BB for κ-casein) over a two-week period in mid-to-late-lactation. On three separate occasions during this period the milks were examined for composition, average casein micelle diameter, rennet coagulation properties, microstructure of the rennet gel obtained during cheese-making and suitability for Cheddar cheese manufacture. While κ-casein variant had no significant effect on the gross composition of the milk, the average casein micelle diameter in the κ-casein BB milk (183.5 nm) was significantly lower than that of the κ-casein AA milk (266.3 nm).

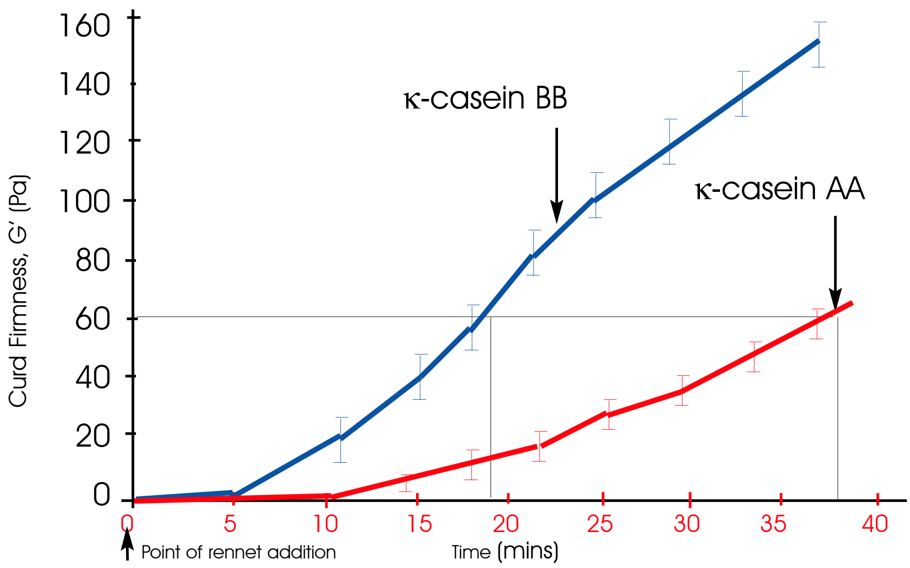
The rennet coagulation properties of the κ-casein BB milk were superior to those of κ-casein AA milk as reflected by shorter gel times, higher curd-firming rates and shorter set to cut times when cutting at a given gel strength when renneting was performed under conditions similar to those used for Cheddar and Mozzarella manufacture (Table 2, Fig. 3).

Table 2. Curd forming characteristics of κ-casein AA, AB and BB variant milks during Cheddar and Mozzarella cheesemaking trials.

	Cheddar		**Mozzarella**		
	AA Milk	BB Milk	AA Milk	AB Milk	BB Milk
Rate of curd firming (per min)	0.08[a]	0.17[b]	0.12[c]	0.19[d]	0.24[d]
Curd firmness at 60 min (mm)	38.33[a]	54.5[b]	48.75[c]	57.63[d]	58.48[d]
Set to cut time (min)	60	30	60	56	53

[a,b,c,d] Values within a row, for each cheese type, without a common superscript, were significantly different (p<0.5).

Fig. 3. The development of curd fimness as a function of time from rennet addition in κ-casein AA and BB variant milks. The set to cut time at a curd firmness of 60 Pa almost doubled from 20 min for the κ-casein BB milk to 38 min for the κ-casein AA milk (Cheddar cheese-making conditions).



The superior rennet coagulation characteristics of the κ-casein BB milk probably reside in its higher level of κ-casein as a percentage of total casein and its lower negative charge. These conditions are conducive to a more compact casein arrangement and a finer gel structure from the κ-casein BB variant milk than from the κ-casein AA milk (*Fig. 4*).

Cheddar cheese production

Cheddar cheese was manufactured at pilot-scale on three separate occasions over a two week period from milk collected from two mid-lactation, Spring-calving, Holstein-Friesian herds (n=11) containing similar casein levels, one producing phenotype AA and the other phenotype BB κ-casein variant milks.
While κ-casein variant did not significantly ($p > 0.05$) influence the casein content or gross composition of milk, κ-casein BB milk had a significantly smaller average casein micelle diameter and superior rennet coagulation properties than that of the AA milk.

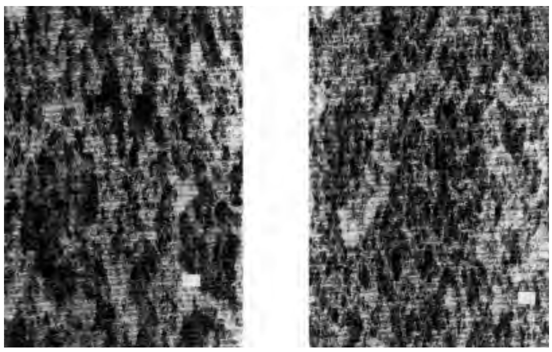


Fig.4. Electron micrographs of rennet gels made from κ-casein AA and BB genetic variant milks. Scale bar:10 μm.
Note: The more compact casein and finer gel structure from the κ-casein BB milk.

Cheddar cheesemaking studies showed that the κ-casein BB milk resulted in significantly higher fat recoveries into cheese and higher actual, and moisture-adjusted cheese yields. The moisture-adjusted cheese yields (MACY) for κ-casein AA and BB milks were 92.5 and 100.1 kg/1000 kg milk respectively (Table 3) equivalent to an 8.2% advantage in favour of BB milk (Table 4).

Cheese produced from κ-casein BB variant milk had higher concentrations of fat and lower protein levels than that produced from the AA variant. The higher fat recoveries, and hence higher yields of the κ-casein BB variant cheese are most likely associated with the finer gel structure (*Fig. 4*). However, κ-casein variant had no significant effect on proteolysis or on the acceptability scores awarded to the cheeses (*Table 3*).

Mozzarella cheese production

Low-moisture, part-skim Mozzarella cheeses were made at pilot-scale (450 kg) on five occasions at weekly intervals from pooled mid-lactation Spring-calving Holstein-Friesian milks containing κ-casein AA, AB or BB genetic variants. Compared with κ-casein A variant milks, the κ-casein B variant milks were associated with higher concentrations of casein ($P<0.001$), whey protein ($P<0.02$), total protein ($P<0.001$), superior curd-forming properties ($P<0.05$) and increased cheese yields ($P<0.05$).

Table 3. The effect of κ-casein genetic variants on the composition of cheesemilk and its suitability for the production of Cheddar and low-moisture part-skim Mozzarella cheese.

	Cheddar		Mozzarella		
	AA Milk	BB Milk	AA Milk	AB Milk	BB Milk
Milk Composition					
Protein (g/kg)	34.4	35.3	35.4^{a}	37.6^{b}	37.5^{b}
Fat (g/kg)	34.7	36.2	29.2^{a}	31.3^{b}	30.9^{c}
Casein (g/kg)	26.3	27	26.4^{a}	28.3^{b}	28.0^{b}
Whey Composition					
Protein (g/kg)	9.6	10	10.3	10.6	10.6
Fat (g/kg)	7.5^{a}	3.8^{b}	5.9^{a}	4.7^{b}	3.5^{c}
Fines (g/kg)	544	324	284	251	64
Cheese Yield					
Actual (kg/1000kg)	93.6^{a}	99.1^{b}	92.3^{a}	99.6^{b}	100.5^{b}
MACY (kg/1000kg)	92.5^{a}	100.1^{b}	91.5^{a}	100.6^{b}	102.5^{b}
MACYPFAM (kg/1000kg)	94.3	99.5	90.2^{a}	93.2^{ab}	95.2^{b}
Recovery in Cheese					
Fat (g/kg/total)	809^{a}	945^{b}	674^{a}	712^{b}	767^{c}
Protein (g/kg/total)	699	729	774	780	775
Texture and sensory properties					
Firmness (N)	199	200			
Fracture stress (kPa)	203	202			
In-house grade	4.26	3.97			
Functionality					
Melt time (sec)			103	104	102
Flowability (%)			43.6	38	38.6
Stretchability (mm)			812	835	814

a,b,c: *Values within a row for each cheese variety, without a common superscript were significantly different: P >0.05.*

MACY: *Moisture adjusted (to 380 g/kg for Cheddar, and 465 g/kg for Mozzarella) cheese yield.*

MACYPFAM: *Moisture adjusted cheese yield per 100kg milk adjusted to 31.2 g/kg protein and 32.5 g/kg fat for Cheddar and to 34 g/kg protein and 28.3 g/kg fat for Mozzarella.*

Grading (Cheddar only): *Cheeses were graded and scored 0 to 8, with 0 denoting rejection and 8 denoting excellence.*

Functional properties (Mozzarella only): *After 10 days storage at 4°C.*

The moisture adjusted cheese yields (MACY) for the κ-casein AA, AB and BB cheeses were 91.5, 100.6 and 102.5 kg/1000 kg milk, respectively (*Table 3*). This was equivalent to an increase of 9.9% and 12%, respectively, in the yields of cheese produced from κ-casein AB and BB milk, compared to κ-casein AA milk (*Table 4*).

Table 4. Percentage yield increase in cheeses made from κ-casein BB and AB variant milks compared to that made from κ-casein AA milk.

Cheese Yield*	**Cheddar**	**Mozzarella**	
	BB milk	BB milk	AB milk
Actual	5.9	8.9	7.9
MACY	8.2	12.0	9.9
MACYPFAM	5.5	5.5	3.3

Actual: = *not adjusted for moisture*
MACY = *Moisture adjusted to 38% for Cheddar and 46.5% for Mozzarella*
MACYPFAM = *Moisture adjusted to 38% for Cheddar and 46.5% for Mozzarella and milk adjusted to 3.12% protein for Cheddar and 3.40% for Mozzarella*

κ-Casein variant had no significant effect on the proteolysis and ripening of uncooked cheese or on the functionality (melt time, flowability and stretchability) of the cooked cheese, during the course of a 90 day storage period at 4°C.

Rennet coagulation properties of late lactation milk

Irish manufacturing milk is produced mainly from Spring-calving herds, fed predominantly on pasture, resulting in a seasonal supply accompanied by significant compositional changes. This is of particular importance in late-lactation when cheesemaking is often terminated due to deteriorating milk quality. In this study the effect of blending late-lactation milk with reconstituted skim milk powders (SMP) made from κ-casein AA, AB and BB milks was investigated. It was demonstrated that blending late-lactation milk with skim milk powder made from κ-casein BB containing milk in particular improved the renneting characteristics of the late-lactation milk (*Fig. 5*). This indicates that it may be possible to extend cheesemaking further into late-lactation in countries such as Ireland where there is a seasonal milk supply.

Fig 5. (A, B, C)

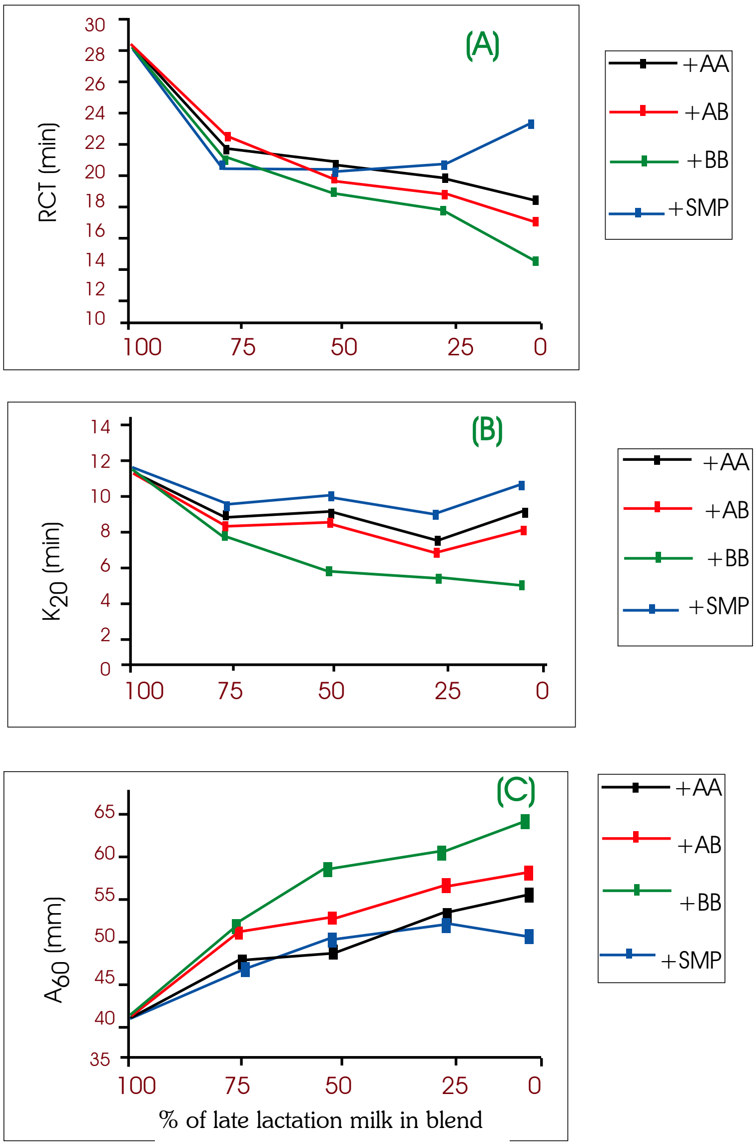


Fig.5. Effect of blending poor quality late-lactation milk with reconstituted skim milk powders made from herd milk containing κ-casein AA, AB, and BB variants on (a) rennet coagulation time (RCT), (b) rate of curd formation ($1/K_{20}$) and (c) curd firmness after 60 min. (A_{60}). Good rennet coagulation properties correspond with short RCT, low K_{20} and high A_{60}. Commercial low heat skim milk powder (SMP) was used as control.

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