CANOLFAN TECHNOLEG BWYD
FOOD TECHNOLOGY CENTRE
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Investigation into the Effect of κ-Casein Milk Gene Variants on Cheese Clotting and Yield

for Rhys Davies Farming Connect, Menter a Busnes

Project No: 17029

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Food Technology Centre
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Cronfa Amaethyddol Ewrop ar gyfer Datblygu Gwledig
Ewrop yn Buddsoddi mewn Ardaloedd Gwledig
European Agricultural Fund for Rural Development:
Europe Investing in Rural Areas

Llywodraeth Cymru
Welsh Government

FARMING connect
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INTRODUCTION

The client has approached the Food Technology Centre (FTC) for assistance with testing the influence of the kappa-casein BB gene in dairy cows on cheese formation and yield. Kappa-casein is one of several molecular types of casein protein present in milk and is the one responsible for the clotting action of rennet during cheesemaking. It can exist as different variant alleles, where AA, AB or BB arrangements of the alleles may be present. Affordable technology now exists for farmers to profile their cattle for kappa-casein genotype, where studies have been published that have determined that cheese yield can be significantly improved up to 10% higher with BB milk compared to AA milk, and the renneting time up to 25% faster. AB milk would be intermediate in properties between AA and BB.

The kappa-casein genotype of cows within a dairy herd in north Wales has been analysed, to categorise the cows according to the presence of either AA, AB or BB genes. These revealed that within the herd, 8 are AA, 15 are AB and 27 are BB. These were selectively milked to provide milk for trials at FTC, investigating any differences in clotting time, clot firmness and yield in cheesemaking.

TRIAL 1 01/10/18 – 02/10/18

The client delivered approximately 10 litres each of 3 different types of milk, containing the AA, AB or BB genes, to FTC on 1st October. The samples were freshly milked that morning from a herd of cross-bred Jersey cows, with just one cow milked to provide each sample. The cows had been selected for similar protein, fat and total solids levels in their milk, however, this was from data taken 3 weeks previously.

The milk was transported in 3 litre sealed plastic bags, floating in ice chilled water drums to cool them. After decanting each milk type into 10 litre buckets, the temperature was around 12°C. Each was stirred and sampled into two 100ml sample pots which were left at room temperature for compositional testing with a FOSS Milkoscan Minor analyser (Fig 1).

Fig 1. FOSS Milkoscan Minor

The results were as follows (Table 1):

Table 1. Milkoscan Analysis of Milk Samples delivered 01/10/18

Sample	Fat	Protein	Lactose	Total Solids (TS)	Solids Non Fat (SNF)	Freezing Point Depression (FPD)
AA sample 1	6.79	4.67	3.42	16.05	9.33	0.49
AA sample 2	6.16	4.73	3.45	15.51	9.41	0.49
AA average	6.48	4.70	3.44	15.78	9.37	0.49
AB sample 1	7.00	4.40	4.03	16.47	9.63	0.55
AB sample 2	6.96	4.40	4.04	16.45	9.64	0.55
AB average	6.98	4.40	4.04	16.46	9.64	0.55
BB sample 1	5.80	4.24	4.10	15.14	9.51	0.55
BB sample 2	5.62	4.22	4.11	14.97	9.50	0.55
BB average	5.71	4.23	4.11	15.06	9.51	0.55

Initial trials were carried out that same day on clotting the AA milk with rennet. After 1.5 hours the samples were only very weakly gelled, so a further test was carried out adding starter culture for 1 hour prior to adding the rennet, as would be more normal in cheesemaking, however this made little difference to the clotting time.

The milk samples were stored chilled in 10 litre buckets overnight. The following day, an experiment was set up to clot all three types of milk staggered at 5 minute intervals and test the coagulation rate and clot firmness by testing portions of the clotting milk at 15 minute intervals to 2.25 hours.

These were stirred thoroughly for a few minutes to evenly distribute the fat, then 2.00kg of each was accurately weighed into 3 litre stainless-steel bain-marie pots. These were stood in water at around 45°C with occasional stirring by spoon until they reached around 33°C. Microbial rennet (Marzyme, purchased from Orchard Valley Dairy Supplies) was added at the equivalent used in most cheesemaking, at 25ml per 100 litres milk, which required 0.5ml per 2 litres. 0.5ml of rennet from a pipette weighed around 0.57g, so to be as accurate as possible, this amount was weighed 3 times on a balance to 4 decimal places in a small plastic weighing boat.

The rennet was added to milk sample AA and a timer started. It was stirred well and 80g portions were then weighed into 100ml glass jars, which were immediately placed in a water bath to maintain their temperature at 32°C, a temperature commonly used in cheesemaking (Fig 2). Rennet was added to the AB milk at 5 mins, and the BB milk at 10 mins, also filling these into 8 jars each.

Fig 2. Glass jars filled with clotting milk, incubated in water bath at 32°C

One jar of each type of milk was then tested at 30 minutes from adding the rennet, and then at 15 minute intervals. TA.XT.Plus Stable Micro Systems Texture Analyser, with a P/0.5R half-inch diameter probe (Fig 3). The test measured force on compression, at speed 0.5mm/s for 20mm distance, with a 0.1g trigger force on touching the surface.

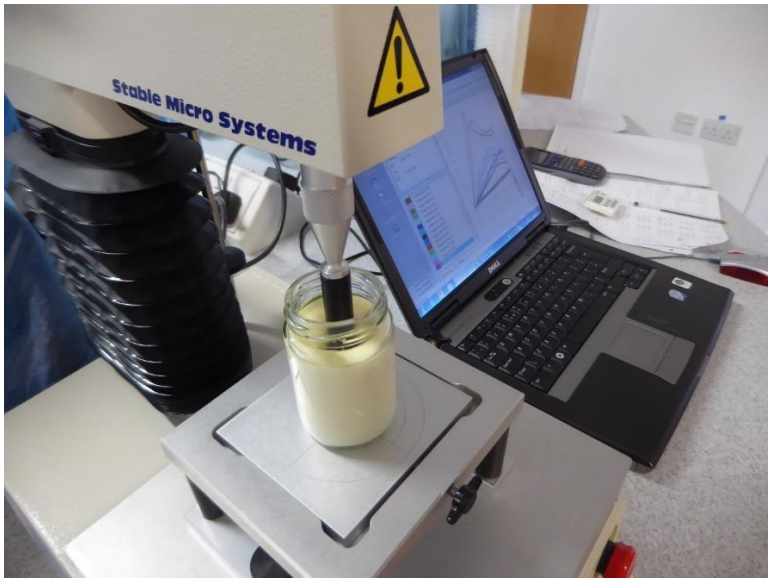


Fig 3. Testing clotted gels with the Texture Analyser

The texture profiles obtained as the probe travelled down through the clotting milk are shown in Figures 4 - 11 below, comparing the 3 types of milk at various time intervals. (These graphs are plotted as Force against Time. The analyser was set up to also record force data as the probe retracted from the sample, giving a return curve when plotting Force against Distance that was not required. As the probe moved down at a constant speed, the graphs shown would be identical if plotted as Force against Distance). Note that these graphs are not all plotted on the same force scale on the Y-axis.

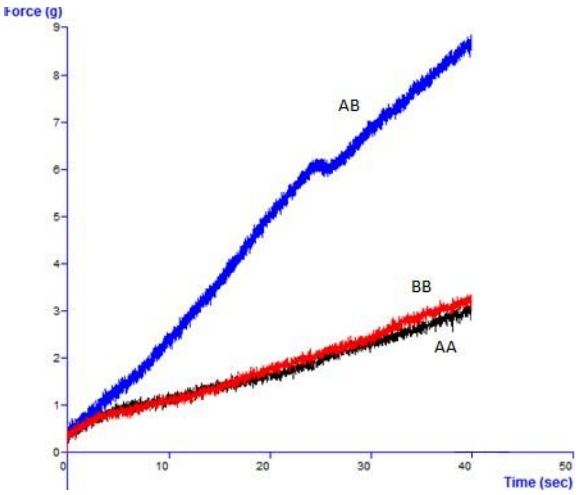


Fig 4. Texture Profiles 0.5 hours

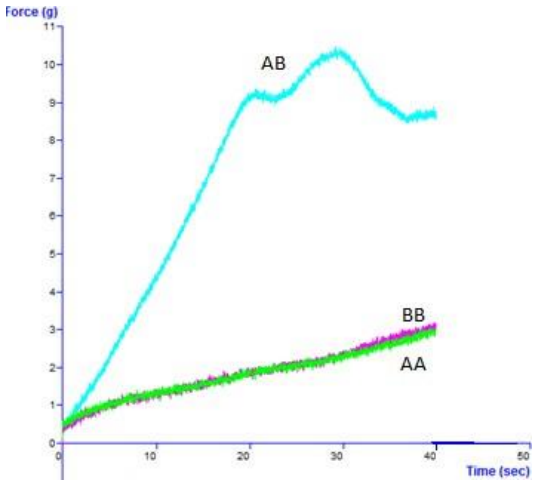


Fig 5. Texture Profiles 0.75 hours

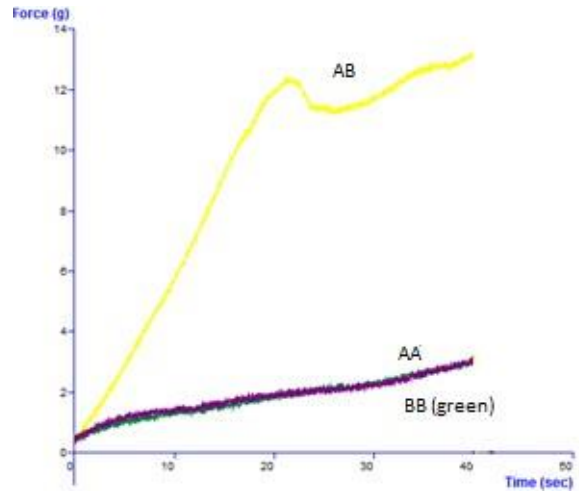


Fig 6. Texture Profiles 1 hour

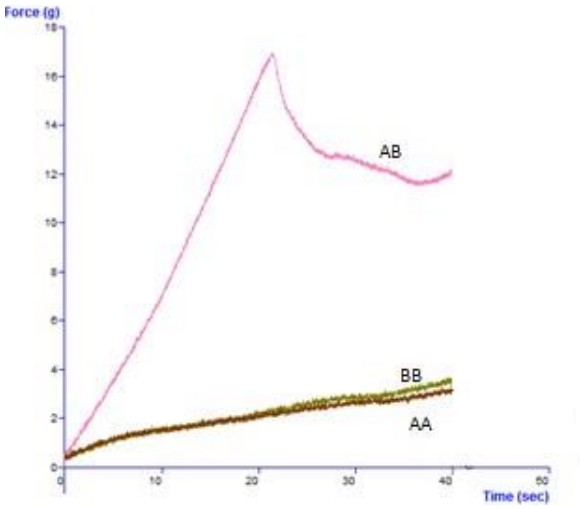


Fig 7. Texture Profiles 1.25 hours

Comparing Figs 4 – 7 above, the AA and BB milks were not yet clotting at up to 1.25 hours. Any small differences in the AA and BB curves are unlikely to be significant. However, what was obvious was the vastly quicker clotting time and firmness of milk AB. At 0.5 hours, the clot is a lot firmer than AA and BB, and is starting to show a slight break point in the curve, which becomes more pronounced from 0.75 hours onwards. This indicates that a weak elastic gel has formed that breaks as the probe moves downwards. The force at the first peak of the graph can be noted as the 'break load' of the gel, and the distance to the break represents the 'elastic limit'. Break load divided by elastic limit (the initial gradient) gives the 'gel strength'.

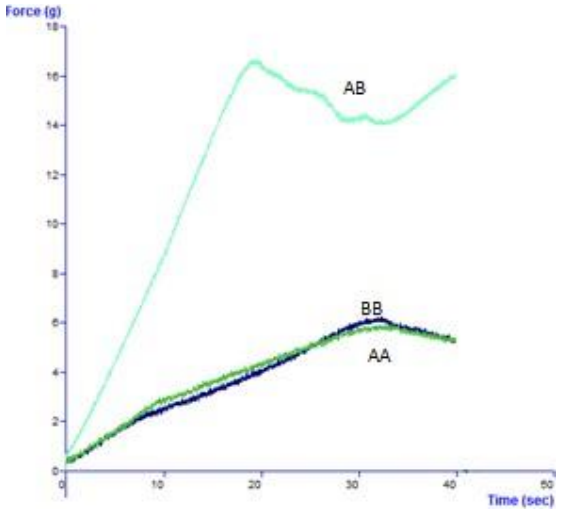


Fig 8. Texture Profiles 1.5 hours

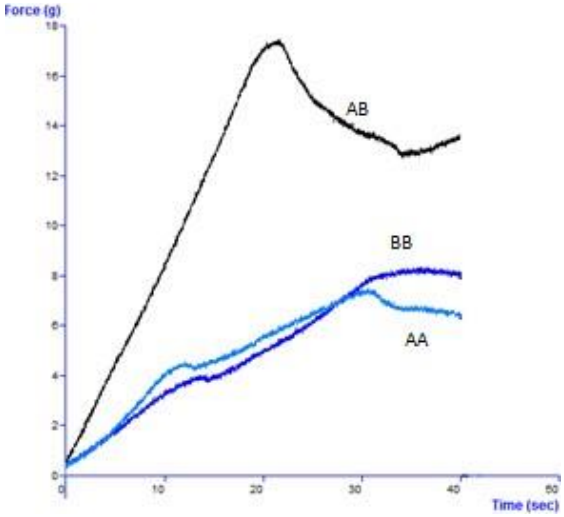


Fig 9. Texture Profiles 1.75 hours

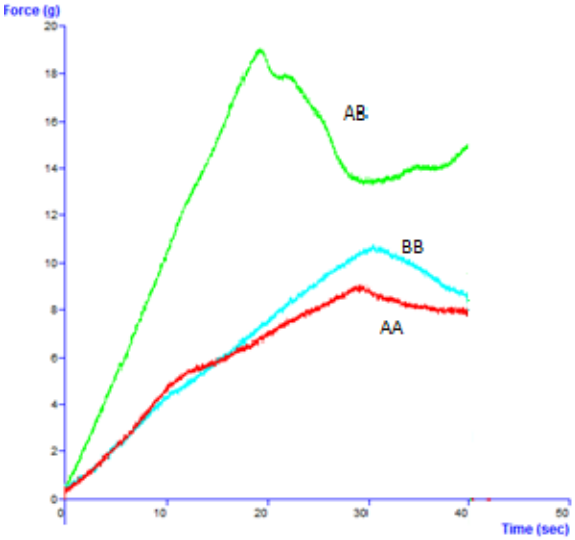


Fig 10. Texture Profiles 2 hours

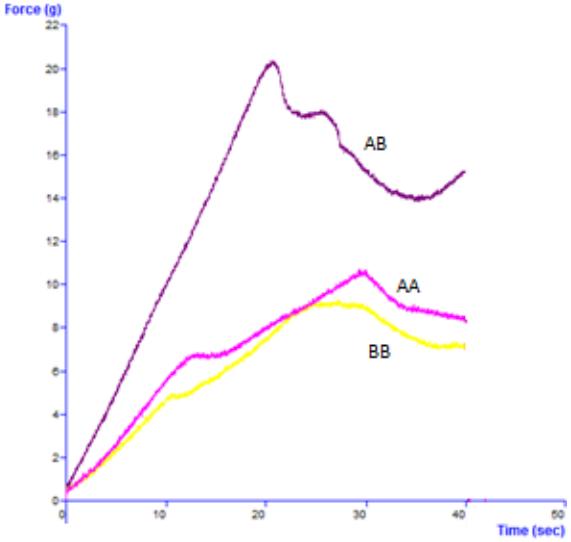


Fig 11. Texture Profiles 2.25 hours

As time progresses from 1.5 hours onwards (Figs 8 – 11 above), sample AB shows little futher increase in clot firmness, and samples AA and BB are starting to show signs of clotting. However, the break loads are far weaker and show a double peak. It is likely that the first peak is due to the probe breaking through a fatty skin that was forming on the surface of the samples. Even by 2.25 hours, the gels are still quite weak compared to AB. It is not possible to significantly differentiate between samples AA and BB, with very similar texture profiles for both these samples on every testing occasion.

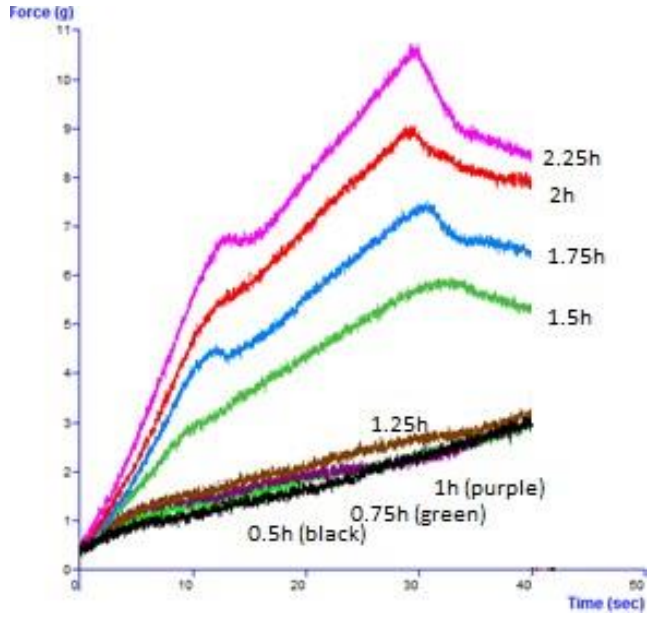


Fig 12. Comparison of all texture profiles for clotting of AA milk, from 0.5 to 2.25 hours

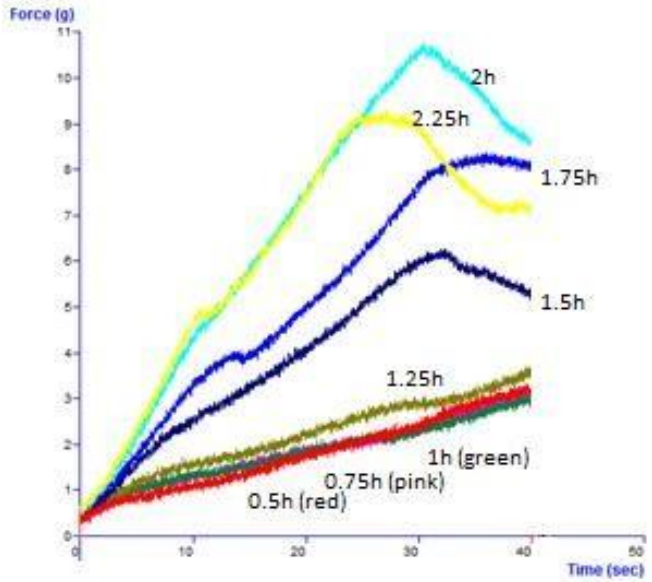


Fig 13. Comparison of all texture profiles for clotting of BB milk, from 0.5 to 2.25 hours

Figures 12 and 13 above compare all the texture profiles on the same graph for samples AA and BB respectively. These profiles are similar, with no significant clotting occurring up to 1.25 hours. The AA samples give weak gels with a slightly cleaner break than BB.

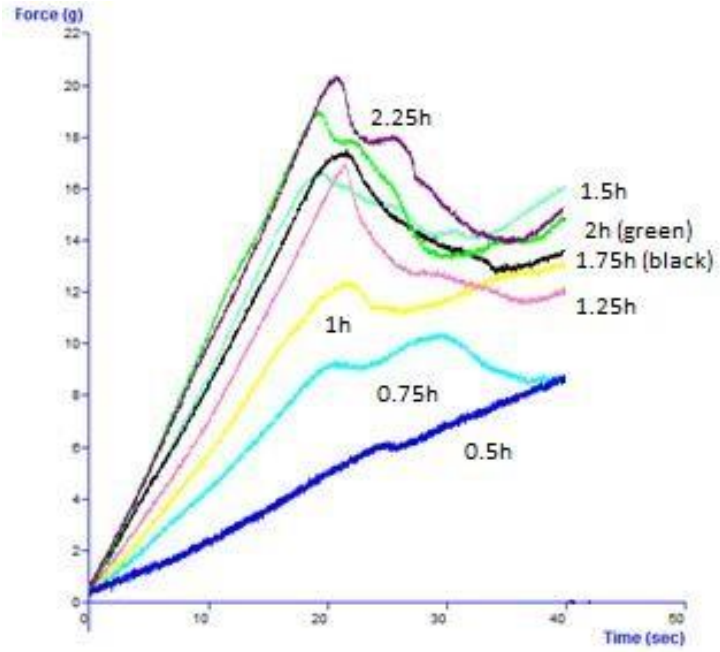


Fig 14. Comparison of all texture profiles for clotting of AB milk, from 0.5 to 2.25 hours

Figure 14 shows the AB milk starting to clot as early as 0.5 hours, forming much stronger gels than with the AA or BB milk (note that graph is on double the force scale as Figs 12-13).

It is likely that the gels may have clotted better if starter culture was added for 1 hour prior to adding the rennet, as is more usual in cheesemaking. This can cause a slight drop in pH that may aid clotting, even though the pH would still be far above the isoelectric point of the milk protein.

TRIAL 2 03/10/18 – 04/10/18

The client delivered a further 40 litres of the 3 different types of milk, containing the AA, AB or BB genes, to FTC on 3rd October. The samples were freshly milked that morning from a herd of cross-bred Jersey cows, with milk from several different cows milked and combined to provide each sample. It was hoped that this would give closer overall values for protein, fat and total solids than in trial 1. The milk was transported in bags as previously. Each type of milk had been mixed thoroughly before filling into the bags, so that it was only necessary to open one bag for sampling into 2 small pots for testing composition on the Milkoscan. These results are given in Table 2.

These results show a similar pattern of fat, protein and total solids levels as in Trial 1 where individual cows were selected, showing that combining milk from several cows has not helped to even out the differences. The AB milk is now even higher in protein and total solids than previously, and although the difference in protein between AA and BB is slightly reduced, the difference in total solids is greater.

Table 2. Milkoscan Analysis of Milk Samples delivered 03/10/18

Sample	Fat	Protein	Lactose	Total Solids (TS)	Solids Non Fat (SNF)	Freezing Point Depression (FPD)
AA sample 1	6.44	4.41	4.11	16.03	9.70	0.55
AA sample 2	6.41	4.42	4.13	16.03	9.71	0.56
AA average	6.43	4.42	4.12	16.03	9.71	0.56
AB sample 1	6.98	4.88	4.01	17.04	10.05	0.55
AB sample 2	6.97	4.89	4.01	17.03	10.07	0.55
AB average	6.98	4.89	4.01	17.04	10.06	0.55
BB sample 1	5.72	4.10	4.12	14.94	9.39	0.55
BB sample 2	5.73	4.10	4.12	14.95	9.39	0.55
BB average	5.73	4.10	4.12	14.95	9.39	0.55

It was decided at this point that there was little to be gained from repeating the laboratory experiments of the previous day, and that it would be of more interest to test clotting times and cheese yields of the AA and BB types of milk, using a cheese-making process in vats. Although BB milk had around 0.3% less protein and 1% less total solids, it would be interesting to see whether or not the BB genes had a positive enough effect for this BB milk to give an equivalent or greater rate of clotting and cheese yield.

Around half of the AA and BB milks were used to make cheese on that same day, and the remaining half used the following day to duplicate the process as closely as possible to give insight into how significant any observed differences may be.

The cheese method selected was the start of the process for making camembert types of cheese, only taking the process up to the point of cutting and draining the curd in the moulds on day one, and weighing them on day 2 after draining overnight, to determine the yield. (To complete the camembert process, these cheeses would then have been placed in a 20% brine solution for around 1 hour, then dipped the following day in a dispersion of *Penicillum candidum*, a white mould. The cheeses would then be ripened at around 10-12°C and 90% humidity for at least 10 days, with frequent turning, wrapped when a white mould coat had formed, then ripened for a further 4 – 8 weeks for the cheese texture to soften and develop more flavours).

In the first cheese trial, 20kg of AA and BB milks were accurately weighed in a stainless steel bucket and poured into two identical 50 litre Labu cheese vats (Fig 15), which had been previously warmed to 32°C with water inside. The vats are oil jacketed and thermostatically controlled. The room was thermostatically heated to 22-24°C. Each milk took around 30 minutes to warm to 32°C. With their temperatures matched as closely as possible, Danisco Probat 222 freeze-dried starter culture was accurately weighed in weighing boats to 4 decimal places and added to both vats at the dosage rate of 10 DCU (Danisco Culture Units) per 100 litres of milk. The AA milk was at 32.8°C and the BB milk at

31.7°C at this point. Both vats were stirred for a few minutes to disperse the cultures, and then stirred occasionally over a 1 hour ripening time, during which the bacteria in the cultures start to grow, converting lactose milk sugar into lactic acid.

Fig 15. Milk added to Labu cheese vat

Rennet (Marzyme vegetarian microbial) was then pre-weighed in small plastic beakers and added to each vat to give the equivalent of 25ml per 100 litres of milk, where 5ml would be required for 20 litres of milk. 5ml of the rennet had previously been shown to weigh 5.7g, so this amount was accurately weighed for each vat. At this point the milk in both vats was at 32.2°C. As rennet is an enzyme its action can be fairly temperature dependent, so it was important to match the temperatures at this stage. The vat heating was turned off to avoid localised heating and convection currents when the thermostat turns on and off, with the vats well insulated to prevent much heat loss.

During clotting, the flocculation time was compared for both vats, which is the point at which the casein micelles are becoming destabilised and starting to aggregate to form a weak gel. A test was used whereby a drop of milk is taken by dipping a finger into each milk in turn, and allowing it to fall into water at around 37°C. If the milk disperses then flocculation has not occurred (Fig 16). When the milk forms solid flakes that sink down to the bottom, the flocculation point has been reached (Fig 17). This was tested repeatedly for each vat.

At 29 mins 30 secs since adding the rennet, the BB milk was showing initial signs of flocculation. At 30 mins 20 secs the AA milk did not show signs of flocculation. At 31 mins 29 secs the BB milk had definitely flocculated. At 32 mins 05 secs the AA milk was showing signs of flocculation, and had definitely flocculated at 32 mins 37 secs. This indicated that the BB milk flocculated around 2.5 minutes before the AA milk. At the point of flocculation there was also an obvious change in the milk on the finger, changing from a thin liquid to something starting to clump together.

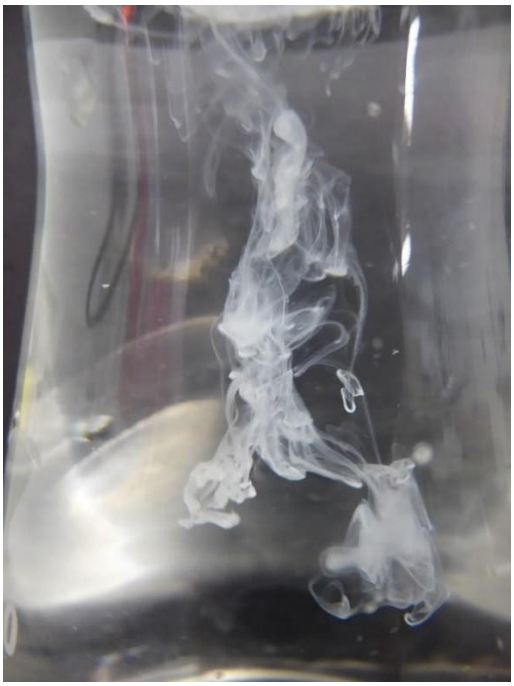


Fig 16. Milk disperses in warm water

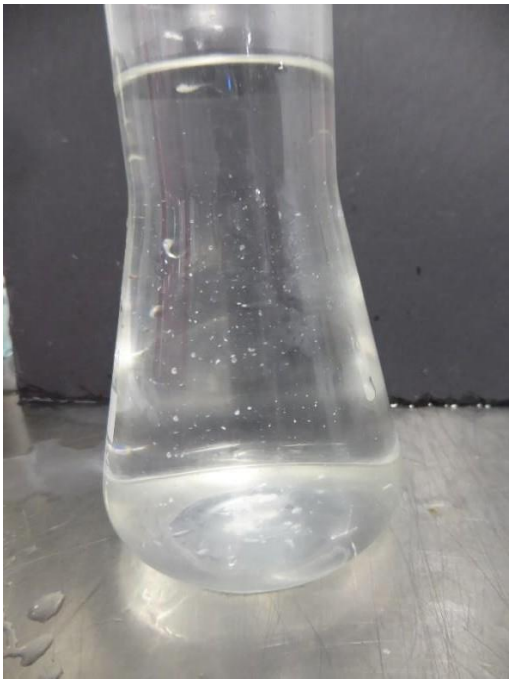


Fig 17. Milk forms flakes that fall to the bottom

Following flocculation, in the second stage of coagulation, the gel becomes progressively harder though cross-linking of the casein micelles. Both vats were tested for final clotting at 60 minutes after adding the rennet, by gently pushing the curd away from the side of the vat with a finger, and by placing a finger inside the clot, turning it horizontally, then flicking the curd apart with the thumb and seeing if the curd splits cleanly. The clotted curd from both types of milk were ready to cut at this time, and the BB clot felt firmer to the touch than the AA clot. The curds were cut into cubes using a set of three curd knives, cutting in 3 stages as shown in Figure 18 below.

Fig 18. Cutting the curds to form cubes

The cut curd was left to settle for 10 mins, then both were gently stirred identically for 2 mins, then settled for 10 mins, stirred for another 2 mins, continuing in this pattern for the next 1 hour. The curd pieces gently firm up and expel more whey during this time (Fig 19). The vat with AA milk was around 0.5°C warmer than the BB milk during this hour, with both dropping to around 29°C.

Fig 19. Curds being stirred

Fig 20. Moulds ready to fill

The curds were then filled into plastic basket moulds (Figs 20 and 21), filling to just above the rim. The curd then soon starts to settle down as the whey drains. The cheeses were turned 3 times by hand during the rest of the day, after15 minutes (Fig 22), after a further 30 minutes and finally after an additional 40 minutes (Fig 23). At this point they were covered with cloth and left in the warm room overnight.

Fig 21. Curds filled into moulds

Fig 22. Cheese turned for the first time

The following day each cheese (Fig 24) was weighed.

Fig 23. Cheese left overnight to drain

Fig 24. Cheese ready for weighing

The weights of each cheese are given below in Table 3. The AA cheeses were generally heavier than the BB cheeses and AA milk gave a 1.3% higher yield of cheese than the BB milk.

Table 3. Weights of AA and BB Cheese

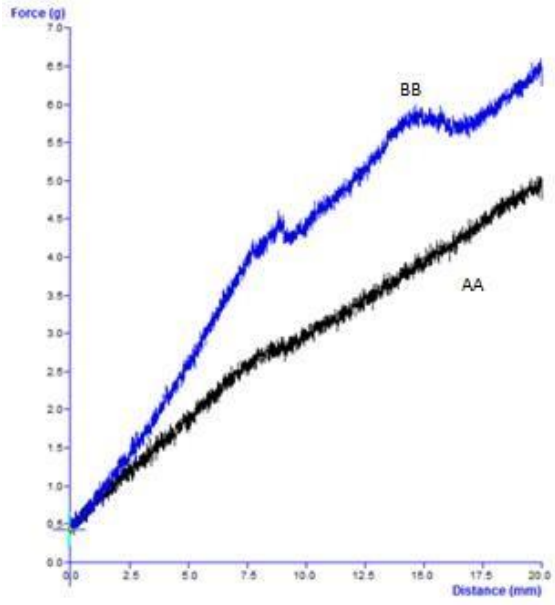
Milk Type	Cheese Weights (g)	Statistics
AA	240.7, 238.1, 234.3, 238.5, 238.5, 228.4, 237.1, 225.2, 234.3, 228.0, 231.4, 235.6, 214.2, 219.3, 224.9, 228.0, 219.9, 214.4, 224.7, 247.6, 232.8, 228.0	22 cheeses Total weight 5063.9g Average weight 230g Yield 25.3%
BB	201.8, 217.5, 219.4, 222.5, 206.7, 223.9, 230.5, 212.2, 227.4, 202.5, 221.9, 213.4, 206.2, 216.1, 211.1, 207.9, 214.5, 202.6, 202.2, 211.7, 208.1, 206.3, 110.4	22.5 cheeses Total weight 4796.8g Average weight 213g* Yield 24.0%

*the average weight excluded the half-cheese

The cheesemaking was repeated on the day that the previous cheeses were weighed. Exactly the same process was followed, swapping over the vats for each type of milk. Only 17kg of each milk was used, instead of 20kg. When the culture was added, AA milk was at 33.3°C and BB at 34.2°C. On adding the rennet, AA was 32.3°C and BB at 32.8°C. To confirm the potential firmer clot seen with BB milk the previous day, it was decided to fill 3 x 80g amounts of renneted milk into glass jars for further gel testing with the Texture Analyser, placing these gels in the lab in a water bath at 32°C.

Flocculation times were again checked for the milk clotting in the vats. At 27mins 40 sec, the BB milk went slightly bitty, and at 28mins 40 sec it was definitely flocculated. The AA milk was starting to go bitty in the water at 30 mins, and was definitely flocculated by 34 mins, around 5 minutes after the BB milk.

The gels in the lab were tested for firmness with the Texture Analyser at around 40 minutes, 40 minutes and 70 minutes after adding the rennet. Due to the time taken to perform each test, the BB gels were tested 2 minutes after the AA gels at each testing occasion.



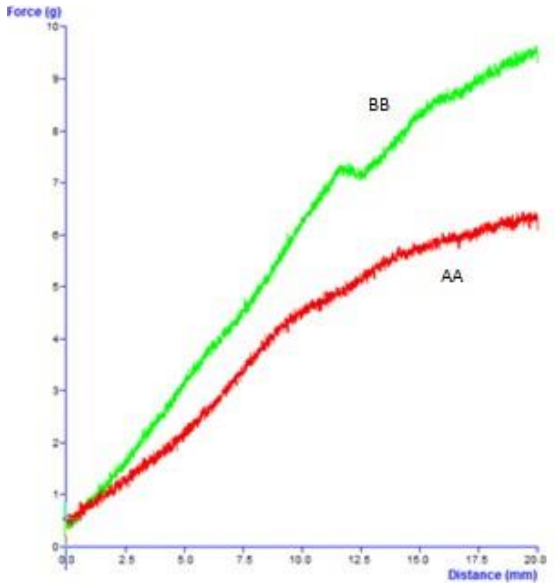


Fig 25. Comparison of texture profiles at ~40 mins Fig 26. Comparison of texture profiles at ~50 mins

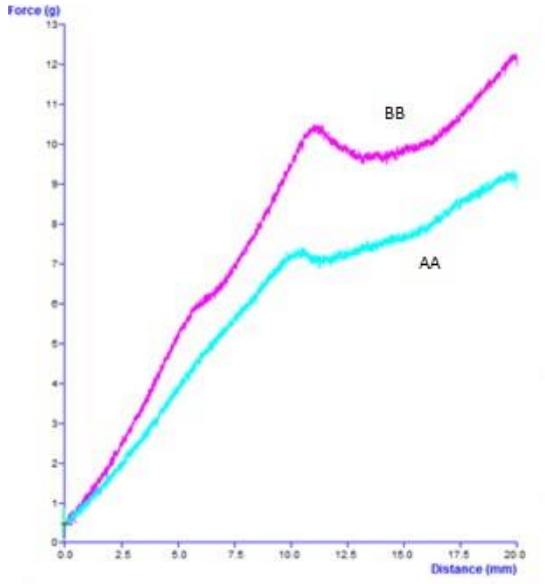


Fig 27. Comparison of texture profiles at ~70 mins

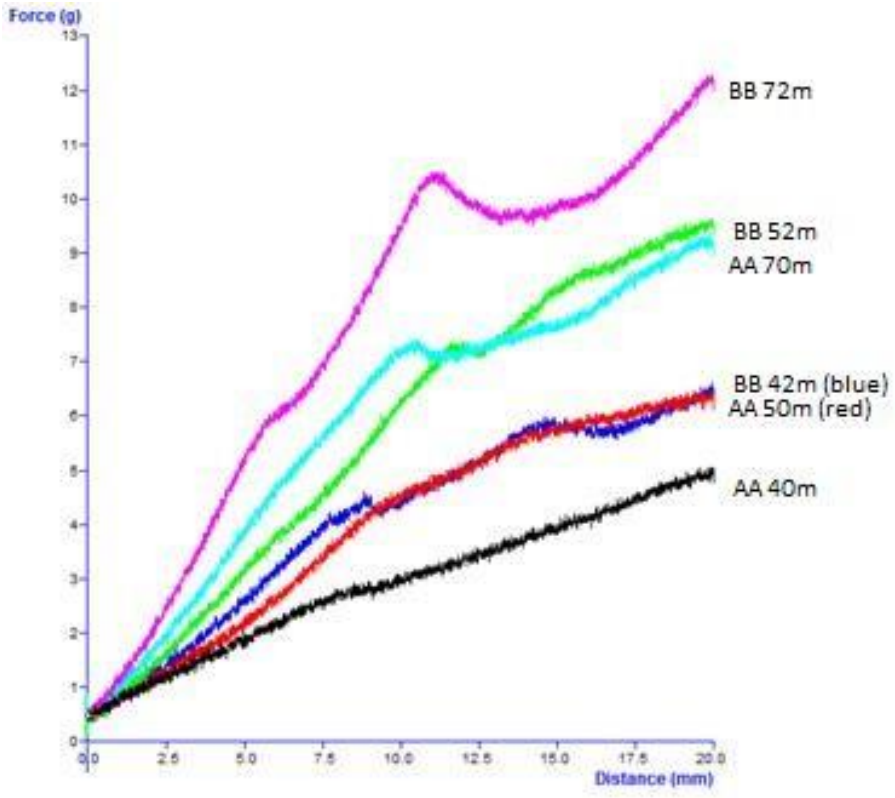


Fig 28. Comparison of all the texture profiles for the AA and BB gels

These gel tests (Figs 25 – 28) confirmed that the BB milk had clotted to a firmer gel than the AA milk at equivalent time intervals. The gels were also firmer than those achieved in the laboratory experiments in Trial 1, possibly due to the ripening time with starter cultures prior to adding the rennet.

The clotted milk in the vats was cut at 65 mins, with the curd in both vats feeling slightly weaker than in the trial the previous day. Towards the end of the 1 hour stirring and settling of the curds, the temperature in the AA vat was 28.5°C and in the BB vat it was 28.9°C.

The cheeses were weighed the following morning, with the results given below in Table 4. The weights of cheese from each type of milk was fractionally higher for the BB milk, at 24.4% yield, compared to 24.1% yield for AA milk. (The yields were calculated from 16.76kg of milk, allowing for that removed for gel tests). The average weights were more comparable this time, at 219g for AA and 221g for BB.

Table 4. Weights of AA and BB Cheese

Milk Type	Cheese Weights (g)	Statistics
AA	219.9, 223.2, 224.1, 219.1, 217.7, 228.3, 214.2, 213.3, 223.8, 215.0, 219.8, 212.5, 212.8, 226.7, 232.7, 209.8, 202.3, 219.8, 104.8	18.5 cheeses Total weight 4039.8g Average weight 219g Yield 24.1%
BB	221.2, 246.2, 237.2, 217.0, 232.4, 209.7, 225.6, 209.9, 209.9, 213.5, 219.9, 213.9, 213.9, 224.8, 220.9, 223.4, 213.7, 221.0, 110.0	18.5 cheeses Total weight 4084.1g Average weight 221g Yield 24.4%

CONCLUSIONS

The protein, fat and total solids levels of the three kappa-casein gene varieties of milk (AA, AB and BB) were not sufficiently matched to be able to rule out clotting effects due to these differences alone. The protein contents in the three types of milk from both of the milk deliveries varied from 4.10 to 4.89%, the fat from 5.71 to 6.98%, and total solids from 14.95 to 17.04%.

The laboratory experiments from the first batches of milk indicated that the rate of clotting and clot firmness appeared to correlate with total solids levels of the milk, with AB being by far the quickest and firmest to clot. To correlate with the kappa-casein gene theory, BB would have been the quickest to clot and given the firmest clot, followed by AB, with AA the slowest and weakest clot. However, it is of note that although the AA milk had higher protein and total solids compared to BB, the BB milk still managed to achieve a very comparable clotting rate and gel strength to the AA milk. This may indicate a positive effect of the presence of the BB gene, however, more trials would be required to verify the significance of this.

For the second batch of milk, the protein and total solids levels of the BB milk was still slightly lower (7% lower) than that of the AA milk. However, in the cheesemaking trials on these batches of milk (using just the AA and BB milk), the BB milk on both occasions indicated a slightly quicker clotting time (around 2.5 minutes quicker on the first occasion and 5 minutes on the second) and a firmer curd clot. However, this did not necessarily translate to a higher yield of cheese, with AA having a 1.3% higher yield on the first occasion, and BB having a 0.3% higher yield on the second occasion. On this small scale and number of trials it is not known how significant these differences are.

These findings necessitate further research into the potentially positive effect of the kappa-casein BB gene. It may be that using milk towards the end of the annual lactation period caused issues with higher than average fat levels and total solids levels. The kappa-casein effects may be more

pronounced in different varieties of cattle, or with specific types of cheese production. For a valid comparison, the milk samples used for analysis would either have to be selected for equal protein, fat and total solids levels, or standardised by industry methods, to eliminate effects due to variance in these levels.

With few studies having been carried out in the UK of the effect of the kappa-casein genotype on cheese production, there is great scope for further research on the potential impact this could have on selective breeding of cattle for improved cheese clotting times and yields.

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