THE EFFECT OF κ-CASEIN GENOTYPE ON THE QUALITY OF MILK AND FRESH CHEESE*

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Samples of evening milk from three groups of Holstein cows with κ-casein genotypes (AA, AB and BB) were taken for analysis and for cheesemaking to evaluate the dependence of some characteristics of milk, fresh cheese and whey on the κ-casein genotype. The milk with κ-casein genotype BB showed the best technological properties (higher dry matter and fat content, higher yield and shorter renneting time) during the cheesemaking, followed by κ-casein AB milk. Milk with κ-casein AA genotype had the lowest dry matter content and the least intensive syneresis. Renneting time of the milk was affected by milk protein content and indirectly correlated with milk fat content. Cheeses with a higher solids non-fat content had higher fat losses into the whey.

proteins; κ-casein; technological properties of milk; yield; cheese

INTRODUCTION

Bovine milk contains on average 36.0 g/l of total proteins of which 29.5 g/l are casein and 6.3 g/l are whey proteins (Whitney et al., 1976; Swaisgood, 1993; Fox, 2009). Knowledge about the genetic polymorphism of the milk proteins and its effects on milk composition is important for efficient milk processing (Hanuš et al., 2000a, b; Zadražil, 2002; Matějíček et al. 2008a, b). Because of its importance to stability of casein complexes, including the stability of whey proteins, κ-casein plays an exceptional role among the caseins. Several studies have been conducted to investigate associations between κ-CN protein variants and milk production and quality characteristics. Of all the genetic polymorphisms of dairy cattle affecting milk composition, the κ-CN genotype is one of the more significant, with associations identified between genotype and lifetime production (Lin et al., 1989), concentrations of individual milk proteins (McLean et al., 1984; Hanuš et al., 2004), protein yield and percentage (Tsiaras et al., 2005) as well as cheese production characteristics including rennet clotting time, curd formation and coagulation strength (Marziali, 1986; Pagnacco, Caroli, 1987; Hanuš et al., 2000a, b; Matějíček et al., 2008a, b).

In the casein micelle, there are only two common variants of κ-CN: A and B, although up to nine other variants have been characterized in bovine species (Farell et al., 2004). Variant A is predominant for most breeds (Zadražil, 2002) and is associated with higher whey protein content and lower casein content (Auldist et al., 2000; Molina, 2006). Studies on specific effects of κ-CN variants showed that milk from variant B cows contains more fat, protein, casein and κ-casein than milk from A variant cows (Bovenhius et al., 1992; Ng-Kwai-Hang, 1997; Bobe et al., 1999; Wedholm, 2006). The B variant association with the casein composition of milk has implications for milk quality for cheese production. Therefore, from the technological point of view, the B allele is preferable, because it improves renneting ability, coagulum quality, syneresis (Böhmová, Černý, 1994) and cheese yield (Dalgleish, 1999; Green, Grandison, 1999; Zadražil, 2002). The B variant has been associated with higher casein percentage (Lundén et al., 1997), higher total solids (McLean et al., 1984) and higher milk yield, fat yield, fat percentage and lactose yield (Tsiaras et al., 2005) than the A variant. Also, Jakob and Puhan (1992) concluded that milk with the κ-casein B allele gives a higher cheese yield because of many factors. These are mainly the higher milk protein content, better renneting abilities, coagulum firmness (thus, lower fineness) and reduced fat falling.

Differences in rennet coagulation properties have also been observed for genotypes of κ-CN. Shorter rennet clotting time, higher curd-firming rates, more intensive coagulum syneresis, higher yield and higher curd firmness have been reported for milk from cows with the BB variant than for milk from cows with the AA variant (Böhmová, Černý, 1994; Walsh et al., 1998; Hallén, 2007). Sherbon et al. (1967) also found that milk from cows with the κ-CN AA genotype has a longer renneting period as well as a lower coagulum tension than milk from cows with the κ-CN AB and BB genotypes.

The aim of this work was to study the effect of κ-casein genotypes on some milk technological characteristics and to confirm or refute the effects of those different milk cheesemaking properties in real samples of fresh cheese prepared on laboratory scale under standard technological

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conditions. The dependence of the whey (as an important whey by-product) characteristics on the κ-casein genotype was monitored as well together with correlations between milk, cheese and whey analytical values. Values are expressed as the average ± standard deviation.

MATERIAL AND METHODS

Two hundred samples of evening milk from Holstein cows were taken for analysis and for cheesemaking. All the cows were fed the same monodiet during the year and the feed portion was determined according to the average group efficiency. The effect of feed on the monitored parameters was therefore eliminated. On average, 0.2 kg grain was fed per 1 litre of milk and the cows received it directly in their mixed feeding ration.

The experimental set consisted of 50 Holstein breed cows which were monitored during their second and third lactation periods. The cows were divided into three groups according to the κ-casein genotype. There were 13 cows with the AA genotype, 18 cows with the AB genotype and 19 cows with the BB genotype.

Fresh cheese was made every month from September to December from the milk of all healthy and milking cows. Monitoring of the overall health status included the udder health check as well. Milk samples (1 l for cheesemaking + 200 ml for analysis) were immediately cooled to below 10 °C and transported to the laboratory. Titratable acidity (TA) was measured in Soxhlet-Henkel units (SH) by the Soxhlet-Henkel method (Czech standard method ČSN 57 0530). Fat (F), protein (P), lactose (L), dry matter (DM) and solids non-fat (SNF) contents were determined with a MilkoScan FT 120 (Foss Analytical A/S, Denmark). Fresh cheese was made from the milk samples. Renneting time (RT) and cheese weight (W) were recorded. Cheese fat content was measured by the Van Gulik method (Czech standard method ČSN 57 0107) and cheese yield (Y) was calculated. DM contents of the cheese and the whey were determined by drying 1–2 g of sample at 110 to 140 °C to constant weight (weight difference less than 0.002 g under infrared light (Precisa HA 300, Switzerland). Fat content (Gerber method in ČSN 57 0530), dry matter and volume of whey (WV) were also measured.

The results were submitted to an analysis of variance (*F*-test) and the means compared using the Tukey test at the 95% level of significance ($p = 0.05$) using the software package Statistica for Windows version 8 (StatSoft Inc.). To determine correlation coefficients (r) between the data *P*-values < 0.05 were regarded as statistically significant. Correlations were described as a weak ($|r| < 0.3$), medium ($|r| = 0.3$ to 0.8) and strong ($|r| > 0.8$).

Cheesemaking procedure. The milk was heated under laboratory conditions for approx. 7 minutes to reach the pasteurisation temperature 72–74 °C with holding time 1–2 seconds and then cooled to 30–32 °C. Mesophilic starter culture (1%) (Laktoflora®, Milcom a.s.) was added and thoroughly mixed with the milk. The mixture was incubated for 30 minutes at 30–32 °C. The rennet (Lactochym®; Milcom a.s.; 75% chymosine and 25% pepsin; strength 1:5 000) was diluted by distilled water (1:10). Two millilitres of this solution was added and mixed well. The coagulation time was from 60 to 120 minutes. The curd was cut as soon as a glass-like flaw was observed. The size of the grains was approximately 1 cm^3. The curd was incubated for 30 minutes and quantitatively transferred into forms for 1 litre of milk. Cheese in the form was inverted and left for 24 h for whey drainage. Cheese yield was calculated using the formula: Y (in DU) = [W (in kg) x DM (in %)]/ milk weight (in kg), where DU are dry matter units coming into the cheese from 1 kg of milk (Böhmová, Černý, 1994).

RESULTS AND DISCUSSION

Most authors (Gonyon et al., 1987; Jakob, Puhan, 1992; Macheboeuf et al., 1993; Böhmová, Černý, 1994; Dvořák et al., 1994) report a higher protein content in milk with the κ-casein BB genotype than in milk with AA or AB genotypes. Our results (Table 1) did not confirm these statistically significant differences in protein content between genotypes. The effect of genetic polymorphism could be influenced and overlapped by many other factors. The most important factors from the breeding point of view are the cow's health and their nutrition (Hui, 1993).

A statistically significant association was observed between genotype and cheese dry matter and fat content (Table 2). The κ-casein BB genotype showed statistically significant effect on the cheese DM ($p = 0.014$) and F content ($p = 0.017$) in comparison with AA genotype. There was also a significant difference in DM between cheeses made from milk from AA and AB genotype cows. The milk from cows with BB genotype also showed statistically significant RT in comparison with AB genotype ($p = 0.019$) and this difference was more distinct in comparison with AA genotype ($p < 0.001$). All these observations are in agreement with literature reports (Sherbon et al., 1967; Hanuš et al., 1995; Zadražil, 2002). The cheese weights were, on average, the highest in case of AA genotype. There were statistically significant differences between milk from cows with AA and AB genotypes ($p < 0.001$) and between milk from AA and BB genotypes ($p < 0.001$). Dry matter content of cheese was the lowest in case of AA genotype and the yield values were not as high as with BB genotype. The BB genotype showed statistically significant effect on the cheese yield in comparison with AA genotype ($p < 0.001$). There was also a significant difference in Y between cheeses made from milk from BB and AB genotype cows ($p = 0.002$). This observation is in agreement with literature reports (Jakob, Puhan, 1992; Böhmová, Černý, 1994).

The lowest whey volumes were from the milk of genotype AA (Table 3). Syneresis was, therefore, the least intensive in the case of the κ-casein AA milk. This observation is also in agreement with literature reports (Böhmová, Černý, 1994).

Table 1. Titratable acidity, fat, proteins, lactose, dry matter and solid non-fat content of milk from cows having different κ-casein genotypes

Month	Genotype	TA (SH)	F (%)	P (%)	L (%)	DM (%)	SNF (%)
9	AA	7.31 ± 0.06	4.88 ± 0.08	3.49 ± 0.07	4.32 ± 0.03	13.23 ± 0.23	8.35 ± 0.32
10	AA	7.31± 0.05	4.32 ± 0.07	3.73 ± 0.08	4.55 ± 0.02	13.15 ± 0.33	8.82 ± 0.34
11	AA	6.68 ± 0.10	3.88 ± 0.09	3.74 ± 0.07	4.59 ± 0.02	12.75 ± 0.37	8.87 ± 0.36
12	AA	7.42 ± 0.06	3.77 ± 0.08	3.56 ± 0.09	4.49 ± 0.03	12.35 ± 0.26	8.59 ± 0.26
9	AB	7.16 ± 0.07	3.90 ± 0.07	3.58 ± 0.10	4.80 ± 0.03	12.82 ± 0.37	8.91 ± 0.39
10	AB	6.98 ± 0.08	4.39 ± 0.10	3.58 ± 0.11	4.76 ± 0.03	13.27 ± 0.38	8.88 ± 0.39
11	AB	7.05 ± 0.07	4.48 ± 0.08	3.83 ± 0.24	4.70 ± 0.04	13.56 ± 0.32	9.07 ± 0.32
12	AB	6.68 ± 0.14	4.47 ± 0.09	3.55 ± 0.09	4.74 ± 0.04	13.31 ± 0.36	8.84 ± 0.34
9	BB	7.24 ± 0.08	4.10 ± 0.12	3.41 ± 0.08	4.91 ± 0.04	12.96 ± 0.38	8.87 ± 0.33
10	BB	6.83 ± 0.13	4.36 ± 0.09	3.52 ± 0.09	4.77 ± 0.04	13.19 ± 0.39	8.83 ± 0.38
11	BB	7.05 ± 0.12	4.30 ± 0.09	3.59 ± 0.09	4.58 ± 0.05	13.01 ± 0.37	8.71 ± 0.34
12	BB	7.42 ± 0.12	3.97 ± 0.10	3.40 ± 0.09	4.74 ± 0.04	12.64 ± 0.39	8.67 ± 0.40

Table 2. Renneting time, dry matter and fat contents, yield and weight of cheeses made with milk from cows with different κ-casein genotypes

Month	Genotype	RT (min)	DM (%)	F (%)	Y (DU)	W (g)
9	AA	155 ± 9.13	34.18 ± 0.88	16.50 ± 1.05	8.27 ± 0.25	290.31± 10.76
10	AA	190 ± 14.94	34.12 ± 0.89	14.00 ± 1.21	9.20 ± 0.21	268.85 ± 11.65
11	AA	155 ± 12.25	33.67 ± 1.11	13.00 ± 1.84	7.52 ± 0.35	223.41 ± 12.25
12	AA	145 ± 13.29	37.05 ± 1.18	17.00 ± 1.76	7.80 ± 0.45	210.63 ± 14.16
9	AB	150 ± 11.97	38.16 ± 1.26	17.50 ± 1.39	8.54 ± 0.45	205.64 ± 13.79
10	AB	160 ± 11.69	40.17 ± 1.64	18.00 ± 1.83	8.55 ± 0.49	212.88 ± 24.99
11	AB	165 ± 10.33	39.17 ± 1.50	18.00 ± 1.85	8.53 ± 0.41	217.63 ± 11.01
12	AB	145 ± 14.26	40.01 ± 1.65	17.00 ± 1.22	8.27 ± 0.46	206.68 ± 10.98
9	BB	140 ± 12.65	38.43 ± 1.41	18.00 ± 2.15	8.12 ± 0.39	253.56 ± 18.38
10	BB	140 ± 10.00	42.03 ± 1.31	19.50 ± 1.38	8.94 ± 0.42	212.75 ± 12.53
11	BB	165 ± 12.52	36.87 ± 1.31	17.00 ± 1.34	8.58 ± 0.31	232.63 ± 8.64
12	BB	140 ± 13.17	44.44 ± 1.28	19.00 ± 1.80	9.88 ± 0.29	222.32 ± 9.88

Table 3. Fat, dry matter content and volume of whey samples

Month	Genotype	F (%)	DM (%)	WV (ml)
9	AA	0.13 ± 0.03	9.31 ± 1.18	630 ± 35
10	AA	0.62 ± 0.12	9.18 ± 1.43	567 ± 88
11	AA	0.52 ± 0.08	8.70 ± 0.89	655 ± 41
12	AA	0.52 ± 0.08	8.81 ±1.21	665 ± 39
9	AB	0.42 ± 0.07	9.20 ± 1.17	750 ± 31
10	AB	0.62 ± 0.08	9.38 ± 1.21	644 ± 34
11	AB	0.71 ± 0.12	9.02 ± 1.08	680 ± 46
12	AB	0.62 ± 0.11	8.57 ± 1.26	680 ± 81
9	BB	0.23 ± 0.08	8.17 ± 1.10	760 ± 55
10	BB	0.33 ± 0.10	8.97 ± 1.78	656 ± 48
11	BB	0.52 ± 0.09	7.53 ± 1.07	685 ± 56
12	BB	0.43 ± 0.11	9.02 ± 1.31	600 ± 35

Among the correlations of all milk, cheese and whey analytical values we confirmed the important contribution of fat to the DM of milk and cheese content (r_{milk} = 0.838; r_{cheese} = 0.851). Renneting time of the milk was affected by the milk protein content (*r* = 0.698) and indirectly correlated with the milk fat content (*r* = –0.580). Cheeses with a higher SNF content had higher fat losses into the whey (*r* = 0.589). These findings are summarised in Table 4 and are in agreement with the literature statements (Hui, 1993; Banks, 1998; Dalgleish, 1999; Robinson, Wilbey, 1999; Fox et al., 2000; Varnan, Sutherland, 2001). The effect of κ-casein genotypes on some milk cheesemaking properties in real samples of fresh cheese prepared on laboratory scale under standard technological conditions was therefore confirmed.

Table 4. The effect of κ-casein genotypes on some milk cheesemaking properties

Genetic variant of κ-casein:	A	B
Dry matter of cheese	*	↑
Fat content of cheese	*	↑
Cheese yield	*	↑
Syneresis	*	↑
Renneting time	*	↓
Volume of whey	*	↑

* = in comparison with

CONCLUSIONS

Milk with the κ-casein genotype BB showed the best technological properties (higher dry matter and fat content, higher yield and shorter renneting time) during cheesemaking, followed by the κ-casein AB milk. Milk with the κ-casein AA genotype showed the least desirable cheesemaking properties (lower dry matter and the least intensive syneresis). Fat in milk and cheese significantly contributed to their dry matter; renneting time of the milk was affected by the milk protein content and indirectly correlated with the milk fat content. Cheeses with a higher solids non-fat had higher fat losses into the whey. The results can be used to select for a protein system genotype with a higher percentage of the dry matter transfer from the milk into the cheese, a higher content of proteins and caseins in the cheese and a suitable coagulation process.

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Vliv genotypu κ-kaseinu na kvalitu mléka a čerstvých sýrů.

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Vliv genetické varianty κ-kaseinu na vybrané technologické vlastnosti mléka, sýrů a syrovátky byl sledován během demonstrační výroby čerstvých sýrů. Analyzované vzorky byly odebrány z večerního nádoje dojnic holštýnského plemene, které byly rozděleny do tří skupin podle genetické varianty κ-kaseinu (AA, AB a BB). Mléko obsahující genetickou variantu κ-kaseinu BB vykazovalo v porovnání s ostatními variantami nejlepší technologické vlastnosti (vyšší sušinu, obsah tuku, vyšší výtěžnost sýrů a kratší dobu srážení) během výroby sýrů. Následovalo mléko s genetickou variantou AB. Mléko s genetickou variantou AA mělo nejnižší obsah sušiny a během výroby sýrů vykazovalo nejméně intenzivní synerézy. Dále byla zjištěna významná závislost obsahu tuku v mléce a sýru na obsahu sušiny. Doba srážení mléka byla ovlivněna obsahem bílkovin v mléce a nepřímo korelovala s obsahem mléčného tuku. Sýry s vyšší tukuprostou sušinou vykazovaly vyšší ztráty mléčného tuku do syrovátky.

bílkoviny; κ-kasein; technologické vlastnosti mléka; výtěžnost sýrů; sýr

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