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The influence of casein haplotype on quality, coagulation, and yield traits of milk from Italian Holstein cows

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ABSTRACT

The aim of this work was to investigate the effect of casein haplotype (CSN1S1, CSN2, and CSN3) on quality, coagulation, and yield traits of milk from Italian Holstein cows. The casein haplotype was determined by isoelectric focusing; milk clotting properties were determined by using a mechanical lacto-dynamographic instrument; and the yields of pressed and *pasta filata* cheeses were expressed as kilograms of cheese per 100 kg of milk processed. Statistical analysis showed a significant effect of the casein haplotype. In particular, *BB-*A^1A^1*-AA* milk showed the highest fat content (4.01 g/100 g), whereas *BB-*A^2A^2*-BB* milk had a higher protein content, the best coagulation characteristics, and the highest yields in pressed and *pasta filata* cheeses, and, consequently, better ability to retain fat and protein in the curd. The results of this study suggest that knowledge of milk protein polymorphisms not only allows the production of milk with specific qualitative and quantitative characteristics, but it could also be used as a specific marker within a breed to identify milk suitable for cheesemaking, which confers an economical advantage for dairy producers.

Key words: casein haplotype, milk quality, coagulation and yield traits, Italian Holstein cows

INTRODUCTION

In many milk-producing countries, such as France, Greece, and Italy, a large part of the total milk produced is used for cheese making. In Italy, many specialized structures in the dairy industry produce different typical and traditional cheeses, in particular Protected Designation of Origin products, such as Grana Padano, Parmigiano Reggiano, Gorgonzola, and Asiago cheeses (Pieri, 2010). Currently, about 70% of product milk in Italy is destined to cheesemaking, and approximately 50% of this fraction is used for Protected Designation of Origin products (Agriculture and Rural Development, 2015). The amount and quality of cheese obtained per volume unit of milk processed are important for the profitability of the dairy industry. Milk coagulation properties (**MCP**) are considered good indicators of both the quality and yield of cheese (Bittante, 2011), and they are commonly measured as rennet coagulation time (**RCT**, min), curd-firming rate (**k_{20}**, min), and curd firmness (**a_{30}**, mm). However, milk clotting properties are affected by various factors, such as total casein and calcium concentrations (Storry et al., 1983), pH (Najera et al., 2003), SCC (Politis and Ng-Kwai-Hang, 1988), genetic polymorphism of milk proteins (Schaar et al., 1985; Mayer et al., 1997; Ikonen et al., 1999), stage of lactation (Okigbo et al., 1985; Ostersen et al., 1997), season (O'Brien et al., 1999), and breed (Auldist et al., 2002, 2004; De Marchi et al., 2008).

Many authors have shown that genetic variants of milk proteins affect both absolute (Ikonen et al., 1997; Hallén et al., 2008) and relative concentrations (Bobe et al., 1999; Heck et al., 2009; Bonfatti et al., 2010) of the individual milk proteins. In particular, the most consistent effect was found for CSN3 (κ-CN) variant B, which has been shown to have a positive effect on κ-CN concentration of milk (Bobe et al., 1999; Hallén et al., 2008; Heck et al., 2009), and it was associated with smaller average casein micelle size (Walsh et al., 1998a), the better coagulating properties, and a higher cheese yield variation in casein micelle size (Mayer et al., 1997; Ng-Kwai- Hang, 1998), whereas the CSN2 polymorphism was found to be related to fat percentage and fat and protein yields (Bovenhuis et al., 1992; Ikonen et al., 1999). However, the effects of casein haplotypes on milk clotting properties and cheese yield were evaluated considering a single locus (Aleandri et al., 1990; Bovenhuis et al., 1992). Many authors (Ojala et al., 1997; Braunschweig et al., 2000) instead suggested that the effects of individual locus are confounded in statistical analyses, even when they are included simultaneously in the model, because their influence on milk traits could be due to the cumulative effect of different casein loci on chromosome 6. Consequently, it is believed that a better estimation of effects of casein

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genotypes is obtained by studying whole combinations of alleles (casein cluster) rather than single alleles due to the tight genetic linkage among casein loci (Gambacorta et al., 1994, 2005; Boettcher et al., 2004).

The objective of this study was to evaluate the effect of casein haplotype on quality, coagulation, and yield traits of milk from Italian Holstein cows.

MATERIALS AND METHODS

Samples

This study was conducted on an intensive farm, consisting of more than 500 Italian Holstein cows, in the countryside of Potenza, southern Italy. Before starting the test, about 250 animals in lactation were identified by isoelectric focusing (**IEF**) to define their haplotypes. Haplotypes were formed by the combination of the individual allelic loci aggregated by CSN1S1, CSN2, and CSN3 (α_{S1}-, β-, and κ-CN, respectively). After definition of individual phenotypes, the cows were grouped by haplotype. Each group included 10 to 12 animals, at an equal stage of lactation (70 to 120 d postpartum), season (spring), and order of birth (third calving). All animals were fed a commercial standard diet according to milk yield. The individual cow milk of the morning milking was collected once and all milk samples were stored at 4°C until analysis.

Sample Preparation for IEF

Individual milk samples, kept at 4°C, were defatted by centrifugation (3,000 × *g* for 30 min at 4°C); the fat layer was solidified at −20°C for 20 min and removed. Casein was prepared by isoelectric precipitation at pH 4.6 with 10% (vol/vol) acid acetic and 1 *M* sodium acetate at room temperature. After centrifugation at 3,000 × *g* for 10 min at 4°C, the casein pellet was washed twice with distilled water and stored at −20°C. The whole casein was dissolved in 9 *M* urea and 1% 2-mercaptoethanol for IEF analysis, according to Aschaffenburg and Drewry (1959).

Genetic Variants of Caseins by IEF

The genetic variants of the different caseins by IEF were determined according to the method of Trieu-Cuot and Gripon (1981). The IEF analysis was performed on polyacrylamide gel (5% acrylamide and 0.15% bisacrylamide) with a thickness of 1 mm and 2% carrier ampholytes to create a gradient of pH 2.5 to 10. The gel was prefocused at a constant value of 0.35 W/mL of gel and at the maximum limit of 1,200 V. The gel was stained in Coomassie Brilliant Blue G-250 according to Blakesley and Boezi (1977). Haplotype frequencies were determined by the number of each haplotype (n_i) divided by the total number of haplotypes (n_{tot}): Frequency (%) = ($n_{i,\ \mathrm{haplotype}}/n_{tot,\ \mathrm{haplotype}}$) × 100. Haplotypes are presented in the order CSN1S1-CSN2-CSN3.

Compositional Analysis

Milk and whey samples were analyzed for DM, total protein (total N × 6.38; by Kjeldahl method), ash (AOAC International, 2000), fat (Röse-Gottlieb method; IDF, 1996), and lactose (IDF, 1974). The pH was measured using a pH meter (model PHM 92, Radiometer, Copenhagen, Denmark) after calibrating with fresh pH 4.0 and 7.0 standard buffers. Milk SCC were determined by fluoro-opto-electronic method (Schmidt-Madsen, 1975), with a Fossomatic 250 (Foss Electric, Hillerød, Denmark).

Analysis of Milk Clotting Properties

Milk clotting properties were determined by mechanical lacto-dynamographic instrument (Formagraph, Foss, Padova, Italy) as previously described by Cipolat-Gotet et al. (2012). In brief, each individual milk sample (10 mL) was heated to 35°C before the addition of 200 μL of the rennet solution [Hansen Naturen Plus 215 (Pacovis Amrein AG, Bern, Switzerland), with 80 ± 5% chymosin and 20 ± 5% pepsin and 215 international milk clotting units (**IMCU**)/mL, which was diluted to 1.2% (wt/vol) in distilled water to achieve 0.0513 IMCU/milk mL]. The lacto-dynamograph recorded the width (mm) of the oscillatory graph every 15 s throughout the extended observation period (min after rennet addition). Traditional MCP parameters were provided directly by the instrument, including RCT (min), k_{20} (min), and a_{30} (mm).

Cheese Yield

Each milk sample was cheesemaking for both *pasta filata* and pressed paste cheeses. Briefly, 2 L of milk was heated to 37°C and coagulated by using lamb rennet paste (177 IMCU/mL; 40.0 mg/kg) for 30 to 40 min. Then, for *pasta filata* cheese, the coagulum was first cut coarsely, heated under whey at 45°C for 2 h, reduced to particles of about 1.5 cm, and held at room temperature until the pH reached approximately 5.3. When the acidified curd was ready, it was manually stretched in hot water (70–80°C). For pressed paste cheese, the coagulum was first cut coarsely, held under whey at 37°C for 2 h, and reduced to particles of about 1.5 cm. Finally, the whey was removed and the curd was pressed. Cheese yield was expressed as kilograms of cheese per 100 kg of milk processed. All cheeses were stored at 10 to 12°C and 75 to 80% relative humidity and weighed after 24 h.

Statistical Analysis

Data were analyzed according to the following linear model (SAS Institute, 1996):

$$y_{ik} = \mu + \alpha_i + \varepsilon_{ik},$$

where y_{ik} is the observation; μ is the overall mean; α_i is the fixed effect of the *i*th haplotype ($i = 1, 2, 3, 4, 5$); and ε_{ik} is the random error. Before setting the values, expressed as percentages, they were subjected to angular transformation. Student's *t*-test was used for all variable comparisons. Differences between means at the 95% ($P < 0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSION

Haplotype Frequencies

In the study population, 5 different casein haplotypes were identified by IEF. The different allelic combinations of loci CSN1S1, CSN2, and CSN3 and their frequencies are reported in Table 1. Haplotypes $BB\text{-}A^2A^1\text{-}AA$, $BB\text{-}A^2A^2\text{-}AA$, and $BB\text{-}A^2A^2\text{-}AB$ showed the highest frequency (>28%), followed by haplotype $BB\text{-}A^2A^2\text{-}BB$ (7.89%), and haplotype $BB\text{-}A^1A^1\text{-}AA$ showed the lowest frequency (<5%). In the Italian Holstein breed, the selection boost carried out only for specific aspects related to some phenotypic characteristics of milk (e.g., fat and protein yields) led to a reduction of the variability of allelic combinations.

Chemical Composition

Casein haplotype is closely associated with milk quality (Ng-Kwai Hang et al., 1986; Boettcher et al., 2004; Gambacorta et al., 2005). The ANOVA showed a significant effect of casein haplotype on all evaluated parameters ($P < 0.01$). The physicochemical composition and SCC of milk with different casein haplotypes is shown in Table 2. Dry matter content ranged between 12.44 g/100 g ($BB\text{-}A^2A^2\text{-}AB$ milk) and 13.01 g/100 g ($BB\text{-}A^2A^2\text{-}BB$ milk); the differences among studied samples were not significant. The $BB\text{-}A^1A^1\text{-}AA$ milk showed the highest fat content (4.01 g/100 g; $P < 0.05$), in agreement with that reported by Ikonen et al. (2001) in Finnish Ayrshire milk. Many studies have highlighted the influence of *CSN2* genotype on fat content: Ng-Kwai-Hang et al. (1986) showed that milk with the A^1A^1 genotype showed a greater fat content, whereas Kiddy et al. (1970) observed a higher fat content in milk with the A^2A^2 genotype. Other authors (Aleandri et al., 1990; Van Eenennaam and Medrano, 1991) revealed no significant difference among casein genotypes for either fat or protein. Relative to the influence of CSN3 genotype on fat and protein, many authors (Ng-Kwai-Hang et al., 1986; Strzalkowska et al., 2002; Ng-Kwai-Hang and Grosclaude, 2003) reported the superiority of variant B compared with variant A. In our study, $BB\text{-}A^2A^2\text{-}BB$ milk showed a higher total protein content (3.61 g/100 g), differing statistically ($P < 0.05$) from that of $BB\text{-}A^2A^2\text{-}AB$ and $BB\text{-}A^1A^1\text{-}AA$ milk. These findings are in disagreement with Matassino et al. (2002), who found that, in Italian Holstein cows, $BB\text{-}A^2A^1\text{-}AA$ milk showed a greater protein content, whereas, in Brown cows, $BB\text{-}A^1A^1\text{-}BB$ milk had the highest protein content. Boettcher et al. (2004) found the highest protein content in $BB\text{-}A^1A^1\text{-}BB$ milk in both Holstein and Brown cows. Many authors also detected a positive correlation between protein content and the *B* allele of CSN3 (Bovenhuis et al., 1992; Bovenhuis and Weller, 1994) and the A^2 allele of CSN2 (Bech and Kristiansen, 1990; Velmala et al., 1995; Freyer et al., 1998). Lactose content was highest in $BB\text{-}A^2A^2\text{-}AA$ and $BB\text{-}A^2A^1\text{-}AA$ milks (4.87 and 4.91 g/100 g, respectively; $P < 0.05$), whereas $BB\text{-}A^1A^1\text{-}AA$ milk showed the lowest content (4.43 g/100 g; $P < 0.05$). Conversely, Matassino et al. (2002) detected the highest lactose content in $BB\text{-}A^2A^2\text{-}BB$ milk. Concerning ash content, $BB\text{-}A^1A^1\text{-}AA$ and $BB\text{-}A^2A^1\text{-}AA$ milks showed the highest content (0.77 and 0.76 g/100 g, respectively; $P < 0.05$). Statistical analysis highlighted a significant effect of casein haplotype on pH, in agreement with Matassino et al. (2002). In particular, the pH value ranged between 6.54 in $BB\text{-}A^2A^2\text{-}AB$ milk and 6.81 in $BB\text{-}A^1A^1\text{-}AA$ milk; significant differences ($P < 0.05$) were found among milks with different casein haplotype. All studied milk samples showed a mean SCC lower than the limit set by the "Hygiene Package" by the Regulation European Commission (Reg. EC 853/2004; 400 × 10^3 cells/mL; Regulation EC 853/2004; EC, 2004). In particular, $BB\text{-}A^2A^2\text{-}AB$

Table 1. Frequencies of the α_{S1}-, β-, and κ-CN haplotypes in Italian Holstein milk

Haplotype (CSN1S1-CSN2-CSN3)	Frequency (%)
$BB\text{-}A^2A^1\text{-}AA$	30.13
$BB\text{-}A^2A^2\text{-}AA$	29.09
$BB\text{-}A^2A^2\text{-}AB$	28.41
$BB\text{-}A^2A^2\text{-}BB$	7.89
$BB\text{-}A^1A^1\text{-}AA$	4.47

Table 2. Physicochemical composition (g/100 g, unless otherwise noted) and SCC of Italian Holstein milk with different casein haplotypes

	Haplotype (CSN1S1-CSN2-CSN3)									
	$BB\text{-}A^{2}A^{2}\text{-}AB$		$BB\text{-}A^{2}A^{2}\text{-}BB$		$BB\text{-}A^{2}A^{2}\text{-}AA$		$BB\text{-}A^{1}A^{1}\text{-}AA$		$BB\text{-}A^{2}A^{1}\text{-}AA$	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM	12.44^{a}	0.36	13.01^{a}	1.11	12.72^{a}	0.52	12.53^{a}	0.68	12.72^{a}	0.39
Fat	3.60^{a}	0.07	3.86^{a}	0.55	3.68^{a}	0.35	4.01^{b}	0.55	3.58^{a}	0.27
Total protein	3.33^{a}	0.18	3.61^{b}	0.12	3.43^{ab}	0.26	3.32^{a}	0.13	3.45^{ab}	0.19
Ash	0.72^{a}	0.03	0.73^{a}	0.04	0.72^{a}	0.02	0.77^{b}	0.08	0.76^{b}	0.04
Lactose	4.78^{a}	0.22	4.79^{a}	0.17	4.87^{b}	0.07	4.43^{c}	0.01	4.91^{b}	0.11
pH	6.54^{a}	0.03	6.66^{b}	0.04	6.74^{c}	0.06	6.81^{d}	0.01	6.75^{c}	0.05
SCC (10^3 cells/mL)	49.00^{a}	4.97	71.75^{b}	19.96	48.83^{a}	17.29	99.50^{c}	2.12	76.20^{b}	24.01

$^{a–d}$Means within a column with different superscripts differ ($P < 0.05$).

and $BB\text{-}A^{2}A^{2}\text{-}AA$ milks showed the lowest SCC (49.00×10^3 and 48.50×10^3 cells/mL, respectively; $P < 0.05$) compared with other studied samples.

Milk Clotting Properties

The MCP parameters of Italian Holstein milk with different casein haplotype are reported in Table 3. Rennet coagulation time, k_{20}, and a_{30} parameters were significantly influenced by casein haplotype ($P < 0.05$), in agreement with results reported by Caroli et al. (2009), who affirmed that both the quantitative (linked to the single genetic mutation) and qualitative variations (linked to different capacity of expression of alleles that control the synthesis of individual caseins and to the interactions among closely associated loci) markedly affect the enzymatic phase and the physicochemical performance of rennet coagulation. Overall, RCT and k_{20} values ranged between 14.45 and 17.78 min, and between 5.83 and 10.92 min, respectively, whereas the a_{30} value ranged between 21.25 and 38.80 mm. All milk samples were classified as type A (optimal milk) according to the classification proposed by Zannoni and Annibaldi (1981). In particular, $BB\text{-}A^{2}A^{2}\text{-}AB$ and $BB\text{-}A^{2}A^{2}\text{-}BB$ milks showed the lowest RCT value ($P < 0.05$); furthermore, the last haplotype ($BB\text{-}A^{2}A^{2}\text{-}BB$) showed the best coagulation characteristics, presenting the lowest k_{20} value ($P < 0.05$) and the highest a_{30} value ($P < 0.05$). In contrast, $BB\text{-}A^{1}A^{1}\text{-}AA$ milk showed the highest k_{20} value ($P < 0.05$) and the lowest a_{30} value ($P < 0.05$), in disagreement with Marziali and Ng-Kwai-Hang (1986), who reported that CSN2 $A^{1}A^{1}$ milk showed the best coagulation characteristics. Our findings are in agreement with those of Summer et al. (2004), who detected that milk with a lower curd-firming time (k_{20}) showed better curd firmness (a_{30}). Within the CSN3 locus, for CSN1S1 variant *BB* and CSN2 variant $A^{2}A^{2}$, $BB\text{-}A^{2}A^{2}\text{-}AA$ milk showed the worst MCP values ($P < 0.05$). The best coagulation characteristics of CSN3 *BB* milk compared with CSN3 *AA* milk were demonstrated by Nuyts-Petit et al. (1997) in Normande cows and by Walsh et al. (1998a) in Irish Holstein cows; this was due to the lower loss of casein and fat of CSN3 *BB* milk during cheesemaking (Hartung and Gernand, 1997; Pabst, 1998; Walsh et al., 1998b). Mariani and Summer (1999) and Mariani et al. (2002) also reported that CSN3 *BB* milk is characterized by a higher CSN3 content and by smaller casein micelles.

Cheese Yield and Whey Composition

Yields of pressed and *pasta filata* cheeses obtained from Italian Holstein milk for the different casein haplotypes is shown in Figure 1. Overall, casein haplo-

Table 3. Milk coagulation properties of Italian Holstein milk with different casein haplotypes[1]

Haplotype (CSN1S1-CSN2-CSN3)	RCT (min)		k_{20} (min)		a_{30} (mm)	
	Mean	SD	Mean	SD	Mean	SD
$BB\text{-}A^{2}A^{2}\text{-}AB$	14.79^{a}	0.41	8.12^{a}	0.73	35.36^{a}	0.89
$BB\text{-}A^{2}A^{2}\text{-}BB$	14.45^{a}	0.61	5.83^{b}	0.44	38.80^{b}	0.87
$BB\text{-}A^{2}A^{2}\text{-}AA$	17.78^{b}	0.77	10.92^{c}	0.40	27.52^{c}	1.11
$BB\text{-}A^{1}A^{1}\text{-}AA$	17.20^{b}	0.79	13.55^{d}	0.78	21.25^{d}	1.34
$BB\text{-}A^{2}A^{1}\text{-}AA$	16.31^{c}	0.74	10.13^{e}	0.47	30.38^{e}	1.37

$^{a–e}$Means within a column with different superscripts differ ($P < 0.05$).

[1] RCT = rennet coagulation time; k_{20} = curd-firming rate; a_{30} = curd firmness.

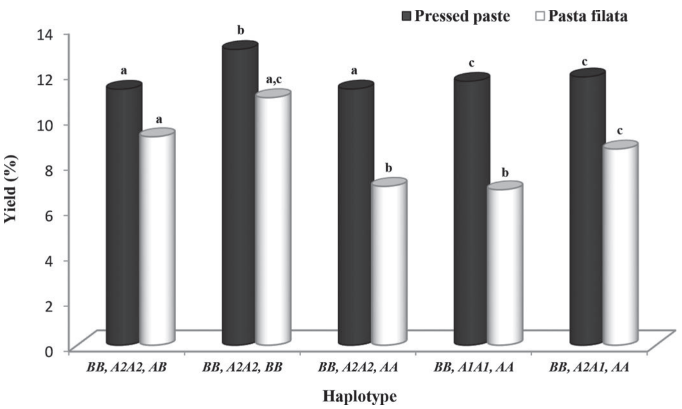

Figure 1. Yield of pressed and *pasta filata* cheeses obtained from Italian Holstein milk for different casein haplotypes (CSN1S1, CSN2, and CSN3, respectively). Means within the same cheese type with different letters (a–c) differ ($P < 0.05$).

type significantly affected cheese yield ($P < 0.01$). In particular, BB-A^2A^2-BB milk showed the highest yield of both pressed and *pasta filata* cheeses (13.05 and 10.93%, respectively), in agreement with that reported by other authors (Marziali and Ng-Kwai-Hang, 1986; Rahali and Ménard, 1991; Nuyts-Petit et al., 1997) who showed, in many pressed cheeses, that the highest yield was obtained from CSN3 variant BB milk. The BB-A^2A^2-AB and BB-A^2A^2-AA milks showed, instead, the lowest yields of pressed cheese (11.30 and 11.31%, respectively; $P < 0.05$), whereas BB-A^2A^2-AA and BB-A^1A^1-AA milks presented the lowest yield of *pasta filata* cheeses (7.03 and 6.88%, respectively; $P < 0.05$). In this study, we also reported fat and protein contents in whey after cheesemaking for the different casein haplotypes (Figure 2), because the whey is considered a good indicator of the milk's ability to transfer its components to cheese (Walsh et al., 1998a; Ikonen et al., 1999; Johnson et al., 2001; Summer et al., 2004). Within the CSN3 locus, for CSN1S1 variant BB and CSN2 variant A^2A^2, BB-A^2A^2-BB whey showed the lowest fat and protein contents (0.90 and 0.63 g/100 g, respectively; $P < 0.05$) compared with BB-A^2A^2-AB (1.13 and 0.73 g/100 g) and BB-A^2A^2-AA (11.16 and 0.76 g/100 g) whey. These findings are in agreement with reports of other authors, who detected that CSN3 AA milk had a higher fat content in whey after processing of Cheddar (Walsh et al., 1998a), Gouda (van den Berg, 1992), and Mozzarella (Walsh et al., 1998b) cheese compared with CSN3 BB milk. Within the CSN2 locus, for CSN1S1 variant BB and CSN3 variant AA, BB-A^1A^1-AA whey showed the highest fat (1.47 g/100 g; $P < 0.05$) and the lowest protein (0.68 g/100 g; $P < 0.05$) content compared with BB-A^2A^1-AA (0.84 and 0.76 g/100 g, respectively) and BB-A^2A^2-AA (1.16 and 0.76 g/100 g, respectively) whey. Marziali and Ng-Kwai-Hang (1986) reported that CSN2 A^1A^1 milk showed a higher capacity to retain protein in coagulum during cheesemaking than did CSN2 A^2A^1.

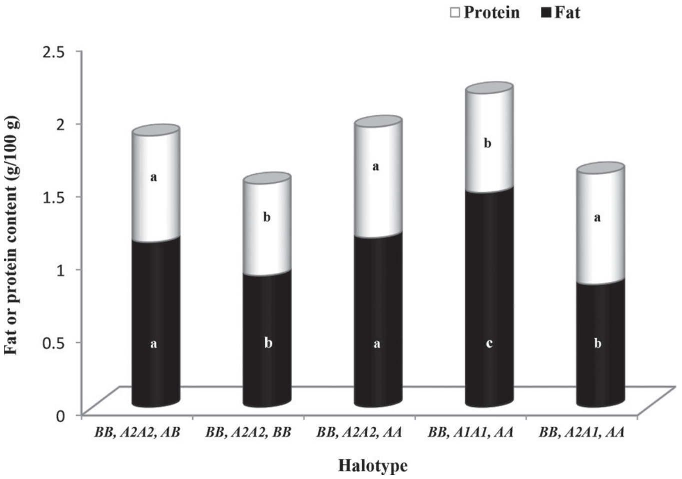

Figure 2. Fat and protein contents in whey after cheesemaking for different casein haplotypes (CSN1S1, CSN2, and CSN3, respectively). Means within the same parameter (fat or protein) with different letters (a–c) differ ($P < 0.05$).

CONCLUSIONS

Casein haplotype influenced both milk composition and cheese yield traits. In particular, BB-A^2A^2-BB milk was particularly suitable for production of pressed and *pasta filata* cheeses, because it showed higher fat and protein contents, the best coagulation characteristics and highest cheese yield, and better ability to retain fat and protein in the curd. Knowledge of milk protein polymorphisms not only allows the production of milk with specific qualitative and quantitative characteristics, but it could also be used as a specific marker within a breed to identify milk suitable for cheesemaking, which confers an economical advantage for dairy producers.

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