J. Dairy Sci. 99:3288–3294
http://dx.doi.org/10.3168/jds.2015-10463

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

Annamaria Perna,[1] Immacolata Intaglietta, Emilio Gambacorta, and Amalia Simonetti
School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Viale dell'Ateneo Lucano 10-85100, Italy

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*^{1}*A*^{1}*-AA* milk showed the highest fat content (4.01 g/100 g), whereas *BB-A*^{2}*A*^{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 ($\mathbf{k_{20}}$, min), and curd firmness ($\mathbf{a_{30}}$, 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

Received September 29, 2015.
Accepted January 20, 2016.
[1]Corresponding author: anna.perna@unibas.it

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 (%) = ($n_{i,\ \text{haplotype}}/n_{tot,\ \text{haplotype}}$) × 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