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The influence of casein haplotype on morphometric characteristics of fat globules and fatty acid composition of milk in Italian Holstein cows

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ABSTRACT

The aim of this work was to investigate the effect of casein haplotypes (α_{S1}-, β-, and κ-caseins) on morphometric characteristics of fat globules and fatty acid composition of Italian Holstein milk. Casein haplotypes were determined by isoelectric focusing; milk fat globule size was measured by using a fluorescence microscope; and fatty acid profile was determined by gas chromatography. Casein haplotype significantly affected the fat globule size, the percentage incidence of each globule size class on total measured milk fat globules, and fatty acid composition. A higher incidence of smaller milk fat globules was associated with the *BB-A^2A^2-BB* genotype (α_{S1}-, β-, and κ-casein haplotypes, respectively), whereas small globules were not detected in *BB-A^2A^1-AA* milk, but that milk had the highest percentage of large globules. A higher content of monounsaturated fatty acids was associated with the *BB-A^2A^2-AB* genotype, whereas higher contents of conjugated linoleic acid and docosahexaenoic acid were detected in *BB-A^1A^1-AA* milk. Our results indicate that casein haplotype could affect fat characteristics and, therefore, the nutritional and technological quality of milk.
Key words: casein haplotype, milk fat globule size, fatty acid profile, Italian Holstein cows

INTRODUCTION

The milk proteins of ruminants have a complex genetic variability. In particular, α_{S1}-, β-, and k-CN have many genetic variants that can result from SNP or from nucleotide deletions or insertions, which affect, at the phenotypic level, both milk composition and cheesemaking properties (Di Stasio and Mariani, 2000; Caroli et al., 2009). Casein genes were the first to be identified and sequenced entirely. Close linkage among the caseins was detected through physical mapping techniques, showing that α_{S1}-CN, β-CN, α_{S2}-CN, and κ-CN are coded by 4 genes (*CSNS1*, *CSN2*, *CSN1S2*, and *CSN*, respectively) tightly linked in a 250-kb cluster on chromosome 6 (Martin et al., 2002). Many authors (Ojala et al., 1997; Braunschweig et al., 2000) have suggested that the effects of an individual locus are confounded in statistical analyses, even when they are included simultaneously in the model, because certain alleles at the casein loci may appear together more or less frequently than expected with a random combination. However, their influence on milk traits could be due to the cumulative effects of different casein loci on chromosome 6. Consequently, the effects of casein genotypes should be estimated using the whole casein cluster instead of single casein loci (Boettcher et al., 2004; Gambacorta et al., 2005; Secchiari et al., 2009; Perna et al., 2013).

Fat is the major substance defining the energetic value of milk and it makes a major contribution to the nutritional properties of milk. Lipids in milk are mainly present as an oil-in-water emulsion in the form of globules with a diameter that ranges between <0.1 and approximately 18 μm, with an average diameter of about 4 μm in cow milk (El-Zeini, 2006; Martini et al., 2013a). The globules consist of a triglyceride core surrounded by a natural biological membrane composed mainly of cholesterol, enzymes, glycoproteins, and glycolipids (Fauquant et al., 2007). About 70% of all fat globules have a diameter of <4 μm, whereas 20% of globules, which represents almost the entire weight of the fat, have diameters between 4 and 6 μm (Barłowska et al., 2011). The milk fatty acids and fat globule size influence the physicochemical, nutritional, and sensorial properties of milk and milk products, as well as their technological ability (Michalski et al., 2003; Fauquant et al., 2005). They, in turn, are affected by various factors, both endogenous (breed, individual milk production, state of health, lactation stage) and exogenous (environmental conditions and farm management, with special reference to the type of diet) (Michalski et al., 2005; Couvreur et al., 2006; Janik et al., 2008; Martini et al., 2013b). The influence of milk protein polymorphisms on morphometric characteristics and the fatty acid composition of milk has been detected in sheep (Martini et al., 2006) and goats (Chilliard et al., 2006; Cebo et al., 2012). In the

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bovine, the connection between milk protein polymorphism and fatty acid composition has been thoroughly investigated (Bobe et al., 2004; Melia et al., 2009). However, little information is reported in the literature on the association between milk protein polymorphism and morphometric characteristics of milk fat globules. Martini et al. (2007) detected relationships among fat globule size and β-CN, κ-CN, and β-LG polymorphisms in Holstein milk. The aim of our study was to estimate the effects of casein haplotypes on morphometric characteristics and fatty acid composition of milk in Italian Holstein cows.

MATERIALS AND METHODS

Animals and Sampling

This study was conducted on an intensive farm, consisting of more than 500 Italian Holstein cattle, in the countryside of Potenza, southern Italy. Before starting the experiment, about 250 animals in lactation were identified by isoelectric focusing (**IEF**) to define their haplotypes. Haplotypes were formed by combining individual allelic loci of α_{S1}-, β-, and κ-CN. After defining individual phenotypes, the cows were grouped by haplotype. Each group consisted of 10 to 12 animals, at similar stages of lactation (70 to 120 d postpartum), season (spring), and parity (third calving). All animals were fed a commercial standard diet according to milk yield. Individual milk samples of cows at the morning milking were collected once and all milk samples were stored at 4°C until analysis; determination of fat globule size was conducted on fresh milk samples. Individual milk samples from each cow were analyzed and each analysis was carried out in triplicate.

Sample Preparation for IEF

Individual milk samples 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 Casein 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 divided by the total number of haplotypes [$\% = (n_{i,\ haplotype}/n_{total,\ haplotype}) \times 100$]. Haplotypes are presented in order for α_{S1}-, β-, and κ-CN, respectively.

Determination of Fat Globule Size

Milk fat globule size were determined as described by Martini et al. (2013b). Image acquisition and processing (100×) were performed using a fluorescence microscope (Axioskop, Zeiss, Germany) equipped with Image J software (National Institutes for Health, Bethesda, MD). After determination of diameter, fat globules were divided into 3 size classes: small globules (diameter <2 μm), medium-sized globules (diameter from 2 to 5 μm), and large globules (diameter >5 μm). In addition, the percentage incidence of each globule class on total measured milk fat globules was calculated separately for haplotype: $\Sigma n_{i\ globules\ (each\ class)}/\Sigma n_{total\ globules} \times 100$.

Determination of Fatty Acid Profile

Total lipids of the samples were extracted using chloroform/methanol (2:1, vol/vol) according to Folch et al. (1957), and FAME were prepared according to the ISO (1978) method. Analysis was performed using a Varian 3400 gas chromatograph (Varian, Turin, Italy), equipped with a split-splitless injector, an Omegawax 250 capillary column (30 m × 0.25 μm i.d. × 0.25 μm film thickness; Thermo Fisher Scientific, Milan, Italy), and a flame-ionization detector. Helium was used as carrier gas at a flow rate of 1.0 mL/min, and the injector and detector temperatures were set at 250°C and 220°C, respectively. The oven temperature program was 50°C for 2 min, increasing at 4°C/min up to 220°C, where it was maintained for 15 min.

Individual FAME were identified by comparing their retention times with those of the corresponding pure standards (Sigma-Aldrich, Milan, Italy). Quantitative analysis was obtained by peak area integration using the Galaxie Chromatography Data System version 1.9.3.2 software (Varian), and results were expressed as percentage of the total fatty acids analyzed. To evaluate the nutritional implications, PUFA:SFA and n-6:n-3 ratios, and atherogenic and thrombogenic indices were