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Original article

Effects of the hot season on milk protein fractions in Holstein cows

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Abstract — Effects of the hot season on milk protein fractions were studied in 40 mid-lactating Holstein cows (141 ± 67 days in milk). The trial was carried out on a commercial dairy herd located in Central Italy (42°15' North, 12°21' East, 227 m of altitude). Twenty cows were monitored for six weeks from March to April; the other 20 cows were monitored for six weeks from June to August. The two groups were balanced for parity, days in milk (DIM), genetic index for milk production, and frequency of κ-casein B and β-lactoglobulin B variants. The diet for the two groups was a base-ration fed as a total mixed ration, plus concentrate given by self-feeders. During the two experimental periods, values of air temperatures and relative humidity were recorded daily and were used to calculate the temperature-humidity index; average feed intake was recorded daily, and rectal temperatures were measured weekly. Milk yield was recorded at both morning (06:00 h) and evening (18:00 h) milkings. Individual milk samples were taken weekly and analysed for concentration of crude protein content, casein number, and protein fractions. During the summer, cows had higher rectal temperatures but lower DMI and milk yield. Moreover, milk from cows in the summer had lower contents of crude proteins and caseins, lower casein number, and higher milk serum proteins. Among caseins, the κ-casein fraction did not differ between the two groups. Conversely, milk from summer cows showed lower contents of α_s-casein and β-casein. The results from the present study indicate that the reduction of milk protein content observed in the summer was due to the reduction in the casein content, which was in turn caused by a reduction in α_s-casein and β-casein contents. These changes might explain the alteration in cheesemaking properties of milk commonly observed during the summer.

summer / dairy cows / milk protein fractions / casein

Résumé — **Effets de la saison chaude sur les fractions protéiques du lait de vaches laitières Holstein**. Les effets de la saison chaude sur les fractions protéiques du lait ont été étudiés chez quarante vaches Holstein en pleine lactation (141 ± 67 jours de lactation). L'expérience a été réalisée sur

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un troupeau commercial de vaches laitières localisé en Italie Centrale (42°15' Nord, 12°21' Est). Vingt vaches ont été contrôlées pendant une période de six semaines au printemps (mars et avril) ; les vingt autres l'ont été pendant une période de six semaines en été (juin à août). Les deux groupes étaient homogènes en ce qui concerne le rang et le stade de lactation, l'index génétique pour la production laitière, ainsi que pour la fréquence des variants B de la κ-caséine et de la β-lactoglobuline. Le régime alimentaire a été le même pour les deux groupes. Il était composé d'une ration complète et de concentré réparti dans des distributeurs automatiques. En été, les vaches ont eu une température rectale plus élevée et une production de lait plus faible, alors qu'elles ont consommé moins de nourriture. Le lait produit a été moins riche en protéines totales et en caséines, et présentait une proportion plus faible de caséines par rapport aux protéines totales, et une proportion plus élevée de protéines du lactosérum. Parmi les caséines, la teneur en κ-caséine ne différait pas entre les deux groupes. En revanche, le lait produit par les vaches en été contenait significativement moins d'α_s-caséine et de β-caséine. Par conséquent, la diminution en été de la teneur en protéines du lait observée dans cette étude est due à la réduction de la teneur en caséines, qui dépend elle-même de la réduction des teneurs en α_s-caséine et en β-caséine. Ces résultats pourraient expliquer l'altération des propriétés de coagulation du lait pour la fabrication de fromages en été.

été / vaches laitières / fractions protéiques du lait / caséines

1. INTRODUCTION

Several factors, such as stage of lactation, parity of cows, milk somatic cell counts, nutrition and genetic variants of caseins can cause variations of relative proportions and contents of milk protein fractions [8, 21, 25], which can significantly affect properties of milk during technological processes [13, 25, 27, 36].

Some authors have reported seasonal changes [21, 40] and effects of air temperatures [20, 31] on milk protein fractions, but these data are not conclusive. Such lack of knowledge on the effects of hot conditions on milk protein fractions is surprising in the light of the severe alterations of the cheesemaking properties of milk and the reduction of cheese yield and alteration of cheese quality occurring during the hot summer months in Italy [28, 29] and in other countries [34, 41].

Therefore, the aim of the present field study was to evaluate the protein fractions in bovine milk produced during the summer and spring in order to find a possible explanation for the alterations of the cheesemaking properties of summer milk in Italy.

2. MATERIALS AND METHODS

2.1. Animals, housing and feeding

The trial was carried out in a commercial dairy herd located in Central Italy (42°15' North, 12°21' East, 227 m of altitude) enrolled in the Italian Dairy Herd Analysis Service, and consisting of 240 Holstein cows. Two groups of 20 lactating cows were selected. The first group was monitored for six weeks during the spring (1st March to 12th April); the second group was monitored for six weeks during the summer (21st June to 2nd August). The two groups were balanced for parity (2.3 ± 1.3 in both groups) and DIM (139 ± 67 vs. 142 ± 66 for the spring and summer groups, respectively). The two groups were also balanced for the genetic index for milk production (481 ± 139 vs. 501 ± 148 for the spring and summer groups, respectively), and frequency of κ-casein B (5 cows in each group) and β-lactoglobulin B variants (4 cows vs. 5 cows in the spring and summer groups, respectively) [10]. The cows selected for the study were kept separate from the main herds to measure group feed intake, and were housed in a cubicle free stall without an external paddock.

The diet consisted of a base-ration fed as a total mixed ration (TMR), plus concentrate given by self-feeders. The TMR was given daily at 09:30 h and offered ad libitum to achieve 5–10% refusals. The diet composition was the same for the spring and summer periods. The composition of TMR is reported in Table I. Concentrate feeding was adjusted according to milk yield (1 kg of concentrate per 3 litres of milk yielded more than 26 $L \cdot d^{-1}$). The concentrate contained 87% dry matter (DM), 21% crude proteins (CP), 4.4% ether extract, 6.9% crude fiber, 9.1% ash, 16% NDF, and 1.82 $Mcal \cdot kg^{-1}$ of NEL on a DM basis.

2.2. Measurements, sampling and laboratory analyses

Temperatures (°C) and relative humidity (%) of the air were recorded at 20 minute intervals, using two electronic data recorders (Data-Hog recorders, Skye Instruments Ltd, Llandrindod Wells, UK). Temperature and relative humidity values were used to calculate the temperature-humidity index (THI) using the formula reported by Johnson [17].

Table I. Ingredients and nutrient composition of the total mixed ratio (TMR) administered during the spring and summer experimental periods.

	TMR
Ingredient composition	
% of DM	
Corn silage	36.18
Alfalfa hay	20.44
Commercial mixed feed(1)	11.44
Ground corn	9.35
Wheat flour middling	6.13
Alfalfa silage	5.87
Soybean meal (49% CP)	5.37
Beet pulp	4.28
Buffer(2)	0.94
Nutrient composition on DM basis	
NEL, $Mcal \cdot kg^{-1}$(3)	1.57
NDF, %	37.8
CP, %	15.6

(1) Contained (on DM basis) 87% DM, 29.9% crude proteins (CP), 5.7% ether extract, 9.8% crude fiber, 14.9% ash, 19% NDF, and 1.67 $Mcal \cdot kg^{-1}$ of NEL.
(2) A mixture of 33.3% $CaCO_3$, 31.7 $Ca_3(PO_4)_2$, 16.7% MgO, 16.6% $NaHCO_3$, and 1.7% $ZnSO_4$.
(3) Net energy content.

Rectal temperatures were measured weekly at 15:00 h using a glass precision thermometer with a prismatic section (Artsana Veterinaria, Como, Italy) having 0.1 °C accuracy of measurement. Daily average feed intake was calculated by weighing the feedbunk refusals at 09:00 h immediately before the total mixed ratio was administered.

Cows were milked twice daily at 06:00 and at 18:00 h. Milk yield was recorded at both milkings and 50 mL individual milk samples were taken at 18:00 h milkings, weekly. Milk sampling lasted 20–25 minutes. Each milk sample was preserved with 0.25 mL of 2-bromo-nitropropane-1,3-diol (Bronopol Tecnico, Formenti, Milano, Italy), and was put into a portable refrigerator at 6–8 °C immediately after sampling. Milk samples were analysed to determine crude protein concentration, somatic cells (SCC) and titratable acidity ($^{o}SH \cdot 50\ mL^{-1}$) within two hours from sampling. An aliquot of each milk sample was stored at –20 °C and analysed within one month to determine casein number (casein/total crude proteins) and protein fractions (caseins (α_s-, β- and κ-casein), whey proteins (α-lactalbumin, β-lactoglobulin) and serum proteins). In this study α_{s1}- and α_{s2}-casein are grouped together and are referred to as α_s-casein.

The concentration of crude protein was measured by infrared spectrophotometry (Milko-Scan-104, Foss Electric, Hillerød, Denmark). Milk SCC were determined by automatic counting (Fossomatic-180, Foss Electric, Hillerød, Denmark), and titratable acidity by an automatic titrator (MicroTT 2050 titrator; Crison, Carpi, Italy). Casein number was determined following the method reported by the Italian Society of Animal Science [3], and protein fractions by electrophoresis on cellulose acetate according to the method described by Davoli

[10]. Serum proteins were determined directly on skimmed milk and on milk serum and were adjusted taking the values of total crude proteins into account. Quantification of the electrophoretically separated proteins was done by densitometry.

2.3. Statistical analysis

Data were analysed as repeated measures using the general linear model procedure of SAS® [37] according to the following model:

$$Y_{ijkl} = \mu + S_i + C_{ij} + W_k + e_{ijkl}$$

where:

Y_{ijkl} = dependent variable,

μ = overall mean of the population,

S_i = mean effect of season (i = 1 to 2),

C_j = mean effect of cows (j = 1 to 20),

W_k = mean effect of the week of sampling (k = 1 to 6), and

e_{ijkl} = the unexplained residual element assumed to be independent and normally distributed.

To isolate the variation due to the seasonal variation of cows, the error term used to test for a season effect was cow within season. Least square means were separated with the PDIFF procedure of SAS® [37]. Significance was declared at $P < 0.05$.

3. RESULTS

3.1. Temperature-humidity index

The average climatic parameters registered during the two seasons are reported in Table II. Mean daytime THI was from 10:00 h to 17:00 h, and mean night-time THI was from 17:20 h to 09:40 h, both during the spring and summer.

During the spring, the THI was always below 72. During the entire summer period, mean daytime THI was above 72, and night-time THI was lower than 72, but it exceeded 72 for seven days during the first three weeks of the trial.

3.2. Rectal temperatures and feed intake

During the summer, cows had mean rectal temperatures that were 0.8 °C higher ($P < 0.01$) than during the spring (39.8 vs. 39.0 °C, respectively).

The base-ration dry matter intake (DMI) during the summer was 19.8% lower ($P < 0.01$) than that recorded during the spring (18.6 vs. 23.2 kg DM·d^{-1}, respectively). Cows during the summer also ingested less concentrate than cows in the spring (–0.96 kg of concentrate per cow on average). That circumstance led to a higher difference in

Table II. Mean value ± SD of climate parameters recorded during the two experimental periods.

		Temperature (°C)	Relative humidity (%)	THI
Spring	Daytime‡	11.6±2.6	66.7±18.5	52.9±4.1
	Night-time†	6.4±3.5	84.2±13.4	43.5±6.0
Summer	Daytime	29.9±2.9	41.8±12.0	79.4±2.7
	Night-time	21.9±4.1	71.0±16.6	69.5±5.1

THI = temperature-humidity index.
‡From 10:00 h to 17:00 h.
†From 17:20 h to 09:40 h.

total feed intake (22%) and to a different forage concentrate ratio between the two groups (48/52 vs. 50/50 in the summer and spring, respectively). Furthermore, summer cows ingested less ($P < 0.05$) energy (Mcal·d^{-1} NEL: –18.0%) and proteins (g crude proteins·d^{-1}: –17.4%) than spring cows.

3.3. Milk yield and composition

Milk yield during the summer was 10% lower ($P < 0.01$) than during the spring (26.7 vs. 29.5 L·d^{-1}, respectively). Milk protein percentages were 9.9% lower ($P < 0.01$) in the summer than in the spring (3.01 vs. 3.31%, respectively).

Milk SCC content was not affected by season (121 000 vs. 154 000 cells·mL^{-1}, in the summer and spring, respectively). Titratable acidity was lower ($P < 0.05$) in the summer than in the spring (3.4 vs. 3.5 °SH·50 mL^{-1}). Casein percentage and casein number were lower ($P < 0.01$) in the summer than in the spring (2.18 vs. 2.58% and 72.4 vs. 77.9% for casein content and casein number, respectively).

Alpha$_s$-casein and β-casein content were lower ($P < 0.01$) in the summer milk, and no difference was found for κ-casein between seasons (Tab. III). The relative percentages of α_s-casein and β-casein were lower ($P < 0.05$), and the relative percentage of κ-casein was higher ($P < 0.05$) in the summer than in the spring milk (37.2, 26.2, and 9.0 vs. 41.1, 29.3, and 7.6% for α_s-, β- and κ-casein, respectively). No differences between the two seasons were detected for α-lactalbumin and β-lactoglobulin contents, but serum protein contents were higher ($P < 0.01$) in the summer than in the spring (Tab. III).

4. DISCUSSION

During the summer, daytime THI was always higher than 72, which is considered the upper critical THI for lactating dairy cows [2]. Moreover, the THI registered during the night-time was near the upper critical THI and probably did not allow cows to completely recover from the heat stress imposed by the daytime THI [15, 16]. Increased rectal temperatures and decreased DMI and milk yield during the summer revealed a condition of heat stress [19, 32]. In contrast, the spring period was characterised by THI values indicating a situation of thermal comfort [18].

Reduction of total milk protein has already been reported by several authors in heat-stressed dairy cows [5, 39]. The reduction of casein content in summer milk has also been reported by some authors [14, 25]. In a previous study we observed lower colostrum casein contents in cattle exposed to a hot environment during the last three weeks of pregnancy [33].

The seasonal effects of photoperiod on the milk protein percentage of lactating cows in hot weather have been reported [1].

Table III. Least square means of milk protein fraction contents (%) in cows monitored during the two experimental periods.

	α_s-CN	β-CN	κ-CN	α-LA	β-LG	Serum proteins	SE
Spring	1.36^B	0.97^B	0.25	0.17	0.38	0.18^A	0.10
Summer	1.12^A	0.79^A	0.27	0.16	0.38	0.29^B	0.08

^{A,B}Means within a column with different superscript letters differ ($P < 0.01$).
CN = casein
LA = lactalbumin
LG = lactoglobulin

To our knowledge, the effect of photoperiod on milk protein fraction contents have not yet been established.

Cow nutrition can affect milk protein [8, 14, 25, 40]. Coulon et al. [8] reported positive effects of the amount and type of energy and nitrogen supply on milk protein content but no or slight effects on the casein/protein ratio. Hermansen et al. [14], Mackle et al. [25] and Todorova [40] found the reduction of caseins by dietary changes consisting of a lower grass availability and consequent lower energy and nitrogen content of the summer diets. Hermansen et al. [14] found a significant positive effect on true protein and casein contents in response to concentrate supplementation. Mackle et al. [25] observed a reduction of casein concentration in cows subjected to restricted pasture intake.

In the present study, the main cause of casein reduction during the summer was the reduction in α_s– and β-casein. These proteins contain high numbers of phosphate groups [38], and the phosphorylation of caseins needs the presence of the γ-phosphate of ATP [30]. As reported before, even though we tried to maintain the ration constant in the two seasons, summer cows consumed less dry matter, protein and energy than spring cows. Moreover, as reported by Mackle et al. [25], feed restriction reduced α_s– and β-casein concentration in grazing cows. Consequently, we can hypothesise that the lower content of α_s– and β-casein in summer milk might be partially due to the reduction in energy and protein availability which usually occurs during heat stress [24, 33] as observed in the present study.

Kroeker et al. [21] described changes in relative percentages of α_s-, β– and κ-casein throughout the year. These authors observed no definitive seasonal trend for the relative percentage of casein fractions, even though they reported a significant effect of month of test for κ-casein. Unfortunately, Kroeker et al. [21] did not report the climatic conditions of their study, thus it is not possible to make any comparison between the studies. Kirchmeier [20] reported an increase of α_s-casein and a reduction of κ-casein when the outdoor temperature increased from 0 to 17 °C and considered the outdoor temperature a limiting factor for the quantity and quality of milk protein synthesis. The comparison of Kirchmeier's study with our study still points to similarities, even in the light of the different environmental conditions.

Casein content [11, 26, 27, 36] and casein fraction changes [13, 35, 36, 38] are strictly related to properties of milk during technical processes. Mariani [26] reported that a variation of 0.10 g of casein per litre of milk corresponds to a variation in cheese yield of 0.27–0.28 kg·100 L^{-1} of milk for aged Parmigiano Reggiano cheese. Moreover, contents and relative proportions of α_s and β-casein, due to their elevated sensitivity towards precipitation by calcium ions, strongly affect renneting properties of milk [38]. In this regard, Pabst [35] reported a positive relationship between the relative proportions of α_s- and β-casein and cheese yield. Remeuf [36] described a positive relationship between β-casein concentration and the relative proportion and coagulation properties of milk. Moreover, Grandison et al. [13] found strong positive relationships between coagulum strength and α_s- and β-casein concentrations, and confirmed that the concentration of α_s-casein may be important to the coagulation process and to curd quality and cheese yield. Alpha$_s$-casein and β-casein, rich in phosphate groups, are the two acidic components of the casein micelles [38]. Thus, the lower contents of α_s- and β-casein of milk yielded during the summer might explain the higher milk pH and the lower milk titratable acidity found in the present study and commonly registered during the hot summer months [6, 7, 28]. Furthermore, the lower α_s- and β-casein contents might explain the lower milk phosphorous content observed by others [12, 22, 23] in milk produced by cows exposed to hot environments.

Therefore, our data characterise a milk with poor clotting properties and poor cheesemaking quality, which can at least partially explain the reported losses in cheese yield and the reported alteration of cheesemaking properties, but not explained by others during the summer [28, 29, 34, 41].

To the best of our knowledge, there is little information on changes of whey fractions and serum proteins during the hot summer months. Muroya et al. [31] reported no effect of heat stress on whey nitrogen. In the present study, we did not observe a seasonal effect on α-lactalbumin and β-lactoglobulin concentrations, but serum proteins increased. Hermansen et al. [14] reported a higher content of whey proteins in milk produced in the late summer and that the result was not related to proteolysis.

An increase in serum proteins is usually associated with the presence of subclinical or clinical mastitis [42], and with higher milk plasmin activity [42]. Since milk SCC indicated that neither spring nor summer cows had mastitis problems, we can exclude mastitis as the possible cause of increased serum proteins in summer cows. Although, we did not measure the plasmin activity in milk, it is known that plasmin activity is strictly related to milk SCC [42] and serious problems with casein proteolysis should begin when milk SCC are higher than 200 000·mL^{-1} [8]. Moreover, plasmin activity of milk is higher in late lactating dairy cows [4, 9]. The low values of SCC and the absence of differences in SCC and lactation stage between the summer and spring cows should exclude proteolysis as the cause of the increased serum proteins in milk produced during the summer. Although, it is not easy to explain the higher contents of serum proteins in summer milk, a concentrating effect of declining milk volume might be the possible cause as reported by Mackle et al. [25].

5. CONCLUSIONS

The reduction of milk protein contents during the hot summer months seems to be attributable to the reduction in casein content, specifically α_s- and β-casein contents. Changes in casein contents and casein fractions might explain the alteration in cheesemaking properties of milk and the reduction in cheese yield reported during the summer in Italy. However, the main factor responsible for changing casein fractions during the summer was not identified.

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