SHORT COMMUNICATION



# Milk of Italian Saddle and Haflinger nursing mares: physico – chemical characteristics, nitrogen composition and mineral elements at the end of lactation

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## ABSTRACT

Aim of this work was to investigate nursing mares milk characteristics at the end of lactation (D) and to make a comparison with milk taken during early lactation (3 to 30 d *post partum*) (E). The survey was carried out on 17 Italian Saddle mares (IS) (193 days in milk; 147 pregnancy days) and on 12 Haflinger mares (H) (174 days in milk; 146 pregnancy days). N fractions were determined by means of Kjeldahl; fat and lactose by means of mid infrared lectures; mineral elements were determined by Atomic Absorption Spectrophotometry; P by colorimetry. Data referring to 58 individual milk samples were analyzed by ANCOVA. Significant variations, similar in both breeds, were observed (E vs D) for density at 15°C (1.036 vs 1.034), pH (6.94 vs 7.24), titratable acidity (3.56 vs 1.70 °SH), fat (1.17 vs 0.76% g/100g of milk), crude protein (2.31 vs 1.68% g/100g), casein number (52.37 vs 46.59) and ash (0.50 vs 0.30% g/100g); similarly (mg/100g) for non casein N (172.31 vs 142.05), non protein N (34.43 vs 29.09), P (61.30 vs 32.48), Ca (112.88 vs 54.42), Mg (8.40 vs 4.38) and K (60.77 vs 41.31). Freezing point, lactose and Na showed no significant variations. At the end of lactation, milk resulted poorer in all main organic components, except lactose, and in all mineral components, except Na. Only freezing point and phosphorus variations differed in IS from H. Breed significantly affected fat, casein N and ash mean contents.

Key words: Mare milk, Physico-chemical characteristics, Nitrogen composition, Mineral elements

### RIASSUNTO

#### IL LATTE DI CAVALLE NUTRICI DELLE RAZZE SELLA ITALIANO E AVELIGNESE: PROPRIETÀ CHIMI-CO-FISICHE, COMPOSIZIONE AZOTATA ED ELEMENTI MINERALI A FINE LATTAZIONE

Lo scopo di questo lavoro è stato di studiare le proprietà del latte di cavalle nutrici a fine lattazione (D) e di fare un confronto con il latte prelevato all'inizio della lattazione (3÷30 d post partum) (E) considerando due diverse razze equine. La ricerca è stata effettuata su 17 cavalle di razza Sella Italiano (IS) (193 giorni di lattazione; 147 giorni di gestazione) e 12 cavalle di razza Avelignese (H) (174 giorni di lattazione; 146 giorni di gestazione). Le frazioni azotate sono state determinate con il metodo Kjeldahl; grasso e lattosio mediante letture nel medio infrarosso; gli elementi minerali sono stati determinati mediante spettofotometria ad assorbimento atomico; P per colorimetria. I dati, riferiti a 58 campioni di latte individuale, sono stati sottoposti ad analisi statistica (ANCOVA). Sono state osservate variazioni significative (E vs D), simili in entrambe le razze, per densità a 15°C (1,036 vs 1,034), pH (6,94 vs 7,24), acidità titolabile (3,56 vs 1,70 gradi Soxhlet-Henkel), grasso (1,17 vs 0,76% g/100 g di latte), proteina grezza (2,31 vs 1,68% g/100g), indice di caseina (52,37 vs 46,59) e ceneri (0,50 vs 0,30% g/100 g); lo stesso si verifica (mg/100g) per N non caseinico (172,31 vs 142,05), N non proteico (34,43 vs 29,09), P (61,30 vs 32,48), Ca (112,88 vs 54,42) e K (60,77 vs 41,31). Punto di congelamento, lattosio e Na hanno mostrato variazioni statisticamente non significative. A fine lattazione, il latte risulta più povero in tutti i principali costituenti organici, eccetto il lattosio, e in tutte le componenti minerali, eccetto il sodio. Soltanto nel caso del punto di congelamento e del contenuto di fosforo le variazioni erano significativamente differenti nel SI rispetto a H. La razza ha mostrato una influenza significativa sui contenuti di grasso, azoto caseinico e ceneri.

Parole chiave: Latte di cavalla, Proprietà chimico-fisiche, Composizione azotata, Elementi minerali

#### Introduction

Mare milk production changes greatly throughout lactation as regards quantity and quality.

Several Authors studied mare milk yield and reported different lactation curves. In nursing mares maximal yield can occur in the first, second or third month. In mares herded without human interference, lactation lasts about 1 year. Drying occurs several weeks to several days before the next foaling (Doreau and Boulot, 1989). In management conditions, horses are usually weaned at five-six months of age, depending on the type of production (Catalano and Martuzzi, 1991). At drying-off, mare pregnancy has usually reached several months.

Late lactation milk has scarce significance for foal nutrition: from the  $3^{rd}$  month of age requirements for maintenance and growth are mainly satisfied by hay and concentrates. Therefore most studies about mare milk deal with the first two or three lactation months (Doreau *et al.*, 1990; Smolders *et al.*, 1990), while those regarding late lactation are few (Bouwman and van der Schee, 1978; Summer *et al.*, 2000; Mariani *et al.*, 2001). Knowledge of the characteristics of mare milk could be interesting from different points of view other than foal nutrition, such as industrial exploitation or human nutrition, due to the recognized properties of the milk as a "functional food" (Salimei *et al.*, 2002).

Many factors affect milk composition; concentrations of the main organic and inorganic milk components vary remarkably during lactation (Doreau and Boulot, 1989; Doreau *et al.*, 1990; Mariani *et al.*, 2001), while the role of breed is not yet clear (Neseni *et al.*, 1958; Doreau *et al.*, 1991; Csapò *et al.*, 1995).

The aim of this study was to observe the characteristics of mare milk at the end of lactation, when the foal is weaned and milk could be used for other purposes, and to make a comparison with early lactation milk characteristics.

The second aim of the study was to investigate if there are significant differences in the modifications in milk between two of the more common Italian horse breeds, bred with different purposes: Haflinger is a medium-small sized, sturdy horse used for light draft and leisure riding. Italian Saddle is an athletic and tall horse, bred for show jumping.

#### Material and methods

Milk characteristics of nursing mares at the end of lactation during initial drying-off were studied (D). The comparison was carried out with early lactation milk yielded from  $3^{rd}$  to  $30^{th}$  d *post partum* (E).

The survey was carried out on 58 individual milk samples from 17 Italian Saddle mares (IS) (drying-off average: 193 days; 147 pregnancy days; age: 6-21 years; parities: 1-16; liveweight: 500-650 kg) and from 12 Haflinger mares (H) (drying-off average: 174 days; 146 pregnancy days; age: 7-15 years; parities: 4-12; live weight: 400-520 kg). Two milk samples were taken from each mare: the first at the beginning of the lactation and the second during the week of foal's weaning. Milk was taken

by hand milking from a single mammary gland, milked as deep as possible, in presence of the foal, that was previously prevented from suckling for 1 - 2 hours by a muzzle.

The mares were fed hay and perennial rye grass in prevalence and lucerne *ad libitum*. Haflinger mares received from 0 to 4 kg of concentrate; Italian Saddle mares from 2 to 5 kg concentrate, with the following average chemical composition (%): dry matter 87.3, crude protein 14.2, ether extract 2.9, crude fiber 8.1, ash 8.2. During the week of drying-off, mares were fed as follows: concentrate was progressively reduced and finally completely removed; hay supply was reduced while straw supply was increased; water intake was reduced; meanwhile the foal was progressively separated from the mother.

Body condition score of the mares at early lactation was 3.25-3.50 and drying-off ranged between 2.75-3.25 (Martin-Rosset, 1990).

On milk the following analyses were carried out: density at 15°C by means of Quevenne lactometer; freezing point by a thermistor cryoscope; pH by a potentiometer; titratable acidity (°SH) with 0.25N NaOH according to Soxhlet-Henkel method (Anon., 1963); fat and lactose by means of mid infrared lectures (Biggs, 1978) with Milko-Scan 134A/B. Nitrogen fractions were determined by Kjeldahl according to Aschaffenburg and Drewry (1959); gross energy (kcal/kg) was calculated with the coefficients reported by Doreau *et al.* (1986), in particular 8.97 for fat, 5.25 for protein and 4.10 for lactose.

Mineral elements were determined by Atomic Absorption Spectrophotometer (Anon., 1982), working on dry ash obtained in muffle furnace at 530°C; P by colorimetry according to Allen (1940).

Data referred to 58 individual milk samples were analyzed by two ways ANCOVA methods, using the GLM procedure and applying the following model:

$$y_{ijkl} = \mu + S_i + B_j + A_{k(j)} + SB_{ij} + b1X1_{ijkl} + \varepsilon_{ijkl}$$

where:  $y_{ijkl}$  = observed values;  $\mu$  = overall mean;  $S_i$  = fixed effect of sampling (i: 1, 2);  $B_j$  = fixed effect of breed (j: 1, 2);  $A_{k(j)}$  = animal (breed);  $SB_{ij}$  = interaction; b1 = coefficient of regression

with feeding (X1, kg);  $\epsilon$  = random error. Breed was tested using mean square for animal (breed) as an error term.

#### **Results and discussion**

Regarding the considered lactation stages there was no difference between the breeds (Table 1). On average, early lactation samples were taken during the first twenty days, late lactation samples after the 180<sup>th</sup> day.

Milk density at 15°C varies significantly and similarly in both breeds and is lower in late lactation (1.036E vs 1.034D) (Table 1). A dilution of milk at the end of lactation was observed also by Mariani *et al.* (2001). No difference was observed between breeds.

Freezing point shows a different variation in the two breeds: in IS it varies from -0.529E to -0.533D, while in H it moves toward water freezing point (from -0.531E to -0.522D); the interaction Breed x Sample is significant (P = 0.028). In any case between the breeds the difference is not significant.

The pH increases significantly (6.94E vs 7.24D), while titratable acidity reduction at the end of lactation end is about 50%. Similarly, several Authors (Johnston *et al.*, 1970; Storch, 1985; Mariani *et al.*, 1993; Martuzzi *et al.*, 1995; Mariani *et al.*, 2001) observed an increase in pH or a decrease in titratable acidity in late lactation.

Fat content is lower in late lactation (1.17E vs 0.76%D) (Table 1), and the content is significantly different between the two breeds: 1.58E vs 1.30D in IS and 0.76 E vs 0.23D in H. As regards fat, a breed effect was observed by Kulisa (1977), while other Authors found no differences (Doreau and Boulot, 1986; Mariani *et al.*, 1996).

Lactose shows an increasing (6.61E vs 6.70D), although not statistically significant difference, in accordance with several studies confirming that mare milk during lactation becomes rich in carbohydrates (Neseni *et al.*, 1958; Ullrey *et al.*, 1966; Intrieri and Minieri, 1969; Bouwman and van der Schee, 1978; Mariani *et al.*, 1993; Sonntag *et al.*, 1996; Mariani *et al.*, 2001). There are also some contrasting indications: e. g. Kulisa (1980), at the 5<sup>th</sup> lactation month, and Krasnova (1962), during

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		Early lact. <sup>1</sup>	Late lact.1	It. Saddle <sup>1</sup>	Haflinger <sup>1</sup>	MSE
Lactation stage	d	19.34	185.48	104.03	100.13	13064.949
Density at 15 °C	2	1.036 ⊧	1.034 -	1.034	1.036	0.0001
Freezing point	Δ°C	0.530	0.528	0.531	0.527	0.0001
рН		6.94 F	7.24 ⊧	7.04	7.13	0.0223
Titratable acidity	°SH	3.56 ⊧	1.70 -	3.10	2.17	0.2743
Fat IR	g/100g	1.17 ⊧	0.76 -	1.44 🗛	0.49 B	0.0989
Lactose IR	w	6.61	6.70	6.40	6.92	0.2418
Crude protein	w	2.31 ⊧	1.68 -	2.18	1.82	0.0526
Total N	mg/100g	362.82 ⊧	263.57	341.11	285.28	1298.4804
Casein N	w	190.50 ⊧	121.52 -	180.05 A	131.97 B	995.6129
Non casein N	w	172.31 ⊧	142.05 -	161.06	153.30	406.4292
Non protein N	w	34.43 ⊧	29.09 -	33.71	29.80	13.2310
Casein number	_	52.37 c	46.59 D	51.29	47.66	39.4326
Gross Energy	kcal/kg	497.96 ⊧	431.08 -	506.47	422.56	1620.6965
(Doreau et al., 1	.986)					
Ash	g/100g	0.50 ⊧	0.30 F	0.46	0.34 B	0.0035
Phosphorus	mg/100g	61.30 =	32.48 -	52.42	41.36	59.4482
Calcium	w	112.88 ⊧	54.42 -	92.39	74.92	129.0147
Magnesium	w	8.40 ⊧	4.38 F	6.70	6.09	0.8733
Sodium	w	20.38	24.49	29.42	15.45	336.4258
Potassium	w	60.77 ⊧	41.31 -	57.14	44.93	79.8484

Table 1.Physico-chmical characteristics, gross composition, nitrogen components and<br/>mineral elements in milk of early and late lactation from Italian Saddle and<br/>Haflinger nursing mares.

<sup>1</sup> least square means.

A, B: P<0.05 referred to differences between breeds

C, D: P<0.01 referred to differences between periods of sampling

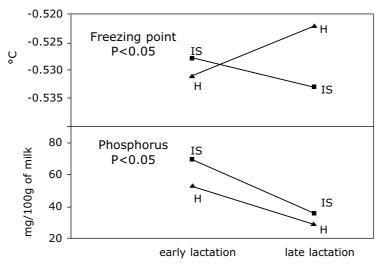
E, F: P<0.001 referred to differences between periods of sampling

 $4^{\rm th}$  -  $6^{\rm th}$  months, observe a decrease in lactose. Lactose decreases slightly from 6.43 to 6.36 g/100g in IS, while it increases from 6.80 to 7.04 in H.

Crude protein (Total N x 6.38) is always lower at the end of lactation (2.31E vs 1.68%D g/100g of milk), in accordance with the observations of Doreau and Boulot (1989) and Mariani *et al.* (2001) regarding late lactation milk. Nitrogen fractions vary significantly: in late lactation non casein N decreases by about 18%; similarly non protein N decreases by 16% and casein number (Casein N x 100/TN) varies from 52.37 to 46.59. The latter variation is in agreement with those reported by Rozhanskii *et al.* (1962) and by Mariani *et al.* (1993). Instead most Authors observe that lactation stage shouldn't have a significant effect on casein number value, excluding colostrum and the short transition phase (Kulisa, 1977; Deskur *et al.*, 1978; Doreau *et al.*, 1990). Minieri and Intrieri (1970), in milk of Haflinger mares from d 3 to d 180 of lactation, found casein number values that fluctuate very little around 67.5. On the other hand, Neseni *et al.* (1958) and Storch (1985) observe more variable values, with a tendency of the casein number to increase in the final phase of lactation.

Casein N content is higher in IS than in H (180.05 in IS vs 131.97 mg/100g in H).

Figure 1. Characteristics that showed different trends between the two horse breeds (IS: Italian Saddle; H: Haflinger).



Gross energy in late lactation milk is 13% lower than in early lactation milk (498E vs 431D kcal/kg). Mariani *et al.* (2001) observed that milk energy value, despite the higher contribution of lactose, decreases progressively and significantly from the 4<sup>th</sup> to 180<sup>th</sup> lactation day; such variation is observed by other Authors as well (Ullrey *et al.*, 1966; Doreau *et al.*, 1988; Burns *et al.*, 1992 and Mariani *et al.*, 1993).

Ash content in late lactation milk is 39.2% lower than that of early lactation (0.503E *vs* 0.306D g/100g). Other Authors observe lower values in late lactation as well (Intrieri and Minieri, 1969; Bouwman and van der Schee, 1978; Martuzzi *et al.*, 1997; Summer *et al.*, 2001). In particular mineral elements vary as follows: P: -47.0%, Ca: -51.8%, Mg: -47.9% and K: -32.0% (Table 1). A lower content of these minerals in late lactation milk was also observed by Summer *et al.* (2001) and Martuzzi *et al.* (1997) and only for Ca and P by Bouwman and van der Schee (1978).

Ash content (g/100g) is significantly different in the two breeds, higher in IS than in H: 0.554 E vs 0.372D in IS and 0.449E vs 0.237D in H. Schryver et al. mention differences among breeds regarding minerals in milk similar to those here described, but it is undetermined if such differences definitely depend on the breed (Schryver et al., 1986). The trend of phosphorus decreasing is different between the two breeds: in IS milk the drop is more accentuated (interaction Breed x Sample: P = 0.050) (Figure 1).

Sodium shows a different variation of content in the milk of the two breeds, although it is not significant: in IS it increases from 23.06 to 35.78 mg/100g, while in H it drops from 17.70 to 13.20. These variations are in accordance with those statistically significant observed regarding freezing point, decreasing when mineral salts increase, which shows a different variation in the two breeds: in IS it decreases from - 0.529E to - 0.533D °C, while in H it moves toward water freezing point (from - 0.531E to - 0.522D °C). Interaction Breed x Sample is significant (P= 0.028). Anyway between the breeds the difference is not significant.

#### Conclusions

As a whole from the nutritional point of view late lactation milk appears to lack interesting qualities, being poorer than early lactation milk in all main organic components except lactose and in all mineral components, except sodium, whose variations between the beginning and the end of lactation were not significant. In particular the halving of phosphorus and calcium contents, the most important elements for bone structure growth, is noteworthy. The slight increase in lactose concentration did not avoid the decrease in energy value of late lactation milk, due mainly to the lower fat content.

Some differences between the milk composition of the two breeds were found: the milk of IS mares resulted richer in fat, ash and casein nitrogen than H mares' milk, and a different trend in freezing point and phosphorus was noticed. Such differences, in any case, do not show a marked superiority of IS milk quality.

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