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Composition of mare's colostrum and milk I. Fat content, fatty acid composition and vitamin contents

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Abstract. Changes in the fat content, fatty acid composition and vitamin contents of mare's colostrum and milk during the first 45 days of lactation were studied. Milk samples (300–800 ml) from 29 lactating mares, were collected daily at the beginning of the lactation and weekly from 5 to 45 days postpartum. Colostrum and early milk samples were obtained by hand, without oxytocin administration, while the foal nursed. Later milk samples were from mixed milk of the totally-milked udder. Each sample was analysed for total solids, fat content, fatty acid

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composition and vitamin contents by conventional methods and Packard gas chromatograph.

The total solids and the fat contents, respectively, of the colostrum and milk were 24.25 to 26.28% and 2.85 to 2.93% on the first day of lactation, 12.15 to 12.78% and 2.05 to 2.17% on the 2nd to 5th days and 10.37 to 10.61% and 1.04 to 1.32% on the 8th to 45th days of lactation. The concentrations of octanoic, decanoic, dodecanoic, miristic and palmitoleic acids increased over time while stearic, oleic, linolic and linolenic acids decreased. The fatty acid composition of mare's milk fat was very different from that of cow's milk fat. Mare's milk fat contained octanoic, decanoic, dodecanoic, linolic, linolenic, stearic, miristic and palmitic acids, respectively, in ratios of approximately 9.6, 3.1, 2.1, 4.4, 224, 0.2, 0.6 and 0.5 times those of concentrations in cow's milk. On the basis of the differences in fatty acid composition, a new method was developed to determine the amount of cow's milk mixed with mare's milk.

Contents of vitamins A, D_3, K_3 and C of colostrum (0.88, 0.0054, 0.043, 23.8 mg/kg) were found to be 1.4 to 2.6 times the levels in normal milk (0.34, 0.0032, 0.029, 17.2 mg/kg). There was no significant difference found between vitamin E contents of colostrum and milk (1.342 and 1.128 mg/kg). Vitamin contents of mare's milk were very similar to those of cow's milk.

1 Introduction

The relative importance of the horse industry in Hungary has changed greatly in recent years due to mechanisation in agriculture. In 2000, there were 70 000 horses in Hungary, with most of the industry being associated with sport horses and slaughter horses. Currently, there is considerable interest in the use of mare's milk for human consumption in Western Europe. It has been suggested that mare's milk may be curative agent for metabolic and allergic diseases and, consequently, the price paid for mare's milk has increased greatly. This suggests a new possibility for producing income from the horse industry. It also suggests that research is needed to evaluate the value of mare's milk as a human food. Most of the earlier research on composition of mare's milk was directed toward evaluating the value of milk as related to nutrition of the foal.

Duration of lactation has been reported to be 5 to 8 months [13, 34] and estimated milk production of mares was 2000–3000 kg [30]. During a single milking, composition of the milk changes, so the mixed milk of the totally-milked udder must be sampled [5, 24]. Butterfat content undergoes the largest change [9, 30], and can be 10 to 20 times more at the end than that at the beginning of milking [8, 9, 12]. Sampling method and milking interval [15, 26] also influence the composition of milk. Most authors suggest that foals should be present at sampling [1, 3, 4, 15, 20, 25, 28, 29, 31, 36]. Some others advise an injection of oxytocin [25, 31].

The composition of mares' colostrum was analysed by several authors [20, 27, 33]. Colostral period of mares was found to be much shorter than that of cows, and the colostrum showed significant differences from normal milk only on the first day after foaling [20, 29, 35]. The dry matter content of mare's milk decreased drastically from colostrum to normal milk, due primarily to a decrease in protein content [17, 29]; the fat content and fatty acid composition of milk fat showed much smaller changes over time [2, 17].

The fat content of mare's milk is very low [10, 19]. However, it can be influenced by environment and ranged form 0 to 7.9% [29, 30]. Analysis of the fatty acid composition of butterfat of mare's milk showed [2, 17, 18, 22] that it contains very small quantities of stearic and palmitoleic acids, and high quantities of linolenic and linolic acids. This could be explained by the fact that unsaturated fatty acids are not hydrogenated in the digestive system and horses consume a very large amount of forage, which is rich in unsaturated fatty acids.

Among the factors influencing milk composition, stage of lactation is the most important, but the stage of lactation may [17] or may not [18] influence the fatty acid composition of milk fat. Most authors [21, 29] have not reported breed to affect milk composition, but Boulot [3] reported a significant effect of breed. Increasing the fat content of feeds did not increase the quantity of milk and caused no change in milk composition [36], which differed from effects on cow's milk [10]. Others found higher milk fat content to be associated with higher fat input [7].

Holmes et al. [16] and Kulisa [22] published data on water soluble vitamin contents of mare's milk. No estimates of fat soluble vitamin contents of mare's milk were found in the literature.

Based on the fact that data on the fatty acid composition of mare's milk are limited and that there are virtually no data comparing the fatty acid composition of mare's milk with cow's milk in the same trial, a study was initiated at the University of Kaposvár to evaluate the composition of mare's milk and cow's milk simultaneously. Additional objectives of the experiment were to evaluate time changes in milk composition from foaling to 45 days after foaling and to obtain more detailed information on the quantities of polyunsaturated essential fatty acids present in mare's milk.

2 Material and methods

In the spring of 2001, the experiments were initiated and milk samples of 29 mares (16 Hungarian Draughts, 4 Haflingers, 6 Bretons and 3 Boulonnais) were collected and analysed. Mares were on pasture of relatively good quality (1 ha per mare-foal/pair), from spring to autumn; supplemental feeding, when needed was 3 kg oats per day. Winter feeding was 3 kg hay, 2 kg concentrate and ad lib. straw daily.

Mares were milked on the following schedule: they were driven in the stable at 06:00 hours. Each mare and foal were tied. First milking was started at 09:00 and finished at 11:00 hours. During milking time, foals were released, but retied following milking. Second milking time was between 11:15 and 12:30 hours. The foals were not retied after the second milking because mares and foals were returned to the pasture until 06:00 hours the following day. Milking of mares was accomplished with a Westfalia milking machine (model RPSZ 400).

Colostrum and milk samples (80–100 ml) were taken directly after foaling and on the second and third days of lactation by hand milking. On the 5^{th}, 10^{th}, 30^{th} and 45^{th} days of lactation, the mixed milk of the totally-milked udder was sampled. Colostrum and milk samples were frozen and stored at $-25\,°C$. At the time of analysis, the frozen material was thawed and mixed. Dry matter of colostrum and milk samples was determined by Hungarian Standard No. 3744–67 by drying to constant weight at $105\,°C$. Fat content was determined by the Gerber method according to Hungarian Standard No. 3703–78.

The fatty acid contents of the milk fat were determined in the form of fatty acid methyl esters by a Packard 419 type gas chromatograph, a flame ionisation detector and a Hewlett-Packard 33900 type electronic integrator. In the quantitative evaluation, the weight percentage proportions of the methyl esters were regarded as equal to the proportions of the corresponding peaks in the chromatogram [6].

To determine vitamins A-, D_3- and E-contents of milk, samples (5 ml) were saponified by alcoholic pirogallol solution and 2.5 ml 80% potassium hydroxide. The resulting material was extracted in an alcohol – n-hexane system. The extract was distilled and diluted in 200 μl methanol; 20 μl of the solution was injected on a 250×5 mm column packed with 10 μm granulation Partisil ODS, and the vitamin concentrations were determined on a Pye UNICAM LC-XP HPLC. Elution was carried out with a 85 : 15 solution of methanol : water at 1.4 ml/minute drift speed. The basis for quantitative evaluation was vitamin standards made by MERCK. Vitamin K_3 was determined on a solution obtained by a chloroform extraction of an alkalescent substrate. The extracted vitamin K was detected on 251 nm. Vitamin C content of milk samples was determined by the method of Radeff [32].

3 Results

Dry matter content and changes associated with stage of lactation (colostrum to 45 days) for mares of the four breeds are shown in *Table 1.* Changes in the fat content of colostra and milks for the same period are in *Table 2*. *Table 3* shows fatty acid contents of milk fat and time changes, expressed as relative percentages of fatty acid methyl esters. Investigations related to comparison of fatty acid composition of mare's milk, cow's milk and blends are summarised in *Table 4.*

Table 1: Dry matter contents of the colostrum and milk of mares (g/100 g milk)

Breed	**No. of mares**	**Days post-partum**		
		0–0.5	2–5	8–45
Haflinger	4	24.25	12.87	10.61
S.D.		4.34	1.49	2.12
Breton	6	24.65	11.93	10.39
S.D.		6.38	2.05	1.24
Boulonnais	3	25.42	12.15	10.37
S.D.		4.12	2.22	1.73
Hungarian Draught	16	26.28	12.78	10.40
S.D.		3.16	1.64	1.57
Mean	29	25.57	12.55	10.42
S.D.		4.10	1.32	1.54

The dry matter content of colostrum immediately after foaling ranged from 14.65 to 29.35%. The mean and standard deviation were 25.57% and 4.10. Due to the large variation, breed differences were not significant ($P \times 0.25$). Dry matter content decreased quickly following foaling, and values found on day 2 differed only slightly from those obtained 5 days after foaling. The dry matter content of transition milk, on days 2 to 5 of lactation, averaged 12.55%. The dry matter content of normal milk obtained on days 8 to 45 averaged

Table 2: Fat contents of the colostrum and milk of mares (g/100 g milk)

Breed	No. of mares	Days post-partum 0–0.5	2–5	8–45
Haflinger	4	2.87	2.05	1.04
S.D.		0.462	0.183	0.610
Breton	6	2.91	2.10	1.32
S.D.		0.381	0.214	0.483
Boulonnais	3	2.85	2.17	1.29
S.D.		0.294	0.331	0.390
Hungarian Draught	16	2.93	2.16	1.26
S.D.		0.455	0.163	0.540
Mean	29	2.91	2.13	1.25
S.D.		0.431	0.189	0.499

10.42% with S.D. = 1.54. There were no significant differences among breeds (P $\times$ 0.25) in the dry matter content of their colostrum or milk samples.

The fat content of colostrum immediately after foaling averaged 2.91% while that of transition milk and normal milk, respectively, averaged 2.13 and 1.25%. There were no significant differences (P $\times$0.25) among breeds in the fat content of their colostrum, transition milk or normal milk. These changes in the fat content of colostrum and milk over time after foaling confirm results of other authors [20, 23]. Those authors who reported an increase in the fat content of colostrum to 48 h [35] or to day 7 of lactation [14] probably made a sampling mistake due to the fact that the udder cannot be easily milked totally immediately after foaling and the fat content increases dramatically during milking. No relationship was found between milk quantity and fat content.

Comparison of the fatty acid composition of butterfat of colostrum and milk showed that the fat of colostrum contained less octanoic, decanoic, dodecanoic, miristic, palmitic and palmitoleic acids than that of normal milk. On the other hand, the fat of normal milk contained less stearic, linoleic and linolenic acids than that of colostrum. There were no significant differences among breeds regarding fatty acid content, and data shown in *Table 3* represent the arithmetic average of 29 mares, ignoring breed.

Table 3: Mean and standard deviations for the fatty acid composition of the lipids in colostrum and milk of mares (Relative percentages of the fatty acid methyl esters)

Fatty acid	Days post-partum							
	Mare						Cow	
	0–0.5		2–5		8–45		5–270	
	x	S.D.	x	S.D.	x	S.D.	x	S.D.
Caprylic acid C 8:0	1.39	0.18	2.56	0.94	2.79	0.91	0.29	0.022
Capric acid C 10:0	5.41	0.47	8.59	2.89	8.05	2.25	2.61	0.219
Lauric acid C 12:0	7.90	1.57	9.89	3.19	8.97	2.10	4.35	0.362
Miristic acid C 14:0	6.30	0.26	9.67	1.89	8.72	1.97	14.00	0.998
Palmitic acid C 16:0	21.32	1.58	25.63	2.99	23.28	3.58	44.06	2.10
Palmitoleic acid C 16:1	2.80	1.97	5.07	1.14	3.96	1.52	2.08	1.009
Stearic acid C 18:0	2.36	0.53	1.63	0.51	1.55	0.79	7.94	1.001
Oleic acid C 18:1n9	17.12	0.21	13.77	5.38	13.72	2.58	17.25	1.533
Oleic acid C 18:1n6	0.78	0.29	0.74	0.21	0.69	0.24	*	
Linoleic acid C 18:2n6	9.78	0.83	6.40	0.90	7.53	1.47	1.72	0.198
γ-linolenic acid C 18:3n6	0.75	0.13	0.51	0.03	0.61	0.19	*	
Linolenic acid C 18:3n3	24.11	2.57	15.53	1.99	20.12	4.12	0.09	0.02

* not determined

Results of these analyses were in agreement with the data in the literature. Exceptions were linoleic acid which was significantly less and linolenic acid, which was significantly higher than data in the literature. These differences can be explained by the composition of the diet of mares. The fatty acid composition of feedstuffs have a greater influence on the fatty acid composition of milk fat in case of horses than of ruminants. Microbial action in the rumen results in more modification of dietary fats than would occur in the horse.

When comparing the fatty acid composition of milk fat of mares at day 45 of the lactation and cows, it can be stated that the fat of mare's milk contains 2.1 times as much dodecanoic acid, 3.1 times as much decanoic acid, 4.9 times as much linoleic acid, 9.6 times as much octanoic acid and 224 times as much linolenic acid as cow's milk fat. On the other hand, the fat of mare's milk contains only 0.62 as much miristic acid, 0.53 as much palmitic acid and 0.2 as much stearic acid as cow's milk fat. The fatty acids which are higher in mare's milk are unsaturated or short chain fatty acids which suggests that mare's milk fat is a more desirable dietary constituents than cow's milk fat. This huge difference between the fatty acid contents of the milk fat of the two species suggested that we could use a function of fatty acid composition to detect the presence of cow's milk in a blend of milks from the two species.

The fatty acid content of cow's milk and mare's milk are shown in the first and last columns of *Table 4*.

A ratio of fatty acid contents, designated as the f-factor, was calculated as the product of fatty acids higher in mare's milk divided by the product of fatty acids higher in cow's milk.

$$f = \frac{\text{caprylic} \times \text{capric} \times \text{lauric} \times \text{linoleic} \times \text{linolenic}}{\text{miristic} \times \text{stearic}}$$

Based on the averages, the f-value for mare's milk was 2257 and that of cow's milk was 0.005. Using day 45 milk of 10 mares and milk of 10 individual cows, f-values were calculated for each individual and the standard deviations were calculated as 112 and 0.0007, respectively, for mares and cows.

Assuming the fat contents of mare's and cow's milks to be 1.5 and 4.0%, respectively, the fatty acid contents of various blends were calculated and entered in *Table 4*. The f-values for 1, 5, 10, 25 and 50% cow's milk were, respectively, 1840, 816, 390, 57, and 5.3. Mare-cow pairs were formed randomly at each blend and 10 f-values were calculated for each blend. These values were used to calculate the standard deviations for each blend, which are shown in *Table 4*. The t-value for 9 degrees of freedom and probability of 0.01 ($t = 3.25$) was used to calculate the 99% confidence band for each blend. Confidence limits = Mean × 3.25 (S.D.). The confidence limits are shown at the bottom of *Table 4* and it can be seen that we could not be 99% certain of detecting adulteration with cow's milk at a level of 1%, but we could be 99% certain of detecting cow's milk at the level of 5%.

All of the above results were based on calculations. The method was tested by creating five blended samples of individual mares and cows at the level of

Table 4: Fatty acid composition of mare's milk blended with different proportions of cow's milk (calculated values)

Fatty acid	Percentage of cow's milk in the blend						
	Mare's milk	**1**	**5**	**10**	**25**	**50**	**Cow's milk**
Caprylic acid C 8:0	2.79	2.73	2.45	2.23	1.62	0.98	0.29
Capric acid C 10:0	8.05	7.92	7.28	6.82	5.42	4.07	2.61
Lauric acid C 12:0	8.97	8.88	8.29	7.93	6.39	5.62	4.35
Miristic acid C 14:0	8.72	8.87	9.24	9.94	11.21	12.56	14.00
Stearic acid C 18:0	1.55	1.70	2.29	3.00	4.55	6.20	7.94
Linoleic acid C 18:2n6	7.53	7.38	6.71	6.21	4.80	3.31	1.72
Linolenic acid C 18:3n3	20.12	19.61	17.40	15.55	10.71	5.56	0.09
f-values							
mean	2257	1840	816	390	57	5.3	0.005
S.D.	112	62.4	16.2	12.8	4.45	0.44	0.0007
99% confidence limits for f [Mean ± 3.25 (S.D.)]:							
Upper limit	2621	2043	869	432	72	6.7	0.007
Lower limit	1893	1778	763	348	43	3.9	0.003

$f = \frac{\text{caprylic} \times \text{capric} \times \text{lauric} \times \text{linoleic} \times \text{linolenic}}{\text{miristic} \times \text{stearic}}$

5% cow's milk. The f-values ranged from 763 to 824 and, when compared with the calibration curve developed from the calculated values, predicted 4.95% cow's milk. Based on the results, the f-value can be used as a means of detecting small quantities of cow's milk blended with mare's milk and the rate of dilution can be quite accurately predicted.

The analysed vitamin contents of colostrum and milk *(Table 5)* showed that colostrum contained 2.6, 1.7, 1.4 and 1.5 times as much vitamins A, D_3, C and K_3, respectively, as mare's milk between 8^{th} and 45^{th} days of lactation. Vitamin E contents of colostrum and milk were similar. Mare's milk contained practically the same amounts of vitamins A, D_3 and K_3 as cow's milk, but

Table 5: Vitamin contents of mare's colostrum and milk

Vitamin (mg/kg)	Days postpartum Mare		Cow
	0–0,5	8–45	5–270
A	0.88	0.34	0.352
D_3	0.0054	0.0032	0.0029
E	1.342	1.128	1.135
K_3	0.043	0.029	0.032
C	23.8	17.2	15.32

vitamin C content was slightly higher.

Since the fat content of cow's milk is 2.5 to 3.0 times as high as that of mare's milk, the concentrations of liposoluble vitamins in milk fat is much higher in mare's milk fat than in cow's milk fat. The values reported in *Table 5* could appear to be the first reported for contents of vitamins A, D_3, E and K_3. Vitamin C was reported to be 14.7 mg/kg [16] which is slightly lower than the 17.2 mg/kg in *Table 5.*

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