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Composition of mare's colostrum and milk II. Protein content, amino acid composition and contents of macro- and micro-elements

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Abstract. Changes in the protein content, protein fractions and amino acid composition of mare's colostrum and milk, and biological value of milk protein during the first 45 days of lactation were studied. Milk samples (averaging 300–800 cm^3) from 29 lactating mares were collected daily at the beginning of the lactation and weekly from the 5th to 45th days post-partum. Colostrum samples were obtained by hand milking without oxytocin administration, while the foals nursed and milk samples

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were obtained from the mixed total daily production. Each sample was analysed for total protein, true protein, whey protein, true whey protein, casein and non-protein nitrogen (NPN) content using a Kjel-Foss nitrogen analyser and for amino acid composition by LKB amino acid analyser. The biological value of the milk protein was calculated on the basis of amino acid composition by the method of Morup and Olesen [27]. Ash and macro and micro-elements contents of colostrum and milk were also determined.

The total protein, whey protein, casein and NPN contents, respectively, were 16.41, 13.46, 2.95 and 0.052% for colostrum immediately after parturition; 4.13, 2.11, 2.02 and 0.043% for milk between the 2^{nd} and 5^{th} days and 2.31, 1.11, 1.20 and 0.031% for milk in the 8^{th} to 45^{th} days of lactation. The ratios of true protein and whey protein to total protein decreased, while the comparable ratios of casein and NPN increased from foaling to 45 days. The amino acid contents of colostrum and milk decreased during the first 45 days of lactation. Most of the essential amino acids (threonine, valine, cystine, tyrosine, lysine) decreased, while glutamic acid and proline increased in the milk protein after parturition. Therefore, the biological value of the milk protein is highest (132.3) immediately after parturition due to very high levels of threonine and lysine. This value decreases in the course of 5 days to 119.7 and to 107.9 on the 45^{th} day of lactation. The essential amino acid composition and biological value of mare's milk protein was much higher than that of bovine milk proteins.

Ash content of colostrum (0.592%) was significantly higher than that of normal milk (0.405%). Calcium content was lowest immediately after foaling (747.7 mg/kg) and reached a maximum at day 5 (953.7 mg/kg). Zinc and copper contents decreased after reaching a maximum on day 5, while manganese content increased to day 5 and maintained that level. The macro- and micro-element contents (mg/kg) of colostrum and milk, respectively, were: potassium, 928.6 and 517.2; sodium, 320.0 and 166.6; calcium, 747.7 and 822.9; phosphorus, 741.7 and 498.8; magnesium, 139.7 and 65.87; zinc, 2.95 and 1.99; iron, 0.996 and 1.209; copper, 0.606 and 0.249 and manganese, 0.0447 and 0.0544.

1 Introduction

The first report in this series [6] reviewed the reasons for initiating studies on the composition of mare's milk and reported dry matter and fat contents of mare's colostrum and milk and the fatty acid composition of milk fat. This report concerns contents of protein and protein fractions of colostrum and milk, amino acid composition of milk and milk protein, biological value of mare's milk protein and contents of macro- and micro-elements of colostrum and milk.

Mare's colostrum was reported to contain more than 10% protein and almost 80% of the protein content was immunoglobulins [9, 16, 28]. Those concentrations decreased rapidly after foaling. Most foals are born with agammaglobulinaemia or hypogammaglobulinaemia, and colostrum with a high immunoglobulin content is needed as soon as possible to endow passive immunity [4, 23, 24].

Following the colostral period, the total protein content of mare's milk (N%×6.38 [28]) was 1.7 to 3% [3, 11, 14, 18, 25, 26, 28, 36, 39]. Casein, as a proportion of total protein, was less than 50% in most cases, while whey protein was more than 50%. In mare's milk, the percentage of non-protein nitrogen (NPN) was surprisingly great compared with other farm animal species, and it may represent 10% of total nitrogen content. The NPN fraction contains much free amino acids [10]. The proteose-peptone fraction of mare's milk was reported to be 0.16–0.19% [37, 42]. After the colostral period, the whey protein fraction of mare's milk contains 11 to 21% immunoglobulin, 2–15% serum albumin, 26–50% α-lactalbumin and 28–60% β-lactoglobulin [25, 35, 42, 43].

There are few reliable data on the amino acid composition of colostrum and milk of mares. The amino acid composition of protein showed little change during the colostral period, and was similar to that of ruminants with the exception of arginine and threonine, and was very similar to that of sow's milk [33]. Others [13, 19, 30] reported that the amino acid composition of mare's milk differed significantly from that of other farm animals due to higher cystine and glycine contents. Mare's milk contains much free serine and glutamic acid but is low in methionine.

Among factors which influence protein composition of mare's milk, stage of lactation is the most important. The protein content of mare's milk decreases rapidly to the second week of lactation and continues to decrease slowly to the end of second month [3, 14, 22]. NPN content did not change during the first two months of lactation [29]. After the colostral period, stage of lactation had no significant effect on either the ratio of milk protein fractions [9, 18, 25, 26], or the total amino acid composition [11].

Most authors [9, 13, 18, 28] reported no breed effect on milk composition, but Boulot [2] reported a significant effect. Though there were considerable individual variations [1, 2, 17, 29], there was a low correlation between milk yield and milk composition.

The protein content of mare's milk decreased when the energy content of feed increased [11], which differs from results experienced with cattle [31]. Several authors [14, 30] have not found a relationship between the nitrogen content of feed and the total protein content of mare's milk, while others reported a dramatic decrease in the levels of protein and NPN in milk if nitrogen content of feed was reduced [10].

Most authors determined only the main components of the protein of mare's milk and very few of them determined the total and free amino acid composition of milk. There is very little information on how protein content and amino acid composition of milk protein change after parturition and during the lactation. There are very few data concerning the free amino acid content of mare's milk and the amino acid composition and biological value of mare's milk protein in comparison with cows' milk and bovine milk protein determined at the same time and by the same method.

Table 1: Macro-element contents of mare's colostrum and milk

Author	Time after foaling (day)	Ash %	K mg/kg	Na mg/kg	Ca mg/kg	P mg/kg	Mg mg/kg
Doreau et al. [11]	7	-	-	-	1350	460	-
	28	-	-	-	1180	420	-
	56	-	-	-	970	360	-
Holmes et al. [15]	28	-	790	-	1060	710	112
	112	-	640	-	1020	630	90
Kulisa [19]	-	0.295	-	-	-	394	29
Linton [20]	28	0.35	-	-	1265	1205	-
	112	0.26	-	-	945	865	-
Oftedal et al. [29]	-	0.42	-	-	-	-	-
Neseni et al. [28]	28	0.45	624	112	847	580	-
	112	0.30	303	75	485	467	-
Schryver et al. [34]	7	0.61	664	237	1345	943	118
	28	0.45	469	161	1070	659	86
	105–119	0.32	341	115	700	540	43
Sutton et al. [38]	1	-	-	-	1000	900	-
	30	-	-	-	1000–1200	500–600	-
Ullrey et al. [39]	28	0.46	580	186	1186	358	65
	112	0.27	370	161	614	216	43

Mineral content of mare's milk has been reported to be lower than that of milk of other farm animals. Ash content *(Table 1)* was generally reported to be 0.3 to 0.5% with extremes of 0.2 and 0.7%. Macro-element contents expressed as mg/kg also varied greatly among the various reports in the literature *(Table 1)* with ranges of 485 to 1350 for calcium, 216 to 1205 for phosphorus, 29 to 118 for magnesium, 75 to 237 for sodium and 303 to 990 for potassium. Mare's milk was found to have 61% of calcium, 31% of phosphorus and 16% of magnesium in colloidal form [8]. Reports of trace elements contents of mare's milk *(Table 2)* are limited in number and the reported concentrations are extremely variable.

Table 2: Micro-element contents of mare's colostrum and milk

Author	Time after foaling (day)	Zn mg/kg	Fe mg/kg	Cu mg/kg	Mn mg/kg
Kulisa [19]	-	0.89	1.46	0.25	-
Lonnerdal et al. [21]	-	1–2	0.3–1.0	0.2–0.4	-
Schryver et al. [34]	7	3.1	-	0.85	-
	28	2.2	-	0.55	-
Ullrey et al. [40]	0	6.4	1.31	0.99	-
	0.5	2.8	0.95	0.83	-
	1	3.6	1.05	0.73	-
	8	3.3	0.88	0.44	-
	35	2.2	0.71	0.25	-
	120	2.4	0.49	0.20	-
Underwood [41]	-	-	-	0.20–0.36	0.05

Bouwman and Van der Schee [3] reported that ash, calcium and phosphorus contents of milk increased from day two to day three of lactation and then decreased to the 28th day of lactation. Calcium content was reported to decline between the 7th and 56th days of lactation [11]. Ash content, most macro-element contents and some micro-element contents were reported to decrease during lactation [20, 34].

2 Material and methods

Protein and protein fraction contents, amino acid composition and biological value of milk and milk protein of colostrum and milk produced up to the 45th day of lactation by 16 Hungarian Draught, 4 Haflinger, 6 Breton and 3 Boulonnais mares were determined. The feeding, milking and sampling techniques were described previously [6].

Milk samples, frozen to −25 °C, were thawed in water of 35 °C and blended. Total protein content and protein fractions of colostrum and milk were measured by the Kjel-Foss nitrogen analyser (protein content = N% × 6.38). Separation of protein fractions was done as described by [5]. The amino acid composition of milk protein was measured by automatic amino acid analyser (type: LKB 4101). Protein was hydrolysed by 6 M HCl, the sulphur-containing cystine was determined in the form of cysteic acid and the tryptophan content of milk protein was determined by the barium-hydroxide hydrolysis method [7]. The biological value of milk protein was calculated by the method of [27] on the basis of amino acid composition.

Ash content was determined by the Hungarian Standard (MSZ-3926/2-76). The macro- and micro-elements which were present in the ash as metallic oxides, were converted to chlorides by hydrochloric acid and taken into solution. The metallic contents were determined by UNICAM Solaar M6 type atomic absorption spectrofotometer. Phosphorus content was determined by Spekol photometer by measuring the blue colour created by ammonium molibdenate.

The composition of cow's milk was determined on samples from 32 cows by the same methods as were used for mare's milk. The cows were under summer feeding conditions, based principally on grass. The sample consisted of 17 Holstein-Friesian sired crossbred cows (62.5% Holstein-Friesian, 25% Jersey and 12.5% Hungarian red spotted) which were in second or third lactation and 15 Hungaro-Friesian cows in first lactation.

3 Results

The concentration of protein and protein fractions, their changes in mare's colostrum and milk to the 45^{th} day of lactation and distribution of protein fractions are shown in *Table 3. Table 4* shows the free amino acid content of mare's colostrum and milk while the total amino acid concentrations in mare's colostrum and milk, expressed as g/100 g fluid and g/100 g protein, are shown in *Table 5.* Ash content and concentrations of macro- and micro-elements are shown in *Table 6.*

There were no significant differences among the four breeds with regard to the protein content of colostrum, transition milk or milk. Breed was found not to influence distribution of protein fractions, amino acid composition, biological value of colostral or milk protein or contents of ash, macro-elements and micro-elements. The results reported are for 29 individual draught mares.

The total protein content of colostrum immediately after foaling ranged from 13.2 and 22.0% and averaged 16.41%. This value decreased to 4.13% in transitional milk (2^{nd} to 5^{th} days) and to 2.13% in milk (8 to 45 days after foaling). Due to the fact that the true protein content was calculated by subtraction of NPN from total protein, the changes in true protein coincided with those of total protein. There were similar large changes in whey protein and true whey protein contents over time. These two components, respectively, decreased from 13.5 to 13.1%, measured immediately after foaling, to 2.1 and 1.8% days 2 to 5 and to 1.1 and 0.9% days 8 to 45.

Corresponding changes in casein and NPN contents were much smaller. The casein content of colostrum was 2.95% immediately after foaling, 2.02% days 2 to 5 and to 1.20% days 8 to 45. The NPN content of colostrum was about 20% higher than that of transition milk and 40% higher than that of normal milk.

The distribution of protein fractions, expressed as percentages of total protein *(Table 3),* changed over time. The true protein content decreased from 97–98% for colostrum to 91% for milk. Contents of whey protein and true whey protein, respectively, decreased from 80 and 82% to 39 and 48%, while casein and NPN contents, respectively, increased from 18 to 52% and from 2.0 to 8.6% during the 45 days after foaling. Most of the observed changes occurred during the first 24 h after foaling, and the remainder of the changes from colostrum to milk occurred gradually over the first five days.

The composition of milk secreted after the 5^{th} day of lactation was almost identical to that of milk on the 45^{th} day of lactation. The authors cited earlier published very few data on colostrum, and these data had much greater standard deviations than we observed. The range of 4.8–25.0% for the protein content of colostrum reported by Linton [20] and the range of 10.6–25.0% reported by Rouse & Ingram [32] indicated great differences among individual mares. The smaller range in present study (13.2 to 22.0%) was probably due to use of consistent sampling methods. We considered first milked colostrum to be only that sample which was taken immediately after foaling before the foal could suckle. If the foal suckles prior to sampling, the sample will be diluted due to initiated milk secretion and the composition is altered significantly. Precise timing of the first sample is much more important for mares than for cows because the quantity of colostrum is much less than that in the mammary gland of cows; therefore, the dilution after sucking is much greater. The value of 16.41% for the total protein content of first milked colostrum measured by us was 2.5% lower than that reported by Ullrey et al. [39] and 5–8% higher than that measured by Sutton et al. [38].

Table 3: Means and standard deviations of protein contents and protein fractions of mare's colostrum and milk (g/100 g milk), and distribution of mare's milk protein fractions as percentages of total protein

Protein fractions	Days postpartum					
	0–0.5		**2–5**		**8–45**	
	x	**S.D.**	**x**	**S.D.**	**x**	**S.D.**
Total protein	16.41	3.21	4.13	0.77	2.31	0.50
True protein	16.08	2.98	3.86	0.72	2.11	0.48
Whey protein	13.46	2.63	2.11	0.61	1.11	0.32
True whey protein	13.13	2.41	1.84	0.50	0.91	0.25
Casein	2.95	0.34	2.02	0.26	1.20	0.14
NPN × 6.38	0.34	0.041	0.27	0.015	0.20	0.052

Protein fractions	Days postpartum		
	0–0.5	**2–5**	**8–45**
Total protein	100	100	100
True protein	97.96	93.44	91.38
Whey protein	82.02	51.09	48.05
True whey protein	79.98	44.53	39.43
Casein	17.98	48.91	51.95
NPN × 6.38	2.04	6.56	8.62

There are no previous reports concerning the protein fractions of colostrum. The results reported here for the protein fractions of milk are difficult to compare with data of authors cited earlier because they reported casein contents ranging from 41 to 65% of the total protein while we obtained a value of 49 to 52%. There are no reports on changes in protein fractions from colostral period to 45th day of lactation. Values observed for the NPN content of mare's milk were slightly higher than those discussed earlier.

The free amino acid content *(Table 4)* of colostrum, with the exception of threonine, serine and glutamic acid, were about twice as high as those of normal milk. When the composition of free amino acids is expressed in percentages, it is clear that colostrum contained approximately five times as much basic (histidine, lysine, arginine) amino acids, and only about 1/3 to 1/2 as much acidic amino acids as normal milk. The concentration of free amino acids in colostrum was 63.68 mg/100 g, which is 19.01% of NPN. These values in case of milk were 31.19 mg/100 g and 15.67%, respectively. Therefore, approximately 16–20% of NPN of colostrum and milk of mares is in the form of free amino acids. It has been reported [19] that mare's milk contains more free serine and glutamic acid than free methionine. The values in *Table 3* would indicate that this statement could be expanded to say that the proportion of all other investigated free amino acids were greater than that for methionine. The sulphur-containing amino acids (methionine and cystine) represented much lower proportions of the free amino acids than any of the other amino acids investigated.

Table 4: Free amino acid contents of mare's colostrum and milk

Amino acid	**Free amino acid content**			
	mg free AA/100 g milk		**g free AA/100 g free AA**	
	colostrum	milk	colostrum	milk
Asp	2.90	0.60	4.6	1.9
Thr	2.90	3.57	4.6	11.5
Ser	5.59	8.97	8.8	28.8
Glu	9.21	9.92	14.5	31.8
Pro	2.50	1.61	3.9	5.2
Gly	4.01	1.01	6.3	3.2
Ala	3.68	0.66	5.8	2.1
Cys	0.53	0.06	0.8	0.2
Val	9.21	1.67	14.5	5.4
Met	0.39	0.03	0.6	0.1
Ile	1.84	0.16	2.9	0.5
Leu	3.75	0.35	5.9	1.1
Tyr	1.38	0.28	2.2	0.9
Phe	1.51	0.57	2.4	1.8
Lys	6.32	0.88	9.9	2.8
His	6.38	0.66	10.0	2.1
Arg	1.58	0.19	2.5	0.6
Totals	63.68	31.19	100.2	100.0

The amino acid compositions of colostrum and milk *(Table 5)* show that changes in amino acid content paralleled those of total protein content relative to time after foaling. It means that each amino acids decrease, without exception, from colostrum to milk (8 to 45 days). For example, threonine content declined from 1.13 g/100 g colostrum to 0.10 g/100 g milk and the corresponding change for glutamic acid was 2.28 to 0.47. When the amino acid composition was expressed as g AA/100 g protein, the changes were much less apparent. Threonine decreased from 6.9 to 4.3 g/100 g protein while glutamic acid increased from 13.8 to 20.1 g/100 g protein. The sum of five essential amino acids (threonine, valine, cystine, tyrosine and lysine) decreased from 26.4 to 21.3 g/100 g protein, while the total of two nonessential amino acids (glutamic acid and proline) increased from 21.9 to 28.5 g/100 g protein.

Table 5: Amino acid composition of mare's colostrum and milk, amino acid composition of colostrum and milk proteins and amino acid composition of cow's milk and cow's milk proteins

Amino acid	**Free amino acid content**						**Cow's milk**	**Cow's milk protein**
	Days postpartum			**Days postpartum**				
	0–0.5	2–5	8-45	0–0.5	2–5	8-45		
	g AA/100 g sample			g AA/ 100 g protein			g AA/100 g milk	g AA/100 g protein
Asp	1.543	0.404	0.246	9.3	9.7	10.4	0.26	7.8
Thr	1.132	0.235	0.101	6.9	5.7	4.3	0.15	4.5
Ser	1.444	0.306	0.147	8.7	7.4	6.2	0.16	4.8
Glu	2.281	0.702	0.474	13.8	16.9	20.1	0.77	23.2
Pro	1.346	0.339	0.197	8.1	8.2	8.4	0.32	9.6
Gly	0.558	0.124	0.045	3.4	3.0	1.9	0.06	1.8
Ala	0.673	0.157	0.076	4.1	3.8	3.2	0.10	3.0
Cys	0.164	0.033	0.014	1.0	0.8	0.6	0.02	0.6
Val	0.853	0.198	0.097	5.2	4.8	4.1	0.16	4.8
Met	0.213	0.054	0.035	1.3	1.3	1.5	0.06	1.8
Ile	0.492	0.132	0.090	3.0	3.2	3.8	0.14	4.2
Leu	1.444	0.388	0.229	8.7	9.3	9.7	0.29	8.7
Tyr	0.771	0.182	0.101	4.7	4.4	4.3	0.15	4.5
Phe	0.738	0.200	0.111	4.5	4.8	4.7	0.16	4.8
Lys	1.444	0.351	0.189	8.7	8.4	8.0	0.27	8.1
His	0.492	0.116	0.056	3.0	2.8	2.4	0.10	3.0
Arg	0.706	0.186	0.123	4.3	4.5	5.2	0.11	3.3
Trp	0.229	0.054	0.028	1.4	1.3	1.2	0.05	1.5
Totals	16.523	4.161	2.359	100.1	100.3	100.0	3.33	100.0

These results seem to contradict data reported by Doreau et al. [11] who reported no change in the amino acid composition of mare's milk protein between the 7th and 56th day of lactation. However, they did not investigate the critical period of the first five days after foaling when the largest changes occurred in our investigation. We did not find a publication which reported data on the amino acid composition of colostrum milked immediately after foaling.

Data for the amino acid composition of milk protein, with the exception of the two sulphur-containing amino acids, agree with results of Doreau et al. [11] and Peltonen et al. [30]. In the case of some amino acids, our results differed from results reported by Kulisa [19] and Sarkar et al. [33].

The biological value of milk protein was calculated by the method of Morup & Olesen [27] based on amino acid composition. The biological value of colostrum milked immediately after foaling (132.3) almost reached the maximum of the method (140), which was due to the very high threonine and lysine contents. During days 2 to 5, this value decreased to 119.7 due to the reduced quantities of essential amino acids. From the 8^{th} to the 45^{th} day, the biological value of milk protein was 107.9. This is a very high biological value compared to that of cow's milk which was 80.2 based on data in *Table 3.* These differences can be explained by the higher proportion of whey protein and higher quantities of essential amino acids, especially threonine, in mare's milk. There are no comparable data in the literature.

Ash content of mare's colostrum averaged 0.592% during the first 48 hours of lactation with a range of 0.515 to 0.804 *(Table 6).* There were no literature values reported prior to day 7 *(Table 1).* Ash content decreased to 0.513% (range 0.499 to 0.542%) on days 3 to 5 and to 0.405% (range 0.301 to 0.479%) in the period 8 to 45 days. The latter value is comparable of the mean of literature values in *Table 1.* The values of 0.61% [34] would seem to be high while values of 0.30% or lower [19, 28, 39] would seem to be low.

All macro-elements except calcium decreased in the colostral period and at the beginning of lactation. Decrease was most evident for magnesium, but significant decrease was also experienced for potassium and sodium. Phosphorus content showed little change prior to 5^{th} day of lactation. Calcium content of mare's colostrum was lowest right after foaling (747.7 mg/kg), reached a maximum of 953.7 mg/kg on the 5^{th} day of lactation and then declined. Ullrey et al. [39] observed maximum calcium content on the 8^{th} day after foaling. Macro-element contents of mare's milk were in good agreement with literature values in *Table 1.*

Time trends for micro-element contents of mare's colostrum and milk showed that zinc and copper decreased continuously, iron decreased after reaching a maximum on the 5^{th} day, manganese increased to the 5^{th} day and then stayed constant. Ullrey et al. [40] published data on micro-element contents of mare's colostrum and reported higher zinc, iron and copper contents of colostrum than we observed in this study. Differences were negligible for later periods of the lactation. Comparison of micro-element contents of mare's milk *(Table 6)* with literature data shows that 1.99 mg/kg for zinc content agrees well with about

Table 6: Ash, macro-element and micro-element contents of mare's milk and cow's

Analysis	Days postpartum							
	Mare						Cow	
	0–2		3–5		8–45		5-270	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Ash, %	0.592	0.091	0.526	0.019	0.405	0.063	0.753	0.031
Macro-elements (mg/kg)								
Potassium	928	75	709	138	517	65	1204	68
Sodium	320	86	177	44	167	72	504	34
Calcium	748	190	953	86	823	125	1287	143
Phosphorus	742	109	638	121	499	83	996	111
Magnesium	140	81	86	15	66	16	139	12
Micro-elements (mg/kg)								
Zinc	2.95	1.36	2.08	0.50	1.99	0.28	5.63	0.19
Iron	1.00	0.54	1.58	0.90	1.21	0.63	1.07	0.32
Copper	0.61	0.30	0.25	0.12	0.23	0.09	0.30	0.06
Manganese	0.045	0.025	0.053	0.022	0.054	0.029	0.093	0.013

half of the literature data, and is slightly lower then the others. Iron, copper and manganese contents were also in general agreement with values shown in *Table 2*.

Cow's milk contained almost twice as much ash, potassium, phosphorus, magnesium and manganese, 50% more calcium, iron and copper and almost three times as much sodium and zinc as mare's milk. The low sodium content of mare's milk is a particularly desirable attribute for a dietary component for cardiovascular and hypertension patients.

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