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EFFECTS OF THE LACTATION PERIOD, BREED AND FEED ON AMINO ACIDS PROFILE OF MARE'S MILK

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ABSTRACT

The effects of the lactation period, breed, and feed on amino acids profile of mare's milk were investigated. The feed contained two major essential amino acids (EAAs) leucine (7.31 – 10.3 g.kg^{-1}) and arginine (6.37 – 9.59 g.kg^{-1}); it also included minor EAAs methionine (2.11 – 3.05 g.kg^{-1}) and histidine (2.48 – 3.60 g.kg^{-1}). Glu+Gln, Asp+Asn, and proline, major nonessential amino acids (NEAAs), constituted approximately 60% of total NEAAs (TNEAAs). The ratio of total EAAs to NEAAs ranged from 1:1.2 to 1:1.4. Amino acids (AA) content throughout all milk samples varied due to mare's different conditions and lactation days. Except for the 1P milk sample, total AA content in the 2 – 8Ps specimens caused by differences in breed oscillated from the 2nd to 28th day of lactation within the following limits: 21.9 – 54.6 g.kg^{-1}, 33.6 – 70.7 g.kg^{-1}, 38.1 – 71.2 g.kg^{-1}, 29.46 – 74.2 g.kg^{-1}, 52.2 – 87.1 g.kg^{-1}, 37.9 – 70.3 g.kg^{-1} and 26.4 – 64.5 g.kg^{-1}, respectively. In relation to TEAAs in milk, the highest EAAs levels were reached in arginine, leucine and lysine ranging between 2.41 – 4.35 g.kg^{-1}, 3.36 – 5.59 g.kg^{-1} and 2.72 – 4.80 g.kg^{-1}, respectively, while the lowest AAs amounts were indicated in histidine and methionine, 0.91 – 1.58 g.kg^{-1} and 1.23 – 2.04 g.kg^{-1} respectively. Total NEAAs content was slightly higher than that of EAAs; the TNEAAs to TEAAs ratio was 1:0.9 proximately. Glu+Gln, Asp+Asn and proline were determined as major NEAAs of milk ranging between, 6.77 – 11.0 g.kg^{-1}, 3.21 – 5.60 g.kg^{-1} and 1.25 – 2.18 g.kg^{-1}, respectively; levels of NEAAs such as cysteine and glycine oscillated between 0.89 – 1.52 g.kg^{-1} and 0.64 – 1.15 g.kg^{-1}, respectively. The average TAAs contents caused by breed differences were 62.8 g.kg^{-1}, 42.8 g.kg^{-1}, 44.7 g.kg^{-1} and 44.8 g.kg^{-1}, respectively, on the 2nd, 5th, 10th, and 28th lactation days.

Keywords: mare's milk; amino acids; minerals; lactation; feed; breed

INTRODUCTION

Milk is a fluid secreted by the female of all species of mammals. There are about 4500 species producing milk to cover the complete nutritional requirements of the neonate of the species, as well as to provide some immunological protection and to satisfy other physiological requirements. The milk of all species is similar but there are significant species-specific differences. Interspecies differences in the quantitative composition of milk probably reflect differences in the metabolic processes of the lactating mother and the nutritive requirements (amino acids, minerals, fatty acid, vitamins, etc.) of the sucking young. In addition to supplying all the nutritional requirements of the neonate, many of the micro-constituents of milk, such as oligosaccharides, immunoglobulins, metal-binding proteins, and enzymes serve protective roles. Because the nutritional and physiological requirements of each species are rather unique, the composition of milk shows substantial inter-species differences **(Fox, 2009; Fox and Mcsweeney, 1998; Wong et al., 1999)**.

Milk of only about 180 species of mammals has been analyzed up to now; the data for only about 50 species are considered reliable **(Fox and Mcsweeney, 1998; Park, 2009)**. Besides being the most important nutritional resource for foals during the first months of life, the mare´s milk is also one of the most important basic foodstuffs for human populations in Kazakhstan, Kirghizia, Tadzhikistan, Uzbekistan and Mongolia. Lactic-alcoholic beverages called Koumiss, Airag and Kumis are traditionally produced through fermentation **(Di Cagno et al., 2004; Montanari et al., 1996; Malacarne et al., 2002; Orskov, 1995; Pikul and Wójtowski, 2008)**. To a lesser extent, horses have been used as dairy herds in Eastern and Central Europe (Belarus, Ukraine, Hungary, and Bulgaria) **(Caroprese et al., 2007; Doreau and Martin-Rosset, 2002; Fox, 2009)**. Regarding Western Europe, where the most important product of equine breeding is represented by the foal, studies on mare's milk have concentrated mainly on the growth and health of the newborn horse, however, in two decades, the mare's milk has been subjected to through research and, as the result, consumed for human health and as a fundamental food **(Park, 2009)**. Besides the composition of mare's milk fat, the properties of its protein fractions and composition of

amino acids suggest that this product is more similar to human milk than to cow's milk. For the above reason, and because of the low cross-reactivity between cow's and mare's milk proteins, a clinical study has suggested that mare's milk could be used as a valid replacement for cow's milk in children with severe IgE-mediated cow's milk protein allergy **(Businco et al., 2000)**. More recently, the consumption of milk and other dairy foods, by virtue of their mineral, bioactive lipids, and protein components, has been shown to help reduce the risk of chronic disease disorders including osteoporosis, hypertension, excess body weight and body fat, dental caries, and some types of cancer **(Csapó et al., 1995; Curadi et al., 2001; Miclo et al., 2007)**.

The nutritional role of mare's milk as a component of the diet has traditionally been evaluated based on its overall contribution of essential and non-essential nutrients forming a high-quality diet for the support of optimal growth and development of the foal. The colostral period of mares is much shorter than that of cows and the colostrum shows significant differences from common milk only on the first day after foaling **(Barello et al., 2008; Huth, DiRienzo and Miller, 2006)**. The nutritional dependence of the foal on mare's milk composition seems to be most marked immediately after birth. In the case of dairy animals, it is well known that nutrients digested by the dairy cows have a significant effect on the maternal milk composition **(Huth, DiRienzo and Miller, 2006)**. For the proper nutritional management of horses (during the lactating period and of foals during the sucking period), it is essential to clarify the relationship between the intake of nutrients and the milk composition in mares. The daily nutrient requirements of lactating mares are very high, and they can be compared with those of racing horses in heavy training. Important nutrients are secreted by the mare to supply her foal with energy, proteins, fats, carbohydrates, vitamins, and minerals for optimal development and growth. To substitute these nutrient losses and at the same time to support maintenance requirements, lactating mares must consume adequate amounts of quality feeds **(Pagan and Hintz, 1986)**. It is known that the intake of minerals and amino acids is particularly important for the growth of foals. The protein requirement of the horse is influenced by the capacity of the protein to satisfy the amino acid needs of the animal. In the horse diet, there are currently four main essential amino acids: lysine, methionine, threonine, and tryptophan. These amino acids are the most important nutrients because limiting their levels will affect the growth and development of the horse.

In the last decade, quite detailed research on equine milk and colostrum composition and, especially studies on proteins, lipids, and minerals have been carried out for the above-mentioned reasons. Many authors have reported data on the profile of essential nutrients such as minerals, amino acids, and vitamins in mare's milk; however, no complex research including all the above factors has been implemented **(Fišera et al., 2018)**. Moreover, there are a few studies of the relationship between changes in mare's milk main components and the above affecting factors such as lactation stage and others.

Scientific hypothesis

Therefore, this study is focused on changes in contents of essential nutrients detected in mare's milk depending on lactation stages, feeding system, and on some breeds. The research aimed to analyze the content of significant nutritional components in mare's milk and lactating mare´s feed as well as to characterize the effects of several factors that affect milk properties such as lactation periods, breed, and mare's diet. More specifically, the aims of the study were i) to analyze changes in the major amino acid composition of mare's milk caused by differences in broodmares and by periods in the first month after parturition, ii) to analyze the major amino acid content and dry matter levels in the lactating broodmares´ feed during the lactation days after parturition and iii) to study the relations between the amino acid composition of milk and feed. The final goal was to get detailed information on the relationship between amino acids detected in mare's milk and their feed. Effects of the lactation period, breed, and feed on amino acids profile of mare's milk were investigated and successively evaluated.

MATERIAL AND METHODOLOGY

Selection of broodmares and broodmares' milk samples

To collect mare's milk and colostrum samples, eight different mature, well-developed, and normal broodmares were selected. All the selected mares were from seven to sixteen years of age (10 ±3 parities), with the live weight between 500 and 600 kg. They were kept indoor and outdoor individually and fed with 1.3 kg wheat bran, 2.1 kg hominly, 5.0 kg oats every day. Water was available from troughs at all times. All mares were vaccinated against influenza and herpes virus per 6 months.

The milk samples (approximately 40 – 50 mL) from selected broodmares were taken into plastic containers on 2, 5, 10, 28, and 56 days postpartum; 31 individual samples were collected in total (Table 1). Before milking, foals were separated from their mothers for approximately 2 hours to prevent suckling. The first milking of mares was carried out with hands as deep as possible. Some mares had not previously been subjected to any milking procedures. The second milking was done accordingly. Collected milk samples were taken directly to the laboratory and then immediately stored and frozen at -20 °C and -25 °C. The samples of feed mixture for the lactating mares (in the amount of approximately 500 g) were collected into polyethylene bags on the days of milk sample collecting altogether 29 samples were collected (Table 1). Then all the samples were taken to the laboratory and stored at room temperature.

All the collected milk samples were being frozen in a freezer at -80 °C for 4 hours, and then they were being lyophilized at 120 Pa and -50 °C for 2 days using vacuum lyophilization (Labicom, Czech Republic). All the lyophilized samples were stored in a fridge at -4 °C before their analysis. All the feed samples were being mixed or ground using a mixer for 3 min and then put into plastic containers. All the grounded samples were stored at room temperature prbeforeheir analysis.

Reagents and solutions: All reagents used were of analytical reagent grade (Sigma-Aldrich, St. Lois, Mo, USA). Concentrated hydrochloric acid 37% (v/v) HCl, 6 mol.L^{-1} HCl, argon gas, 0.1 mol.L^{-1} HCl, loading buffer of pH 2.2, 30% (v/v) H_2O_2, 85% (v/v) HCOOH.

Equipment: The contents of amino acids in all samples were determined by using an AAA 400 Amino Acid Analyzer (INGOS, Prague, Czech Republic), a liquid chromatograph designed for the analysis of amino acids on an ion-exchanger column with post-column derivatization by means of ninhydrin and for the determination of biogenic amines. A thermoblock (Labicom, Olomouc, Czech Republic), a rotary evaporator (Heidolph Instruments GmbH + Co. KG, Kelheim, Germany) and an oil bath (Memmert GmbH + Co. KG, Schwabach, Germany) were used for acid and alkaline oxidizing hydrolyses of all milk and feed samples.

Procedures

Analysis of dry matter

Dry matter (DM) was determined according to the AOAC standard procedure **(AOAC, 1990)**. Contents of dry matter were calculated according to the following equation DM (%) = $m_2.100/m_1$, where DM (%) is the percentage of a total dry matter, m_1 is sample weight before DM determination and m_2 is sample weight after drying.

Analysis of amino acid contents

The contents of amino acids in all samples were determined by using an AAA 400 amino acid analyzer (INGOS, Prague, Czech Republic). An amino acid analyzer is a special compact liquid chromatograph designed for the analysis of amino acids on an ion-exchanger column with post-column derivatization by means of ninhydrin and for the determination of biogenic amines.

The acidic hydrolyses of all milk and feed samples were performed with few modifications under hydrolysis time and temperature conditions according to **Buňka et al. (2009)** and **Buňka, Hrabě and Kráčmar (2004)**. Briefly, the lyophilized milk (50 – 60 mg) and dried feed samples (80 – 90 mg) were accurately weighed in screw-capped test tubes for acidic hydrolysis (capacity of 20 mL) with Teflon caps. 15 mL HCl (6 mol.L^{-1}) solutions were added to every tube with a sample. Afterward, the tubes were being purged by blowing with argon gas for approximately 20 seconds, and then the vacuum-sealed tubes were being heated in a thermoblock at 117 ±1 °C for 23 hours. The temperature of the thermoblock was independently controlled by using a thermometer immersed in a test tube filled with silicone oil (the test tube was placed in the thermoblock). After hydrolysis, hydrolyzed samples were filtered using a paper filter and washed with 0.1M HCl. Then the filtrates were firstly dried using a rotary evaporator at 50 °C and 90 rpm and evaporated secondly and thirdly after a double washing with distilled water (approximately 25 mL) until we obtained a dried mass. The dried mass was dissolved in loading buffer with pH 2.2 and made up to the mark in a 25 mL volumetric flask. Then 1.5 mL of this solution was put into a plastic container and filtered through a 0.45 μm filter and subsequently placed in a fridge until loaded to the amino acid analyzer. All analyses were performed in triplicate for every sample.

In order to determine sulphur-containing amino acids, all samples were hydrolyzed separately with HCl (6 mol.L^{-1}) after oxidizing the samples with performic acid according to methods elaborated by **Buňka, Hrabě and Kráčmar (2004)** and **Amarakoon (2009)** with few modifications. Performic acid was prepared in the ratio of 1:9 from 30% H_2O_2 and 85% HCOOH. After incubation at room temperature for 2 hours, the performic acid was being kept in a fridge at 4 ±1 °C for approximately 30 seconds. The lyophilized milk (50 – 80 mg) and dried feed samples 1000 – 8000 mg) were accurately weighed and put into a 250 mL Erlenmeyer flask. 15 mL of performic acid was added to each sample and then it was being cooled at 4 ±1 °C at least for 16 hours for sample oxidizing. After oxidation, 1 – 2 mL of concentrated HCl was added to the oxidized samples and incubated in the hood for approximately 30 min until chlorine gas was removed. Then 150 mL of 6 mol.L^{-1} HCl was added to the oxidized samples and heated in an oil bath at 117 ±1 °C for 23 hours. After hydrolysis, the samples were filtered with a paper filter, dissolved in 0.1 mol.L^{-1} HCl and made up to the 250 mL mark in a volumetric flask. 25 mL of the diluted sample was transferred with a 25 mL pipette into a flask and then the hydrochloric acid was evaporated in the rotary evaporator after washing it twice with distilled water until a dried mass was obtained. Then the dried mass was dissolved in loading buffer with pH-2.2 and made up to the mark in a 25 mL volumetric flask. Then 1.5 mL of this solution was put into a plastic container after filtering through a 0.45 μm filter and then placed in a fridge before loading it to the amino acid analyzer. All analyses were performed in triplicate for every sample.

Determination of amino acids

A standard mixture of 17 analyzed amino acids (aspartic acid + asparagine, threonine, serine, glutamic acid + glutamine, proline, glycine, alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, histidine, lysine, arginine, methionine, and cystine) was obtained from INGOS (Prague, Czech Republic). The content of amino acids was determined by an AAA 400 device according to the manufacturer´s program. Then the column was being regenerated with 0.2 mol.L^{-1} NaOH for 10 min and stabilized for further 17 min with buffer A. The temperature of the column was set to 60 °C for a specific time slot (0 – 60 min and 90 – 102 min) and to 74 °C in the mean time (60 – 90 min). The following flow rates were employed: 0.3 mL.min^{-1} for buffers and 0.2 mL.min^{-1} for ninhydrin reagent. Each hydrolysate was analyzed in duplicate.

Statistical analysis

All the contents of total amino acids in all the analyzed samples were expressed as mean ±SD and calculated based on retention time obtained in chromatograms **(Buňka, Hrabě and Kráčmar, 2004; Ingos Ltd., 2006)**. The data were evaluated by summary statistics and the variance was calculated using an ANOVA procedure performed using Statistica® 12.0 software (StatSoft, USA). All data obtained by the AAA 400 were expressed as the mean levels in fresh milk using the following equation

TA = $A_0.\varepsilon_0$ = (L-C)/(D-C), where TA is amino acid content in fresh milk, A_0 is amino acid content of lyophilized milk, ε_0 is correlation coefficient, L is fresh milk weight, D is lyophilized milk weight and C is container weight.

RESULTS AND DISCUSSION

Dry matter in the feed

The dry matter (DM) content in all feed samples for the lactating mares is shown in Table 2. The percentage of DM content varied slightly in individual feed samples due to preparation conditions of feed mixture and because of the ratio of forages in the feed mixture. The average DM content oscillated between 88.6% and 90.6%. According to some researchers **(Looper et al., 2001),** the DM content in selected high-quality feed types for the lactating mares such as grains, hay, and chaff ranged from 87 to 91 % (w/w). The above results comply with NRC **(Pagan and Hintz, 1986)**. DM content in all the feeds, therefore, is in accordance with the standard level of DM in feed and forage for the lactating mare.

Amino acid composition of feed and milk

The amino acid content in the feed

In the experiment, the essential amino acids and non-essential amino acid profiles of each investigated feed for the lactating mare were determined. All the data are expressed as mean ±SD in g.kg^{-1} (p <0.005) and they also depict considerable variations in the amino acid content in feeds. The protein nutritive value is given by its amino acid profile. The protein contents in horse feeds such as in grains, hay, chaff, pasture, or in others range from 10 to 25% of total weight depending on the type of feeds **(Pagan and Hintz, 1986)**. Out of 20 amino acids, a human being or an animal can synthesize only nine. The remaining amino acids should be provided in their food. Arginine is regarded as a semi-essential amino acid **(Boisen, Hvelplund and Weisbjerg, 2000)**.

As shown in tables, AA contents in feeds for the lactating mares differed, which could have been caused by some of the above mentioned factors. Generally, NEAA glutamic acid (Glu+Gln) content (18 – 21 g.kg^{-1}) reached the highest values while the EAA methionine and histidine levels
(2.5 – 3.6 g.kg^{-1}) dropped the lowest among all feeds. EAA arginine and leucine, NEAA aspartic acid (Asp+Asn) and proline content in all feeds were higher than levels of other AAs, 7 – 9 g.kg^{-1}, 8 – 10 g.kg^{-1}, 7 – 9 g.kg^{-1} and 8 – 9 g.kg^{-1} respectively. In the animal diet, there are currently recognized four main essential amino acids: lysine, methionine, threonine, and tryptophan, which are the most important nutrients; limiting their levels will affect the growth and development of the horse **(Saastamoinen and Koskinen, 1993)**. Particularly in the horse, the daily lysine requirement for the lactating mare ranges between 13.8 – 16.9 g per 100 kg of body weight **(National Research Council. 2007)**. The contents of these above AAs in feeds were, except for tryptophan, which was not analyzed, relatively lower than the amounts of the others. Lysine levels ranged between 3.0 – 4.5 g.kg^{-1}, methionine amounts oscillated between 2.5 – 3.6 g.kg^{-1} and threonine content ranged between 3.4 – 4.0 g.kg^{-1}.

Regarding other AA such as EAA isoleucine, valine and phenylalanine, NEAA cysteine, glycine, serine and tyrosine, their levels did not differ much ranging between 3.4 g.kg^{-1}, 4.8 – 6.2 g.kg^{-1}, 4.7 – 6.0 g.kg^{-1}, 3.4 – 3.8 g.kg^{-1}, 5 – 6 g.kg^{-1}, 4.5 – 5.2 g.kg^{-1} and 3.4 – 7.0 g.kg^{-1}, respectively. According to some reports, tyrosine and cysteine are regarded as semi-essential amino acids, since they can be synthesized exclusively from methionine and phenylalanine, respectively **(Boisen, Hvelplund and Weisbjerg, 2000)**. Total NEAA amounts (approximately 50 – 66 g.kg^{-1}) in all feeds on all days were slightly higher than total EAA levels (approximately 40 – 51 g.kg^{-1}). The highest total EAA content reached a maximum (51.7 g.kg^{-1} in 5F) on the 2nd day while the lowest value was found in 1F on the 2nd day (40.5 g.kg^{-1}). In the case of NEAA, the highest amount increased to 66.8 g.kg^{-1} in 7F on the 2nd day and the lowest level was 50.4 g.kg^{-1} in 2F on the 5th day. The above amounts were comparable to levels found in some types of feeds such as oats, barley, sorghum and wheat **(Awadalkareem, Mustafa and El Tinay, 2008; Pagan and Hintz, 1986)**. The protein requirement of the horse is influenced by how well the protein provides for the amino acid needs of the animal; some EAAs are limiting essential amino acids in grass-based feeding programs. It has been reported that daily protein intake of the lactating mare averages at 1493 g.day^{-1} **(Pagan and Hintz, 1986)**. Levels of TAAs in examined feeds ranged between 92 – 116 g.kg^{-1}; their amount was higher than that in some species of oats and there was an agreement with their amount contained in good quality feed for animals **(Biel, Bobko and Maciorowski, 2009)**.

Average amino acid composition of feed

The average content of all AAs examined in 1F – 8F on different lactation days is summarized in Table 3. The average content of each amino acid was calculated in relation to amounts of the AA revealed in all feed on the 2nd, 5th 10th and 28th, days respectively. As shown in this table, all AA contents, except for EAA leucine and methionine and for NEAA proline, on the 28th day and in feeds on the 5th and 28th days slightly decreased. It was observed that the AA contents in feeds reached the highest levels on the 2nd day while the lowest amounts were detected in feeds on the 5th day. Nevertheless, EAA phenylalanine content on the 10th day was higher than the amount of the others. The contents of arginine and leucine, the major EAAs in feed, grew highest ranging between 7.70 – 8.42 g.kg^{-1} and 8.66 – 9.35 g.kg^{-1}, respectively.

Other EAA contents were two and three times lower when compared to major EAA amounts. Moreover, Glu+Gln, proline, alanine and Asp+Asn dominated among the NEAA in feeds ranging between 18.5 – 19.9 g.kg^{-1}, 7.31 – 8.30 g.kg^{-1}, 6.22 – 6.52 g.kg^{-1} and 7.90 – 8.36 g.kg^{-1}, respectively. Contents of the other AA did not differ mutually; methionine and histidine levels in feed scored lowest. Therefore, the total EAA content in all feeds was 1.2 times lower than NEAA content; the average ratio of EAA to NEAA was 1:1.2 (44.1 – 47.0 g.kg^{-1}: 56.4 – 61.2 g.kg^{-1}). It was proved that all feeds for the lactating broodmares were rich in EAA.

Table 1 Milk and feed sample characteristics.

Milk	Days postpartum					Feed	Days postpartum				
	2nd	5th	10th	28th	56th		2nd	5th	10th	28th	56th
1P	+	+	+	+	-	1F	+	+	+	+	+
2P	+	+	+	+	+	2F	+	+	+	+	-
3P	+	+	+	+	-	3F	+	+	+	+	-
4P	+	+	+	+	-	4F	+	+	+	+	-
5P	+	+	+	+	-	5F	+	+	+	-	-
6P	+	+	+	-	-	6F	+	+	+	-	-
7P	+	+	+	-	-	7F	+	+	+	-	-
8P	+	+	+	+	-	8F	+	+	+	-	-

Note: + sample was taken; - sample was not taken.

Table 2 The dry matter content of feed (%, w/w).

Feed	Dry matter (mean value ±SD)				
	2nd	5th	10th	28th	56th
1F	89.38 ±0.19	89.32 ±0.15	89.04 ±0.51	88.96 ±0.66	89.49 ±0.51
2F	90.56 ±0.67	88.64 ±0.34	89.54 ±0.37	89.36 ±0.55	n.a.
3F	90.32 ±0.54	89.55 ±0.86	88.69 ±0.98	89.04 ±0.22	n.a.
4F	88.89 ±0.42	89.14 ±0.51	89.06 ±0.20	88.90 ±0.08	n.a.
5F	89.64 ±0.40	88.99 ±0.70	88.65 ±0.68	n.a.	n.a.
6F	90.12 ±0.39	88.80 ±0.57	88.81 ±0.58	n.a.	n.a.
7F	90.47 ±0.31	89.37 ±0.37	88.81 ±0.53	n.a.	n.a.
8F	90.00 ±0.63	88.80 ±0.39	88.7 ± .51	n.a.	n.a.

Note: F – feed samples, SD – standard deviation, n.a. – not analyzed.

Table 3 The average content of each amino acid in 1F – 8F samples feed.

Amino acid (g.kg^{-1})	1F – 8F							
	2nd day		5th day		10th day		28th day	
	mean	SD	mean	SD	mean	SD	mean	SD
Arginine	8.42	0.82	7.91	0.89	8.18	0.84	7.70	0.74
Histidine	3.15	0.32	2.83	0.29	2.95	0.30	2.88	0.29
Isoleucine	4.09	0.36	3.91	0.38	4.04	0.40	3.91	0.27
Leucine	9.35	0.77	8.66	0.80	8.98	0.90	8.99	0.71
Lysine	4.31	0.42	4.00	0.50	4.18	0.47	3.77	0.35
Methionine	2.49	0.26	2.32	0.09	2.38	0.09	2.38	0.21
Phenylalanine	5.45	0.51	5.32	0.60	5.49	0.62	5.32	0.47
Threonine	3.89	0.32	3.63	0.41	3.77	0.38	3.69	0.35
Valine	5.87	0.55	5.55	0.56	5.73	0.61	5.57	0.46
TEAA	**47.02**	**4.34**	**44.13**	**4.51**	**45.70**	**4.61**	**44.21**	**3.85**
Alanine	6.52	0.52	5.99	0.60	6.22	0.59	6.16	0.56
Asp+Asn	8.36	0.71	7.90	0.81	8.13	0.81	7.91	0.61
Cysteine	3.57	0.13	3.52	0.13	3.52	0.22	3.46	0.18
Glu+Gln	19.94	1.45	18.45	1.66	19.23	1.69	18.75	1.54
Glycine	5.69	0.50	5.26	0.57	5.48	0.53	5.29	0.52
Proline	8.30	0.56	7.31	0.44	7.67	0.33	7.79	0.53
Serine	4.89	0.36	4.55	0.48	4.72	0.45	4.61	0.42
Tyrosine	3.96	1.31	3.37	0.43	3.47	0.40	3.40	0.44
TNEAA	**61.24**	**5.54**	**56.35**	**5.12**	**58.45**	**5.03**	**57.36**	**4.79**
TAA	**108.26**	**9.88**	**100.48**	**9.63**	**104.16**	**9.65**	**101.57**	**8.63**

Note: TEAA – total essential amino acid content, TNEAA – total non-essential amino acid content, TAA – total amino acid content.

The ratio of AA in some feeds for animals such as in oats grain was reported as 1:2.8 (27 g. (16 g N)$^{-1}$: 75 g.(16 g N)$^{-1}$) **(Biel, Bobko and Maciorowski, 2009)**. TAA levels in feeds ranged from 100.48 g.kg^{-1} to 108.26 g.kg^{-1} depending on feeding days. Overall, these AA concentrations in all feeds were considerably higher than those in daily feed intake of lactating mares **(Pagan and Hintz, 1986)**.

The amino acid content in milk

The variations in the amino acid contents in milk samples from the eight lactating mares on several days after parturition are shown in Table 4, as a representative example. As shown in this table, the AA contents in 1P on all days were three times lower than those in other mares while there were significant differences in AA contents in all milk. It was, perhaps, due to sample processing and the mare's physiological conditions. It was observed that the highest AA contents were reached in all milk on the 2nd day of lactation; afterwards, all AA contents dropped considerably on the 5th day. It corresponded to the end of the colostral period.

In many papers, the lactation period is the most significant factor influencing the composition of all mammalian milk, in particular of horse milk; protein content in colostral period is five times higher than that in milk period, due to providing the foal with all nutrients and immune substances during the first few days **(Kráčmar et al., 2005)**. The colostral period of mares was found to be significantly shorter than that of cows; quality of colostrum showed significant differences from that of normal milk only on the first 2 days after foaling **(Salamon et al., 2009)**. The AA contents in 2P, 3P, 4P, and 8P on the 10th day of lactation was increased significantly and then decreased gradually. In regard to 1P, 5P, 6P and 7P, the AA content was continuously decreasing till the 10th day of lactation, although on the 28th day it increased slightly again. These changes agreed with reports on mare's milk composition during the lactation period.

Some authors reported that after parturition, the AA content in mare's milk was decreasing continuously **(Csapó et al., 2009)**. Leucine, lysine, arginine and valine contents in all mares' milk samples were higher than those of other EAAs, ranging within 4.4 – 6.7 g.kg^{-1}, 3.7 – 6.5 g.kg^{-1}, 4.2 – 5.8 g.kg^{-1} and 2.7 – 4.9 g.kg^{-1}, respectively; the leucine content was the highest one while histidine content (1.2 – 2.2 g.kg^{-1}) was the lowest one in all milk samples. In the case of NEAA, Glu+Gln, Proline and Asp+Asn, their contents in milk ranged between 8.8 – 15.8 g.kg^{-1} and 4.7 – 7.6 g.kg^{-1} respectively; they reached the highest levels while glycine and alanine contents oscillating between 0.9 – 1.5 g.kg^{-1} and 1.3 – 1.9 g.kg^{-1}, dropped lowest in all milk samples. The above AA contents profile agreed with that foundby **Matsui, Inoue and Asai (2003)**.

Some authors reported that when the AA composition was expressed as AA g.100g^{-1} protein, the changes were noticeably less apparent; it was due to high amount of free AA in milk during the colostral period; after the colostral period, total free AA concentration decreased to half value from 0.6 g.kg^{-1} to 0.3 g.kg^{-1}. The contents of free AAs in colostrum, except for threonine, serine, and glutamic acid levels, were about twice as high as those in normal milk **(Csapó-Kiss et al., 1995)**. Overall, AA contents in 6P on lactation days were higher than those in the others; oscillations in 3P, 4P, 5P, 7P, and 8P AA during lactation days were comparable to each other and no significant difference was observed. Although the AA amounts in all milk on different lactation days differed from each other, they are suggested to depend on feed and breed differences.

Average amino acid composition of milk

Further, Table 5 shows the average total AA content in milk related to differences between eight broodmares, which were fed with above mentioned good quality feeds during lactation, relative to 2nd, 5th 10th and 28th days of lactation. It means that the content of each amino acid decreased, without any exception, considering the change of colostrum to milk (2nd to 28th day), although, on the 10th day, a slight increase of all AA contents in milk was observed in milk.

Generally, after the 2nd and 5th day of lactation, there was a slight difference in AA content which agrees with the data reported by some authors who suggested no changes in the amino acid composition of mare's milk proteins between the 8th and 45th day of lactation **(Csapó-Kiss et al., 1995; Davis et al., 1994)**. In the diet of the horse and the newborn foal, lysine, methionine, threonine, and tryptophan are considered the most important substances to affect its growth and development **(Saastamoinen and Koskinen, 1993)**. Lysine was detected to be one of the major EAAs in milk while methionine and threonine content were the lowest ones. The content of major EAAs arginine, leucine, lysine, isoleucine and valine on the 2nd day of lactation follow: 4.35 g.kg^{-1}, 5.59 g.kg^{-1}, 4.80 g.kg^{-1}, 3.01 g.kg^{-1} and 3.49 g.kg^{-1}, respectively. On the 28th day of lactation, their levels decreased to 2.41 g.kg^{-1}, 3.36 g.kg^{-1}, 2.72 g.kg^{-1}, 1.79 g.kg^{-1} and 2.10 g.kg^{-1}, respectively.

Regarding the oscillation of NEAA amounts, the pattern similar to that of EAAs was observed; the major NEAA Glu+Gln levels decreased from 11.0 g.kg^{-1} to 6.7 g.kg^{-1} and Asp+Asn content dropped from 5.6 g.kg^{-1} to 3.21 g.kg$^{-1,}$ etc. The decrease of AA content in milk between the 2nd and the 28th day ranged between 30 % and 40 %; it was comparable to a decrease of AA content studied by **Csapó et al. (2009)**. Total EAA content reached 30.3 g.kg^{-1}, 20.5 g.kg^{-1}, 21.2 g.kg^{-1} and 17.7 g.kg^{-1} on the 2nd, 5th, 10^{th,} and 28th days respectively, showing values lower than total levels of NEAA.

Therefore, the average ratio of total EAA to total NEAA content on lactation days was calculated as 1:1.1, which is in disagreement with the 1:2.2 value found by **Csapó et al. (2009)**; moreover, the ratio in colostrum was reported as 1:2 **(Lehtola and Saastamoinen, 2010)**, which was influenced by breeds and their feed. On the other hand, total EAA content was significantly higher, and the total NEAA was lower than the values mentioned in the literature. Total AA contents during lactation days reached 62.8 g.kg^{-1}, 42.8 g.kg^{-1}, 44.7 g.kg^{-1} and 37.1 g.kg^{-1} respectively; they decreased throughout lactation days analogically to those of EAAs and NEAAs.

Table 4 Amino acid content in 1P sample milk.

Amino acid (g.kg^{-1})	1P									
	2nd day		5th day		10th day		28th day		*2 – 28th days*	
	Mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	***mean***	***SD***
Arginine	1.28	0.05	0.82	0.02	0.77	0.04	1.02	0.01	*0.97*	*0.23*
Histidine	0.50	0.01	0.40	0.01	0.29	0.02	0.39	0.01	*0.39*	*0.09*
Isoleucine	0.99	0.02	0.76	0.03	0.58	0.03	0.73	0.04	*0.77*	*0.17*
Leucine	1.92	0.06	1.47	0.03	1.14	0.08	1.47	0.12	*1.50*	*0.32*
Lysine	1.51	0.00	1.18	0.05	0.87	0.05	1.05	0.04	*1.16*	*0.27*
Methionine	0.72	0.02	0.54	0.03	0.41	0.04	0.48	0.02	*0.54*	*0.13*
Phenylalanine	0.89	0.05	0.64	0.07	0.50	0.05	0.70	0.06	*0.68*	*0.16*
Threonine	0.75	0.02	0.66	0.01	0.46	0.03	0.62	0.03	*0.62*	*0.12*
Valine	1.17	0.06	0.93	0.05	0.74	0.09	0.90	0.02	*0.93*	*0.18*
TEAA	**9.74**	**0.28**	**7.40**	**0.32**	**5.76**	**0.43**	**7.37**	**0.35**	***7.57***	***1.67***
Alanine	0.74	0.02	0.52	0.01	0.40	0.02	0.56	0.04	*0.55*	*0.14*
Asp+Asn	1.84	0.06	1.45	0.09	1.00	0.07	1.37	0.05	*1.42*	*0.34*
Cysteine	0.54	0.05	0.41	0.02	0.32	0.02	0.38	0.01	*0.41*	*0.09*
Glu+Gln	3.49	0.09	3.01	0.08	2.28	0.12	3.23	0.29	*3.00*	*0.52*
Glycine	0.40	0.01	0.29	0.03	0.21	0.01	0.29	0.01	*0.30*	*0.08*
Proline	1.60	0.02	1.41	0.10	1.05	0.11	1.43	0.01	*1.37*	*0.23*
Serine	1.06	0.06	0.88	0.02	0.63	0.05	0.88	0.05	*0.86*	*0.18*
Tyrosine	1.01	0.01	0.70	0.02	0.53	0.02	0.74	0.09	*0.75*	*0.20*
TNEAA	**10.69**	**0.32**	**8.68**	**0.38**	**6.42**	**0.41**	**8.88**	**0.56**	***8.67***	***1.78***
TAA	**20.42**	**0.59**	**16.08**	**0.70**	**12.18**	**0.84**	**16.25**	**0.91**	***16.23***	***3.45***

Note: TEAA – Total essential amino acid; TNEAA – Total non-essential amino acid; TAA – Total amino acid. In italic – Average content of each amino acid at total lactation days.

Table 5 Average content of each amino acid in 1P – 8P samples milk.

Amino acid (g.kg^{-1})	1P – 8P							
	2nd day		5th day		10th day		28th day	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Arginine	4.35	1.35	2.72	0.95	2.91	1.21	2.41	1.12
Histidine	1.58	0.51	1.06	0.33	1.08	0.47	0.91	0.44
Isoleucine	3.01	0.97	2.09	0.66	2.17	0.89	1.79	0.83
Leucine	5.59	1.81	3.87	1.19	4.05	1.64	3.36	1.51
Lysine	4.80	1.55	3.20	1.03	3.27	1.34	2.72	1.33
Methionine	2.04	0.64	1.59	0.50	1.43	0.66	1.23	0.55
Phenylalanine	2.82	0.92	1.87	0.63	1.97	0.83	1.63	0.77
Threonine	2.59	0.85	1.75	0.56	1.74	0.69	1.49	0.71
Valine	3.49	1.13	2.37	0.76	2.57	1.06	2.10	0.94
TEAA	**30.28**	**9.75**	**20.51**	**6.60**	**21.19**	**8.78**	**17.65**	**8.18**
Alanine	2.18	0.68	1.40	0.47	1.47	0.59	1.25	0.56
Asp+Asn	5.60	1.75	3.76	1.17	3.87	1.61	3.21	1.46
Cysteine	1.52	0.47	1.18	0.38	1.03	0.41	0.89	0.39
Glu+Gln	11.00	3.65	7.64	2.37	8.18	3.24	6.77	2.93
Glycine	1.15	0.35	0.75	0.24	0.76	0.31	0.64	0.30
Proline	4.99	1.64	3.54	1.03	3.92	1.63	3.17	1.28
Serine	3.30	1.03	2.24	0.69	2.33	0.93	1.95	0.87
Tyrosine	2.76	0.83	1.81	0.58	1.92	0.80	1.58	0.73
TNEAA	**32.51**	**10.39**	**22.33**	**6.93**	**23.47**	**9.53**	**19.46**	**8.50**
TAA	**62.79**	**20.14**	**42.84**	**13.53**	**44.67**	**18.30**	**37.11**	**16.68**

Note: TEAA – Total essential amino acid; TNEAA – Total non-essential amino acid; TAA – Total amino acid.

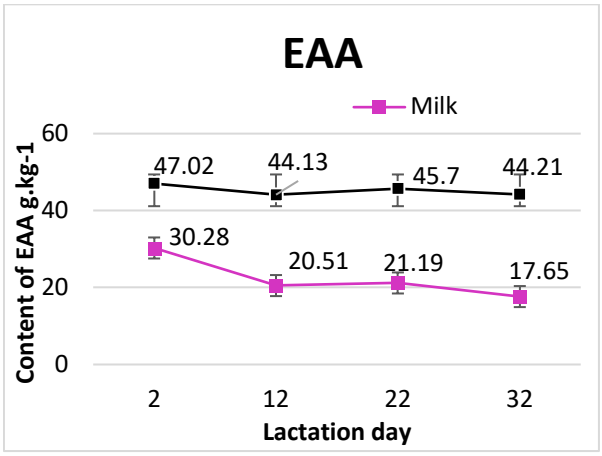

Figure 1 Relation between average total content of essential amino acids in milk and feed.

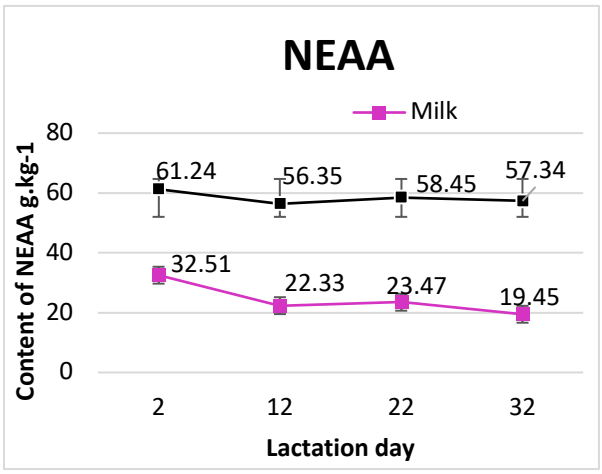

Figure 2 Relation between average total content of non-essential amino acids content in milk and feed.

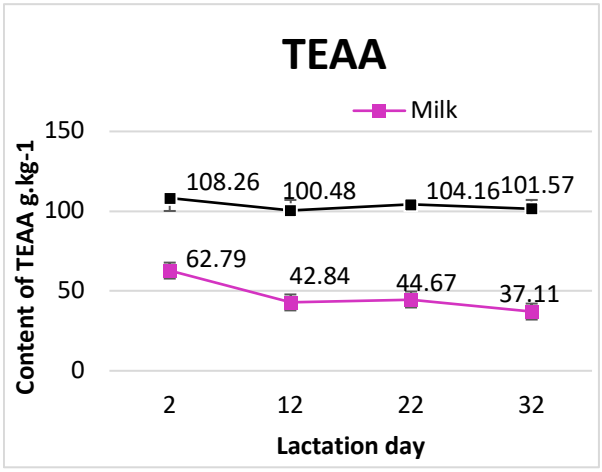

Figure 3 Relation between average total amino acid content in milk and feed.

Some authors reported total AA concentration in mare's colostrum to reach its peak in the first 24 hours after parturition; it ranged from 160 to 170 $g.kg^{-1}$ dropping then to 40 – 42 $g.kg^{-1}$ on the 2 – 5th days, while it decreased to its minimum value of 20 – 25 $g.kg^{-1}$ on 8 – 45th days of lactation **(Csapó-Kiss et al., 1995)**. Equine, milk on the 1st and 2nd day postpartum is considered as initial milk which shows both colostrum and milk properties; therefore, the TAA contents on the 2nd and 5th lactation days did not vary significantly. Nevertheless, TAA levels on the 10th and 28th days were considerably higher than the contents reported above. It has been found by several authors that there were no significant differences in the amino acid composition between the breeds but that total AA content depends on the feeding system and breeds **(Pagan and Hintz, 1986; van den Berg, 2009)**.

The relation between amino acid levels in milk and feed

Supplementary feed and nutrition are considered one of the most important factors that play a crucial role in enhancing the production of milk and its composition. Based on the contents of AA in all feeds and milk, which are given in Table 3 and Table 5, the relations between average contents of each AA in the milk of eight broodmares and studied feeds for the lactating mares on the 2nd, 5th, 10th and 28th days of lactation are summarized in Figure 1, Figure 2 and Figure 3.

Significant relationships between all the compared data were observed; it was indicated that the deviations in levels of each amino acid in milk correlated mutually with those found in feeds on all days of lactation, with the exception of the 2nd day. All amino acid contents on the 2nd day of lactation were considerably higher than AA levels on other days; the above was due to the end of the colostral period **(Csapó et al., 2009)**. Particularly in EAA arginine, isoleucine, leucine, lysine, and phenylalanine, in NEAA Asp+Asn, Glu+Gln, proline and serine, the above tendency was pronounced significantly.

From the 5th to 28th lactation days, all amino acid contents in milk slightly decreased. It was reported that total AA content on the 8 – 45th days was twice lower than that on the 5th lactation day **(Csapó-Kiss et al., 1995)**. In contrast, this study demonstrated that on the 10th lactation day all the AA concentrations, except for EAA lysine, methionine, threonine and for NEAA glycine, increased slightly while all amino acid concentrations in feeds increased.

Methionine, lysine and phenylalanine are the most limiting crucial amino acids contained in animal feed; some feeds slightly improve their amount in milk **(Abu-Ghazaleh, Schingoethe and Hippen, 2001)**. With the exception of methionine, these AA contents were found to be adequate for the lactating mares in all the feeds. Regarding feeds during all lactation days, AA contents in feed were found to be in an adequate amount to cover daily intake of the lactating mare. As reported in the literature, by increasing the level of energy in feed and of the proportion of protein concentrate in the diet, mare's milk amount increased, but milk protein content decreased by 4 $g.L^{-1}$. Moreover, the protein supplementation in the diet showed inconsistent effects on milk protein content.

Additionally, as researchers used a supplement of protein concentrate in a hay-based diet, milk protein content

increased from 17 to 22 g.kg^{-1} **(Martuzzi and Doreau, 2006)**. Further, ratios of total EAA and NEAA content in milk to that in feed on the lactation days obeyed the following values 1:1.5, 1:2, 1:2 and 1:2.4, and 1:1.9, 1:2.5, 1:2.5 and 1:2.8, respectively. In the case of TAA content, the ratio was 1:1.7, 1:2.4, 1:2.4, and 1:2.7. On the other hand, these ratios on the 5th and 10th days of the milk period were consistent while that relating to the 28th day of lactation slightly increased. It has been reported that the ingestion of high-quality protein increases the AA concentration in mare's milk **(Glade and Luba, 1990)**. Thus, the AA concentrations in milk found in this study were significantly related to AA concentrations in the feeds.

CONCLUSION

All results of this research indicated significant relations and changes in major minerals and amino acid composition of different lactating mares' milk throughout the lactation period and in feeds used to feed the mares.

The average DM content of the feed ranged within 89.2 ±0.72 % w/w (p <0.05). Total amino acid contents in all feed generally ranged from 84.4 g.kg^{-1} to 117.0 g.kg^{-1} and the highest total EAA content was 51.7 g.kg^{-1} in 4F on the 10th day while the lowest EAA content dropped to 38.4 g.kg^{-1} in 2F on the 5th day; the major EAAs were represented by leucine and arginine, whose levels ranged within 7.3 – 10.3 g.kg^{-1} and 6.37 – 9.59 g.kg^{-1}, respectively. Methionine and histidine were found minor EAAs with levels oscillating between 2.11 – 3.05 g.kg^{-1} and 2.48 – 3.60 g.kg^{-1}, respectively. In the case of NEAA, their levels were lower compared to those of EAAs contents, which ranged between 66.8 g.kg^{-1} and 50.7 g.kg^{-1}. Glu+Gln, Asp+Asn, and proline were the major NEAAs which represented approximately 60% of TNEAA. The ratio of total EAA to NEAA ranged from 1:1.2 to 1:1.4. Generally, average total AA content in feeds for the lactating mares was found at 105.0 g.kg^{-1}.

According to results obtained, the AA content in all milk samples varied, which was caused by differences in mares and by variations throughout lactation days. The AA contents in 1P milk were three times lower (12.2 – 20.4 g.kg^{-1}) compared to other mares. With exception of 1P, total AA content in 2-8 Ps caused by breed differences ranged 21.9 – 54.6 g.kg^{-1}, 33.6 – 70.7 g.kg^{-1}, 38.1 – 71.2 g.kg^{-1}, 29.5 – 74.2 g.kg^{-1}, 52.2 – 87.1 g.kg^{-1}, 37.9 – 70.3 g.kg^{-1} and 26.4 – 64.5 g.kg^{-1}, respectively, from the 2nd to 28th day of lactation. The average decrease of total amino acid content reached 45.5% from the initial milk period to the 2nd day of the milking period. The average total AA contents caused by breed differences on the 2nd, 5th, 10th and 28th lactation days reached levels at 62.8 g.kg^{-1}, 42.8 g.kg^{-1}, 44.7 g.kg^{-1} and 44.8 g.kg^{-1}, respectively on the 2nd, 5th, 10th and 28th lactation days. During the lactation period, total AA composition did not change, but their amount significantly altered. Concerning TEAA in milk, the highest levels of EAAs were represented by arginine, leucine, and lysine, whose amounts ranged between 2.41 – 4.35 g.kg^{-1}, 3.36 – 5.59 g.kg^{-1} and 2.7 – 4.8 g.kg^{-1}, respectively while the lowest EAAs were exemplified by histidine and methionine, whose levels oscillated between 0.9 – 1.6 g.kg^{-1} and 1.2 – 2.0 g.kg^{-1}, respectively. The total NEAA content was slightly higher than that of EAA; the TNEAA to TEAA ratio was found at 1:0.9 proximately. The major NEAAs in milk were represented by Glu+Gln, Asp+Asn, and proline, ranging between 6.8 – 11.0 g.kg^{-1}, 3.2 – 5.6 g.kg$^{-1,}$ and 1.3 – 2.2 g.kg^{-1}, respectively. In contrast, levels of NEAA cysteine and glycine ranged within 0.9 – 1.5 g.kg^{-1} and 0.6 – 1.2 g.kg^{-1}, respectively.

Significant relationships between all the compared data were observed; it was indicated that the deviations in each amino acid level in milk were accordingly correlated with those found in feed on all days of lactation, except the 2nd one. Overall, the ratios of total EAA and NEAA content in milk to that in feed throughout the lactation period follow 1:1.5, 1:2, 1:2 and 1:2.4; and 1:1.9, 1:2.5, 1:2.5 and 1:2.8, respectively. In the case of TAA content, the ratio was 1:1.7, 1:2.4, 1:2.4, and 1:2.7.

Further studies on mare's digestion of proteins, levels of free amino acids, and minerals in feed and on changes in the nutrients in blood and milk of the lactating mare are suggested to clarify changes in milk composition depending on feeding systems.

REFERENCES

Abu-Ghazaleh, A. A., Schingoethe, D. J., Hippen, A. R. 2001. Blood amino acids and milk composition from cows fed soybean meal, fish meal, or both. *Journal of Dairy Science*, vol. 84, no. 5, p. 1174-1181. https://doi.org/10.3168/jds.S0022-0302(01)74578-X

Amarakoon, R., Kráčmar, S., Hoza, I., Budinský, P. 2009. The effect of Cooking on nutritive quality of selected legumes. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 57, no. 5, p. 13-18. https://doi.org/10.11118/actaun200957050013

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edition, Washington DC. 18 p.

Awadalkareem, A. M., Mustafa, A, I., El Tinay, A. H. 2008. Protein, Mineral content and amino acid profile of sorghum flour as influenced by soybean protein concentrate supplementation. *Pakistan Journal of Nutrition*, vol. 7, no. 3, p. 475-479. https://doi.org/10.3923/pjn.2008.475.479

Barello, C., Perono Garoffo, L., Montorfano, G., Zava, S., Berra, B., Conti, A., Giuffrida, M. G. 2008. Analysis of major proteins and fat fractions associated with mare's milk fat globules. *Molecular Nutrition and Food Research*, vol. 52, no. 12, p. 1448-1456. https://doi.org/10.1002/mnfr.200700311

Biel, W., Bobko, K., Maciorowski, R. 2009. Chemical composition and nutritive value of husked and naked oats grain. *Journal of Cereal Science*, vol. 49, no. 3, p. 413-418. https://doi.org/10.1016/j.jcs.2009.01.009

Boisen, S., Hvelplund, T., Weisbjerg, M. R. 2000. Ideal amino acid profiles as a basis for feed protein evaluation. *Livestock Production Science*, vol. 64, no. 2-3, p. 239-251. https://doi.org/10.1016/S0301-6226(99)00146-3

Buňka, F., Hrabě, J., Kráčmar, S. 2004. The effect of sterilisation on amino acid contents in processed cheese. *International Dairy Journal*, vol. 14, no. 9, p. 829-831. https://doi.org/10.1016/j.idairyj.2004.02.008

Buňka, F., Kříž, O., Veličková, A., Buňková, L., Kráčmar, S. 2009. Effect of acid hydrolysis time on amino acid determination in casein and processed cheeses with different fat content. *Journal of food Composition and Analysis*, vol. 22, no. 3, p. 224-232. https://doi.org/10.1016/j.jfca.2008.10.023

Businco, L., Giampietro, P. G., Lucenti, P., Lucaroni, F., Pini, C., Di Felice, G., Iacovacci, P., Curadi, C., Orlandi, M. 2000. Allergenicity of mare's milk in children with cow's milk allergy. *Journal of Allergy and Clinical Immunology*, vol. 105, no. 5, p. 1031-1034. https://doi.org/10.1067/mai.2000.106377

Caroprese, M., Albenzio, M., Marino, R., Muscio, A., Zezza, T., Sevi, A. 2007. Behavior, Milk Yield, and Milk Composition of Machine-and Hand-Milked Murgese Mares. *Journal of Dairy Science*, vol. 90, no. 6, p. 2773-2777. https://doi.org/10.3168/jds.2006-603

Csapó, J., Salamon, S., Lóki, K., Csapó-Kiss, Z. 2009. Composition of mare's colostrum and milk II. Protein content, amino acid composition and contents of macro- and micro-elements. *Acta Universitatis Sapientiae, Alimentaria*, vol. 2, no. 1, p. 133-148. Available at: http://www.acta.sapientia.ro/acta-alim/C2-1/alim2-11.pdf

Csapó, J., Stefler, J., Martin, T. G., Makray, S., Csapó-Kiss, Z. 1995. Composition of mare's colostrum and milk. Fat content, fatty acid composition and vitamin content. *International Dairy Journal*, vol. 5, no. 4, p. 393-402. https://doi.org/10.1016/0958-6946(94)00008-D

Csapó-Kiss, Zs., Stefler, J., Martin, T. G., Makray, S., Csapó, J. 1995. Composition of mare's colostrum and milk. Protein content, amino acid composition and contents of macro and micro-elements. *International Dairy Journal*, vol. 5, no. 4, p. 403-415. https://doi.org/10.1016/0958-6946(94)00014-G

Curadi, M. C., Giampietro, P. G., Lucenti, P., Orlandi, M. 2001. Use of mare milk in dediatric allergology. Processings of the Associazione Scientifica di Produzione Animale XIV Congress, Frenze, June 12-15, vol. 14, p. 647-649.

Davis, T. A., Nguyen, H. V., Garcia-Bravo, R., Fiorotto, M. L., Jackson, E. M., Reeds, P. J. 1994. Amino acid composition of the milk of some mammalian species changes with stage of lactation. *British Journal of Nutrition*, vol. 72, no. 6, p. 845-853. https://doi.org/10.1079/BJN19940089

Di Cagno, R., Tamborrino, A., Gallo, G., Leone, C., De Angelis, M., Faccia M., Amirante, P., Gobbetti, M. 2004. Uses of mares' milk in manufacture of fermented milks. *International Dairy Journal*, vol. 14, no. 9, p. 767-775. https://doi.org/10.1016/j.idairyj.2004.02.005

Doreau, M., Martin-Rosset, W. 2002. Dairy animals - Horse. In Roginski, H. *Encyclopedia of Dairy Science*. New York, USA : Academic Press, vol. 2, p. 630-637. ISBN 978-0-12-227235-6. https://doi.org/10.1016/B0-12-227235-8/00115-2

Fišera, M., Valášek, P., Kráčmar, S., Kubáň, V., Burešová, P., Velichová, H., Fišerová, L. 2018. Influence of composition of feed and lactation period on mineral composition of Mare's. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 216-225. https://doi.org/10.5219/894

Fox, P. F. 2009. Milk: an Overview. In Thompson, A., Boland, M., Singh, H. *Milk Proteins. From Expression to Food*. Cambridge, USA : Academi Press, p. 1-54. ISBN 978-0-12-374039-7. https://doi.org/10.1016/B978-0-12-374039-7.00001-5

Fox, P. F., Mcsweeney, P. L. 1998. *Dairy Chemistry and Biochemistry*. London, UK : Blackie Academic and Professional. ISBN: 0-412-72000-0.

Glade, M. J., Luba, N. K. 1990. Benefits to foals of feeding soybean meal to lactating broodmares. *Journal of Equine Veterinary Science*, vol. 10, no. 6, p. 422-428. https://doi.org/10.1016/S0737-0806(06)80136-X

Huth, P, J., DiRienzo, D. B., Miller, G. D. 2006. Major Scientific Advances with Dairy Foods in Nutrition and Health. *Journal of Dairy Science*, vol. 89, no. 4, p. 1207-1221. https://doi.org/10.3168/jds.S0022-0302(06)72190-7

Ingos Ltd. 2006. *Automatic amino acid analyser AAA* 400: User manual, Prague, Czech Republic.

Kráčmar, S., Kuchtík, J., Baran, M., Váradyovaá Z., Kráčmarová, E., Gajdůšek, S., Jelínek, P. 2005. Dynamics of changes in contents of organic and inorganic substances in sheep colostrum within the first 72 h after parturition. *Small Ruminant Research*, vol. 56, no. 1-3, p. 183-188. https://doi.org/10.1016/j.smallrumres.2004.06.012

Lehtola, K., Saastamoinen, M. T. 2010. Mare's milk composition in two breeds during different stages of lactation. *Book of Abstracts of the 61st Annual Meeting of the European Association for Animal Production*, 23-27 August, Heraklion, Greece, 16, p. 40. ISBN 978-90-8686-152-1. https://doi.org/10.3920/978-90-8686-708-0

Looper, M. L., Stokes, S. R., Waldner, D. N., Jordan, E. R. 2001. Managing milk composition: Feed additives and production enhancers. Available at: http://www.aces.nmsu.edu/pubs/_d/d-106.html

Malacarne, M., Martuzzi, F., Summer, A., Mariani, P. 2002. Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *International Dairy Journal*, vol. 12, no. 11, p. 869-877. https://doi.org/10.1016/S0958-6946(02)00120-6

Martuzzi, F., Doreau, M. 2006. Mare milk composition: Recent findings about protein fraction and mineral content. In Miraglia, N., Martin-Rosset, W. *Nutrition and Feeding of the Broodmare*. Wageningen, Netherland : Wageningen Academic Publishers, p. 65-76.

Matsui, A., Inoue, Y., Asai, Y. 2003. Diurnal Variations in Milk Amino Acid Concentrations in the Horse. *Journal of Equine Science*, vol. 14, no. 4, p. 101-109. https://doi.org/10.1294/jes.14.101

Miclo, L., Girardet, G. M., Egito, A. S., Mollé, D., Martin, P., Gaillard, J. L. 2007 The primary structure of a low-M_r multiphosphorylated variant of β-casein in equine milk. *Proteomics*, vol 7, no. 8, p. 1327-1335. https://doi.org/10.1002/pmic.200600683

Montanari, G., Zambonelli, C., Grazia, L., Kamesheva, G. K., Shigaeva, M. K. 1996. Saccharomyces unisporus as the principal alcoholic fermentation microorganism of traditional koumiss. *Journal of Dairy Research*, vol. 63, no. 2, p. 327-331. https://doi.org/10.1017/S0022029900031836

National Research Council. 2007. Nutrient Requirements of Horses, 6th rev.edition, , Washington DC., USA : National Academy Press, p. 2-48. ISBN 978-0-309-10212-4. https://doi.org/10.17226/11653

Orskov, E. R. 1995. A Traveller's view of Outer Mongolia. *Outlook on Agriculture*, vol. 24, no. 2, p. 127-129. https://doi.org/10.1177/003072709502400211

Pagan, J. D., Hintz, H. F. 1986. Composition of milk from pony mares fed various levels of digestible energy. *The Cornell Veterinarian*, vol. 76, no. 2, p. 139-148. Available at: https://hdl.handle.net/2027/coo.31924053530501?urlappend=%3Bseq=162

Park, Y. W. 2009. *Bioactive Components in Milk and Dairy Products*. USA : Wiley-Blackwell Ltd. 439 p. ISBN-13: 978-0-8138-1982-2.

Pikul, J., Wójtowski, J. 2008. Fat and cholesterol content and fatty acid composition of mares' colostrums and milk during five lactation months. *Livestock Science*, vol. 113, no. 2-3, p. 285-290. https://doi.org/10.1016/j.livsci.2007.06.005

Saastamoinen, M. T., Koskinen, E. 1993. Influence of quality of dietary protein supplement and anabolic steroids on muscular and skeletal growth of foals. *Animal Science*, vol. 56, no. 1, p. 135-144. https://doi.org/10.1017/S0003356100006255

Salamon, R. V., Salamon, S., Csapó-Kiss, Z., Csapó, J. 2009. Composition of mare's colostrum and milk I. Fat content, fatty acid composition and vitamin contents. *Acta Universitatis Sapientiae, Alimentaria*, vol. 2, no. 1, p. 119-131. Available at: http://www.acta.sapientia.ro/acta-alim/C2-1/alim2-10.pdf

van den Berg, M. 2009. Feeding the Lactating Mare. *Horses and People Magazine*, p. 12-14. Available at: https://www.mbequineservices.com/feeding-the-lactating-mare/

Wong, N. P., Jenness, R., Keeney, M., Marth, E. H. 1999. Fundamentals of Dairy Chemistry. 3rd ed., Gaithersburb, USA : Aspen Publishers. ISBN: 0-8342-1360-5. Available at: https://www.academia.edu/19097830/Fundamentals_of_Dairy_Chemistry_3rd_ed_-_Noble_P._Wong_Aspen_Publishers_Inc._1999_

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