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Original scientific paper

Influence of feed for horse nutrition on the chemical parameters and fatty acid composition of mare's milk

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ABSTRACT

The aim of this research was to determine the influence of horse feed on selected nutritionally important components of mare's milk with a focus on fat content: total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), stearic fatty acid, n-3 PUFA, n-6 PUFA, linoleic (LA) and α-linolenic (ALA) fatty acids. Also, the chemical parameters of the fatty acid composition of feed for horses (briquettes and meadow hay) were examined. Research results showed that complete feed contains a higher crude protein and fat content than hay. In addition, there was a difference in the composition of fatty acids in the milk of mares fed meadow hay compared to briquettes. Accordingly, the diet of mares has an influence on the chemical and fatty acid composition of milk, but it is not the only factor that has an influence.

1. Introduction

Mare's milk is highly appreciated, due to its unique nutritional profile. The chemical composition of mare's milk is similar to human milk, allowing its use in infant feeding as a substitute for human breast milk. Researchers from other countries have conducted a significant number of studies in the last few years, in order to examine the nutritional composition, the presence of bioactive components and certain therapeutic and preventive properties, showing a strong similarity between human breast milk and mare's milk. Mare's milk is attracting increasing interest from consumers due to its high content of vitamins and minerals, better digestibility and lower content of fat in comparison with cow's milk (*Sheng and Fang*, 2009).

Mare's milk is characterized by a high lactose content, while the fat and protein content is low (*Jastrzębska et al.,* 2017). The composition of fatty acids in milk fat is very diverse, due to the presence of over 400 fatty acids (*Trbovic et al*., 2018). The main nutritional components that are important for human nutrition and have an impact on human health are the fatty acids present in mare's milk. Although the fat content of mare's milk is low, the content of polyunsaturated fatty acids (PUFA) is high (*Shaikh et al.*, 2022). The composition of mare's milk is influenced by genetic, physiological and nutritional factors as well as environmental conditions. The aim of this the research was to determine the influence of horse feed on selected nutritionally important components of mare's milk with a focus on fat content: total fat, saturated fatty acids (SFA), monounsaturat-

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ed fatty acids (MUFA), polyunsaturated fatty acids (PUFA), stearic fatty acid, n-3 PUFA, n-6 PUFA, linoleic (LA) and α-linolenic (ALA) fatty acids.

2. Materials and methods

The feed for the horses included two rations, briquettes (oats, corn and bran) and a ration of meadow hay. After mares consumed the feed, milk samples were taken to examined the chemical parameters and composition of fatty acids in the milk. Samples of the rations were also tested (granules and meadow hay) for the same parameters. In this study, samples of mare's milk collected during a period of 6 months of lactation were examined. Milk was from mares of the Friesian horse breed.

ISO standard methods were used to determine chemical parameters (moisture, fat, crude protein, ash, crude fibre, calcium and phosphorus) in horse feed (briquettes and meadow hay). Moisture was determined by drying the sample to a constant mass (ISO 6496:1999), fat content was determined after acid hydrolysis in a Sohlet apparatus (ISO 6492:1999), crude protein, were determined using the Kjeldahl method on the apparatus (Kjeldahl 8400, Foss, Denmark), ash was determined by burning the sample in a muffle furnace (ISO 5984:2022), crude fibre were determined by the intermediate filtration method (ISO 6865:2000), calcium by volumetric and phosphorus by spectrophotometric method (ISO 6490-1:1985, ISO 6491:1998, respectively).

The following chemical parameters were tested in mare's milk samples: lactose content by high-performance liquid chromatography, protein content according to Kjeldahl and fat content by the Gerber method (ISO 22662:2013; ISO 8968-1:2014; ISO 19662:2018, respectively).

2.1. Fatty acid analysis by capillary gas chromatography

In this analysis, fatty acid derivatives, fatty acid methyl esters (FAMEs) (Christie et al. 2001) were detected by gas-liquid chromatography (GLC, Shimadzu 2010, Japan) combined with a flame ionization detector and a capillary HP-88 column (length 100m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperatures were maintained at 250°C and 280°C, respectively. Nitrogen was used as the carrier gas at flow rate of 1.87 mL min^{-1}. The injector split ratio was set to 1:50 and injection volume was 1 μL. In order to achieve complete separation of the examined compounds, a programmed column oven temperature starting at 50°C and ending at 230°C was applied. The total analysis time was 63.12 min. The chromatographic peaks in the samples were identified by comparing FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, PA) and to which a mixture of 5 mg mL^{-1} conjugated linoleic acid (CLA) was added (O5632, Sigma Aldrich).

Nitrogen-free extractives as a measure of the soluble carbohydrates in the feed, such as percentage of starch and sugar, were calculated according to the equation:

$$NFE = 100 - (\text{moisture} + \text{protein} + \text{total fat} + \text{ash} + \text{fibre})$$

3. Results and discussion

Complete feed (briquettes) had a higher crude protein content than meadow hay, while the moisture content was similar, as shown in Table 1. Briquettes contained more fat, less cellulose and more nitrogen-free extractives (carbohydrates) compared to meadow hay. Also, the composition of fatty acids of hay and complete food differed. Table 1 shows that meadow hay contained more MUFA, while briquettes contained more SFA and PUFA. Of PUFA, linoleic acid (C18:2n-6) was more abundant in briquettes than in meadow hay, while linolenic acid (C18:3n-3) content was higher in meadow hay, although it was present in a significant amount in briquettes.

The nutritional content of mare's milk is shown in Tables 2 and 3, where it can be seen that the protein and fat content was slightly higher in the milk of mares fed with meadow hay compared to concentrated food (briquettes), while the lactose content was lower. Also, some authors (*Doreau et al.,* 1992) reported that mare's milk contains more protein and fat and less lactose when mares had more hay in their diet compared to concentrated feed. Additionally, observed by (*Barłowska et al.*, 2023), significantly increased content of dry matter, fat, lactose and ash occur in the milk of mares that do not have access to pasture, while the protein content of milk is increased in mares that have access to pasture.

The content of palmitic acid (C16:0) and palmitoleic acid (C16:1) was higher in mare's milk than in complete feed and meadow hay, shown in Tables 4 and 5. The reason for this is the fact that these fatty acids are synthesis products in mare's milk (*Djordjevic et al.*, 2019). Linoleic acid (C18:2n-6) was twice

Table 1. Chemical composition and composition of fatty acids in meadow hay and briquettes

Parameters	Meadow hay, g/100 g	Briquettes, g/100 g
Crude proteins, g	4.52	11.94
Moisture, g	10.30	11.02
Crude total fat, g	0.85	4.40
Crude ash, g	7.99	3.57
Crude fibre, %	30.57	10.24
Ca, g	0.68	0.25
P, g	0.21	0.50
NFE, g	53.76	62.40
Fatty acid composition		
C16:0	23.94	15.02
C18:0	3.52	1.49
C18:1n-9	10.05	30.70
C18:2n-6	23.59	49.09
C18:3n-3	5.63	2.12
SFA	5.90	17.40
MUFA	36.55	30.87
PUFA	35.82	51.92

NFE – nitrogen-free extractives; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

as abundant in complete feed and meadow hay than in mare's milk, which indicates that linoleic acid is incorporated unchanged into mare's milk. The content of linoleic acid (C18:2n-6) was higher in the milk of mares fed with bulky and concentrated feed, while the content of α-linolenic acid (C18:n3-3) was lower, established by *Naert et al.,* (2013), which coincides with our research.

In our study, the content of SFA in mare's milk was increased, and the content of PUFA was decreased in relation to the diet (meadow hay and briquettes). The content of MUFA was lower in mare's milk compared to meadow hay, and higher compared to in complete feed. Also, some authors (*Barłowska et al.*, 2023) reported higher levels of SFA in milk from mares with access to pasture, and lower levels of MUFA and PUFA. These differences in results can be attributed to the influence of other factors such as lactation of horse breeds and others.

The content of linolenic acid was higher in the milk of mares that had meadow hay in their diet, shown in Table 4. Also, greater amounts of α-linolenic (C18:3 n-3) acid were in the milk of mares that had access to pasture (*Barłowska et al.*, 2023). Linoleic acid (C18.2n-6) was higher in the milk of mares fed complete feed (briquettes), shown in Table 5, as well as in milk from mares without access to pasture (*Barłowska et al.,* 2023).

Table 2. Chemical composition of mare's milk (fed with meadow hay)

Nutrient	100 g
protein, g	2.67
fat, g	1.10
lactose, g	5.78
energy value, kcal	39.79
energy value, kJ	169.14

Table 3. Chemical composition of mare's milk (fed with Briquettes)

Nutrient	100 g
protein, g	2.15
fat, g	0.63
lactose, g	6.90
energy value, kcal	36.56
energy value, kJ	155.33

Table 4. Fatty acid composition of mare's milk (fed with meadow hay)

Fatty acids	Mare's milk
C4:0	No data
C6:0	No data
C8:0	1.02
C10:0	3.42
C12:0	4.22
C14:0	6.27
C15:0	0.49
C16:0	25.23
C16:1	4.89
C17:0	0.47
C18:0	3.55
C18:1TRANS-11	NO DATA
C18:1CIS-9	19.81
C18:2N-6	8.10
C20:0+C18:3N-6	NO DATA
C18:3N-3	19.50
C9T11CLA	NO DATA
C20:2N-6	0.15
C20:4N-6	NO DATA
C20:5N-3	NO DATA
C22:6N-3	NO DATA
SFA	45.69
MUFA	26.06
PUFA	28.57

Table 5. Fatty acid composition of mare's milk (fed with briquettes)

Fatty acids	Mare's milk
C4:0	0.08
C6:0	0.22
C8:0	2.07
C10:0	4.09
C12:0	3.28
C14:0	4.49
C15:0	0.22
C16:0	22.50
C16:1	7.37
C17:0	0.16
C18:0	1.23
C18:1TRANS-11	0.20
C18:1CIS-9	34.36
C18:2N-6	13.36
C20:0+C18:3N-6	0.06
C18:3N-3	3.12
C9T11CLA	0.50
C20:2N-6	0.35
C20:4N-6	0.10
C20:5N-3	0.06
C22:6N-3	0.07
SFA	38.34
MUFA	47.23
PUFA	14.44

Values represent mean ± SEM, n – number of samples; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

4. Conclusion

Our results showed that mare's milk is rich in linoleic and linolenic acids, which are necessary for the growth and development of the nervous system in humans. This indicates the importance of mare's milk in human diets. Variations in the composition of milk should be minimal in order to talk about the impact of mare's milk on human health. In our study, it can be seen that horse feed has an effect on the chemical composition and fatty acid composition of mare's milk. In addition to nutrition, many other factors affect the composition of milk, such as genetics, lactation, breed and environmental conditions. Accordingly, it is necessary to examine the influence of other factors on the composition of mare's milk.

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