Journal of Dairy Research (2008) **75** 302–309. © *Proprietors of Journal of Dairy Research 2008*
doi:10.1017/S0022029908003336 *Printed in the United Kingdom*

Fat content and fatty acids profile of colostrum and milk of primitive Konik horses (*Equus caballus gmelini* Ant.) during six months of lactation

Jan Pikul[1], Jacek Wójtowski[2]*, Romualda Danków[1], Beata Kuczyńska[3] and Jacek Łojek[4]

[1] Department of Dairy Technology, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
[2] Department of Sheep, Goat and Fur Animals Breeding, Poznan University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland
[3] Department of Cattle Breeding, [4] Department of Horses Breeding, Warsaw Agricultural University, Ciszewskiego 8, 02-766 Warsaw, Poland

Received 21 August 2007; accepted for publication 11 March 2008

The effect of the stage of lactation, the number of foals and age of the mare on changes in the fat content and fatty acid composition of colostrum and milk of primitive Konik horses was investigated. Colostrum and milk samples from 12 lactating mares were collected at the beginning of lactation, on the days 1 and 2 after foaling and then, starting from the first month of lactation, at 4-week intervals up to the sixth month of lactation. Significant differences were observed in fat content as well as the composition of some analysed fatty acids between colostrum and milk of mares of the Konik breed. The number of foalings and the age of mares did not have a statistically significant effect on the fat content in milk and had only a slight effect on the fatty acid composition. Milk produced by mares of the Konik breed is characterized by a considerable content of polyene fatty acids with 18 carbon atoms, a low ratio of n-6 fatty acids to n-3 fatty acids as well as low, highly advantageous values of atherogenic and thrombogenic indices.

Keywords: Mare, colostrum, milk, lactation stage, fat content, fatty acids profile.

Mares' colostrum and milk constitute food products of unique nutritional properties used more and more frequently as natural pharmacological agents to assist the treatment of various diseases of thegastrointestinal tract, including chronic inflammatory conditions of the intestinal wall epithelium in Crohn's disease, of the immune system, neurodermatosis and psoriasis (Kahle, 2001; Ellinger et al. 2002; Orlandi et al. 2003; Fökel et al. 2007). To a considerable extent they owe their dietary and salubrious properties, to their fat composition which depends, among other factors, on the level of nutrition, the lactation period as well as the breed of horse (Doreau & Martuzzi, 2006; Pikul & Wójtowski, 2008). Numerous publications deal with fatty acid profiles of different, highly specialized horse breeds e.g., Breton, Boulonnais, Haflinger, Hungarian Draught, Wielkopolska and Thoroughbred (Csapó et. al. 1995; Kny, 1998; Orlandi et al. 2002; Pikul & Wójtowski, 2008). However, until now there have been no publications discussing the fatty acid composition of milk of primitive horse breeds closest to their wild ancestors.

The primitive Konik horse (*Equus caballus gmelini* Ant.) is the only primitive horse breed originating directly from wild horses called Tarpans. From their wild ancestors, Konik horses inherited exceptional endurance, resistance to diseases and adaptability to hard conditions of life (Vetulani, 1925, Frąckowiak & Komosa, 2006). The domestic population of Konik horses (conservation breeding) is estimated at 450 mares and 110 stallions, of which 8% are kept under free-roaming conditions.

The aim of this study was to characterize the fat content and composition of colostrum and milk of primitive Konik mares during six months of lactation, depending on different numbers of foals and mares' age and to discuss several parameters that could be of interest in realtion to human nutrition.

*For correspondence; e-mail: jacwoj@neostrada.pl

Material and Methods

Animals, feeding and milk sample collection

The experiment was carried out on milk obtained from 12 mares of the Konik breed kept under stable conditions at the Experimental Station of the Polish Academy of Sciences in Popielno (Białowieska Primeval Forest, East-North Poland). Mares foaled during the period from March to May. The average bodyweight was 364 kg (±6·2 kg, SE) and the body condition score ranged from 3·5 to 4·0 (measured on a 0–5 scale). All the experimental animals were reared under identical environmental conditions. Until May, mares were kept in stables with an outdoor yard, whereas from the beginning of May until the end of October, they were kept on pasture (paddocks). After foaling, until the beginning of May, mares were fed about 5–6 kg hay and about 1·5 kg oats. From the middle of May until the end of October mares remained on pasture, feeding on green forage only. Foals were also kept on the paddock near their mothers. Mares were hand-milked, without oxytocin injection. Colostrum was collected twice: the first time during the period from 18 to 24 h after foaling and the second time, from 42 to 48 h post partum. Milk for analyses was collected in the amount of 80–120 ml a day. Milk samples were collected from the first to the sixth month of lactation, always twice i.e., on the 5th and 6th day of each month from each mare individually, at 4-week intervals. Mares were always milked at the same time of the day i.e., between 8.00 and 9.00, 1 h after separating foals from their mothers. During milking, foals remained in visual and tactile contact with their mothers.

Experimental procedures

Fat content as well as fatty acid composition were determined in the collected samples of colostrum (n=24) and milk (n=144). Fat from colostrum and milk was extracted by the standard procedure of Folch et al. (1957) using a mixture of chloroform and methanol in a ratio of 2:1. Fatty acid methylation was performed according to the transesterification method by Kramer et al. (1997). The identification with fatty acid standards and quantitative determination of individual fatty acids in crude fat was conducted in a Hewlett Packard 5890 GC with HP Chem software, and a flame-ionization detector and DB-23 column, which was covered with a polar stationary phase of 50% cyanopropyl-methylpolysiloxane, 60 m in length, 0·25 mm in diameter and 0·25 µm thick. The separation was performed at pre-programmed temperature: 130 °C for 1 min; 130–170 °C at 6·5 deg C/min; 170–215 °C at 2·75 deg C/min; 215 °C for 12 min; 215–230 °C at 20 deg C/min and 230 °C for 3 min. Other parameters were: carrier gas (He) pressure, 125 kPa (18 psi); split sample injector (50:1); injector temperature, 220 °C; and detector temperature, 240 °C. Flow rate of the carrier gas was to 25 cm/s. Atherogenic (AI) and thrombogenic indices (TI) were calculated using equations described by Ulbricht & Southgate (1991):

$$AI = [C\ 12{:}0 + (4{*}C\ 14:0) + C\ 16:0]/[MUFA + (n\text{-}6) + (n\text{-}3)]$$

$$TI = (C\ 14{:}0 + C\ 16:0 + C\ 18:0)/0{\cdot}5{*}MUFA) + (0{\cdot}5{*}n\text{-}6) + (3{*}n\text{-}3) + (n\text{-}3/n\text{-}6).$$

Where MUFA = monounsaturated fatty acids.

In the equations, the 14:0 fatty acid is considered to be 4-times more atherogenic than the other fatty acids; thus a coefficient of 4 has been assigned to it. Polyunsaturated (PUFA) n-6 and MUFA have been assigned coefficients of 0·5 since they are less antiatherogenic than PUFA n-3, which has been assigned a coefficient of 3.

Statistical analysis

Results were processed by the GLM procedure of SAS (SAS®, version 6.12, 1996). The following effects were included in the analyses of variance:the effect of the type of milk: colostrum and milk (1, 2); stage of lactation: day (1, 2) or month (1 to 6); number of foals: (1, 2); age of mare: (1, 2). Differences between animals were not significant. Differences between means were determined with the Duncan test and significance was declared at $P<0{\cdot}05$.

Results

Fat content of colostrum and milk

The content of fat in mares' colostrum was statistically significantly higher than in milk (Table 1). Mares' milk collected in the 5th and 6th months of lactation contained significantly less fat than milk produced in the 1st month of lactation. Statistically significantly more fat was found in colostrum collected from mares that had foaled more than 5 times in comparison with mares that had foaled fewer times (Table 2). The number of foalings did not have a statistically significant influence on fat content in mares' milk. The age of the examined mares i.e., mares older than or younger than 10 years, failed to have a statistically significant effect on fat content either in the colostrum or milk (Table 3).

Fatty acid profile of colostrum

The analysis of fatty acid composition of colostrum obtained from experimental mares shows that saturated fatty acids constituted nearly 50% of all determined fatty acids (Table 1), of which acid 16:0 was found in the highest quantities (about 26%). The proportion of middle-chain saturated fatty acids constituted about 14% and the dominant acids in this group were 14:0, 12:0 and 10:0. No short-chain saturated fatty acids were detected in the colostrum. Most of the unsaturated fatty acids in colostrum were monoenoic acids, primarily the 18:1 *cis*-9. PUFA

https://doi.org/10.1017/S0022029908003336 Published online by Cambridge University Press

Table 1. The fat content (g/100g) and fatty acid profile (% of fatty acids) of mares' colostrum during two lactation days and milk during six lactation months

	Lactation stage												Effect of:	
	Colostrum (d)				Milk (months)									
Fatty acids†	1	2	1–2	SED‡	1	2	3	4	5	6	1–6	SED‡	Colostrum: milk	Lactation stage
Total fat	2·47	2·07	2·27	0·13	1·93ABa	1·57	1·43a	1·48	1·34A	1·31B	1·51	0·07	***	**
8 : 0	2·40	2·16	2·28	0·20	3·24	3·76abc	3·25	2·62a	2·59b	2·62c	3·01	0·12	*	*
10 : 0	5·47	5·65	5·56	0·65	7·14Aa	7·38BCb	6·98Dcd	4·95Bc	5·22abc	3·84ACD	5·92	0·27	NS	***
10 : 1	0·55	0·87	0·71	0·16	1·11	1·45	1·36	1·26	1·63	1·09	1·32	0·09	*	NS
12 : 0	6·05	6·52	6·29	0·68	7·64a	7·91b	8·16Acd	6·54c	6·59d	6·09Aab	7·15	0·21	NS	*
14 : 0	6·86	7·45	7·15	0·38	7·52	7·30	7·65	7·17	7·30	7·16	7·35	0·14	NS	NS
14 : 1	0·41	0·48	0·45	0·05	0·50Aa	0·52b	0·53c	0·59d	0·89Abcd	0·79a	0·64	0·04	NS	*
16 : 0	26·20	25·81	26·00	0·82	21·59	20·46	21·33	22·12	21·98	22·57	21·68	0·23	***	NS
16 : 1 cis-7	0·69	0·62	0·65	0·07	0·68	0·51	0·53	0·33	0·39	0·49	0·49	0·04	NS	NS
16 : 1 cis-9	4·64	5·68	5·16	0·36	5·04ab	4·76AB	5·10cd	5·53e	6·95Aace	6·94Bbd	5·72	0·23	NS	**
18 : 0	2·51	2·16	2·34	0·20	1·37	0·86	0·96	0·92	0·83	0·90	0·97	0·07	**	NS
18 : 1 *cis*-9	21·88	20·16	21·02	1·45	16·53	16·43	15·40	16·40	16·72	17·58	16·51	0·46	NS	NS
18 : 1 *cis*-11	1·58	1·40	1·49	0·11	0·89	0·71	0·90	0·81	0·88	0·95	0·86	0·04	**	NS
18 : 2 n-6	8·61	7·97	8·29	0·39	6·86	6·78	6·84	7·69	6·20	7·78	7·02	0·22	NS	NS
18 : 3 n-3	11·75	12·74	12·25	1·27	19·52	20·76	20·71	22·58	21·38	20·59	20·92	0·42	***	NS
CLA	0·00	0·00	0·00	0·00	0·01AB	0·02Ca	0·02Db	0·06Aabc	0·06BCDd	0·03cd	0·03	0·00	*	***
20 : 4 n-6	0·42	0·33	0·37	0·03	0·34A	0·40a	0·28B	0·41b	0·40c	0·60ABabc	0·41	0·03	NS	**
SFA	49·49	49·75	49·62	1·20	48·51ab	47·66c	48·33d	44·33a	44·52	43·18bcd	46·09	0·58	NS	*
MCSFA	13·92	14·33	14·13	1·47	18·02Aab	19·04BCc	18·39Dde	14·11Bad	14·40bce	12·55ACD	16·09	0·55	NS	***
LCSFA	35·57	35·42	35·49	0·84	30·48	28·62	29·94	30·22	30·11	30·63	30·00	0·28	***	NS
UFA	50·51	50·25	50·38	1·20	51·49ab	52·34c	51·67d	55·67a	55·48	56·82bcd	53·91	0·58	NS	*
MUFA	29·74	29·21	29·47	1·63	24·76	24·38	23·82	24·92	27·45	27·83	25·53	0·55	NS	NS
PUFA	20·78	21·04	20·91	1·21	26·73	27·96	27·85	30·75	28·04	28·99	28·39	0·50	NS	NS
FA n-6	9·02	8·29	8·66	0·39	7·22	7·21	7·14	8·17	6·65	8·40	7·46	0·23	NS	NS
FA n-3	11·75	12·74	12·25	1·27	19·52	20·76	20·71	22·58	21·38	20·59	20·92	0·42	***	NS
UFA : SFA	1·04	1·04	1·04	0·05	1·09A	1·11a	1·09B	1·27	1·26	1·33ABa	1·19	0·03	NS	**
MUFA : SFA	0·62	0·61	0·61	0·04	0·53a	0·52b	0·50c	0·57	0·62	0·65abc	0·57	0·02	NS	*
PUFA : SFA	0·43	0·43	0·43	0·03	0·56	0·59	0·59	0·70	0·63	0·68	0·63	0·02	NS	NS
PUFA : UFA	0·42	0·43	0·42	0·03	0·52	0·54	0·54	0·55	0·51	0·51	0·53	0·01	NS	NS
FA n-6 : n-3	1·07	0·85	0·96	0·12	0·43	0·35	0·35	0·36	0·32	0·42	0·37	0·02	***	NS
18 : 2/18 : 3 ratio	1·03	0·82	0·92	0·12	0·41	0·33	0·34	0·34	0·29	0·39	0·35	0·02	***	NS
AI	1·18	1·24	1·21	0·04	1·15	1·10	1·16	1·03	1·04	1·01	1·08	0·02	NS	NS
TI	0·64	0·61	0·62	0·02	0·39	0·35	0·37	0·35	0·36	0·37	0·37	0·01	***	NS

† Fatty acids = SFA (saturated fatty acids) = sum of MCSFA+LCSFA; MCSFA (middle-chain saturated fatty acids), = sum of 8 : 0+10 : 0+12 : 0; LCSFA (long-chain saturated fatty acids), = sum of 14 : 0+16 : 0+18 : 0; UFA (unsaturated fatty acids) = total sum of MUFA+PUFA; MUFA (monounsaturated fatty acids) = sum of 10 : 1+14 : 1+16 : 1 *cis*-7+16 : 1 *cis*-9+18 : 1 *cis*-9+18 : 1 *cis*-11; PUFA (polyunsaturated fatty acids) = sum of 18 : 2+CLA+18 : 3; FA n-6 (fatty acids) = sum of 18 : 2+20 : 4; FA n-3 (fatty acids) = 18 : 3; AI = atherogenic index calculated using equation described by Ulbricht & Southgate (1991); TI = thrombogenic index calculated using equation described by Ulbricht & Southgate (1991)

‡ SED = Standard error of difference across treatment means

Differences between means in rows marked with the same letters are statistically significant at: capital letters $P<0{\cdot}01$, small letters $P<0{\cdot}05$

Other differences are statistically significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$, NS, not significant

Table 2. The influence of number of foalings on fat content (g/100g) and fatty acid profile (% of total fatty acids) of mares' colostrum and milk

	Colostrum			Milk			
Fatty acids†	NF1§	NF2§	SED‡	NF1§	NF2§	SED‡	Effect no. of foalings
Total fat	2·17A	2·41A	0·13	1·49	1·44	0·07	**
8 : 0	1·96a	2·72a	0·20	2·85	3·24	0·12	*
10 : 0	4·64	6·85	0·65	5·42a	6·62a	0·27	**
10 : 1	0·60	0·86	0·16	1·31	1·32	0·09	NS
12 : 0	5·33	7·63	0·68	6·88a	7·53a	0·21	*
14 : 0	6·71	7·78	0·38	7·18a	7·58a	0·14	*
14 : 1	0·41	0·50	0·05	0·67	0·60	0·04	NS
16 : 0	26·85	24·82	0·82	21·91	21·35	0·23	NS
16 : 1 *cis*-7	0·71	0·57	0·07	0·56a	0·38a	0·04	*
16 : 1 *cis*-9	5·47	4·73	0·36	5·68	5·78	0·23	NS
18 : 0	2·52	2·08	0·20	0·93	1·03	0·07	NS
18 : 1 *cis*-9	23·70a	17·27a	1·45	16·87	16·00	0·46	*
18 : 1 *cis*-11	1·59	1·35	0·11	0·88	0·83	0·04	NS
18 : 2 n-6	8·56	7·91	0·39	6·94	7·14	0·22	NS
18 : 3 n-3	10·56	14·60	1·27	21·44	20·20	0·42	NS
CLA	tr	tr	0·00	0·04	0·03	0·00	NS
20 : 4 n-6	0·40	0·33	0·03	0·44A	0·35A	0·03	***
SFA	48·01a	51·87a	1·20	45·18a	47·36a	0·58	***
MCSFA	11·93	17·20	1·47	15·15	17·40	0·55	**
LCSFA	36·08	34·68	0·84	30·03	29·96	0·28	NS
UFA	51·99a	48·13a	1·20	54·82a	52·64a	0·58	***
MUFA	32·47a	25·28a	1·63	25·97	24·91	0·55	NS
PUFA	19·52a	22·84a	1·21	28·85	27·73	0·50	NS
FA n-6	8·96	8·24	0·39	7·42	7·53	0·23	NS
FA n-3	10·56	14·60	1·27	21·44	20·20	0·42	NS
UFA : SFA	1·10a	0·95a	0·05	1·23a	1·14a	0·03	**
MUFA : SFA	0·69a	0·50a	0·04	0·58	0·54	0·02	*
PUFA : SFA	0·41	0·45	0·03	0·64	0·60	0·02	*
PUFA : UFA	0·38	0·48	0·03	0·53	0·53	0·01	NS
FA n-6 : n-3	1·11	0·76	0·12	0·37	0·38	0·02	NS
18 : 2/18 : 3 ratio	1·06	0·73	0·12	0·34	0·36	0·02	NS
AI	1·14a	1·32a	0·04	1·05a	1·12a	0·02	***
TI	0·67	0·56	0·02	0·36	0·38	0·01	NS

† see Table 1 for abbreviation key
‡ SED as for Table 1
§ NF1, NF2 = number of foalings (NF1 $\leqslant 5$, $n=7$; NF2 >5, $n=5$)

constituted a considerable (about 21%) proportion of fatty acids in colostrum, of which 18 : 3 was the dominant acid. The ratio of the n-6 family fatty acids to those of the n-3 family in colostrum was about 0·9 and this can be attributed mainly to the proportion of 18 : 2 and 18 : 3 acids. Out of the 16 detected fatty acids, statistically significant differences were observed between colostrum and milk for 6 acids, namely 8 : 0, 16 : 0, 18 : 0, 18 : 1 *cis*-11, 18 : 3 and conjugated linoleic acids (CLA). Calculated AI and TI values in mares' colostrum were 1·21 and 0·62, respectively.

Fatty acid profile of milk during six lactation months

A significant effect of lactation period on fat composition was found for the ratio of the following seven fatty acids: 8 : 0, 10 : 0, 12 : 0, 14 : 1, 16 : 1 *cis*-9, CLA and 20 : 4 (Table 1). Saturated fatty acids constituted approx. 45% of all fatty acids found in mares' milk, of which approximately 30% were long-chain acids, with 16 : 0 predominating (about 22%) followed by 14 : 0 (over 7%). No short-chain saturated fatty acids were detected in mares' milk. Unsaturated fatty acids made up about 55% of all fatty acids found in mares' milk, of which MUFA constituted about 25%, with 18 : 1 *cis*-9 constituting over 16%. PUFA constituted over 8% of all fatty acids in mares' milk. This group of acids was dominated by 18 : 3 (approx. 21%) and 18 : 2 (nearly 7%). Fatty acids from the n-3 family made up nearly 21% of all fatty acids. The ratio of n-6 to n-3 acids, on average, amounted to 0·37. Mean AI and TI values in mares' milk were 1·08 and 0·37, respectively. With the lengthening of the lactation period, the proportion of saturated acids (mainly medium-chain length) decreased statistically significantly, while that of 14 : 1 and 16 : 1 *cis*-9 acids

https://doi.org/10.1017/S0022029908003336 Published online by Cambridge University Press

Table 3. The influence of mares' age on fat content (g/100g) and fatty acid profile (% of total fatty acids) of mares' colostrum and milk

	Colostrum			Milk			
Fatty acids†	A1‡	A2‡	SED§	A1‡	A2‡	SED§	Effect of mares' age
Total fat	2·31	2·24	0·13	1·44	1·56	0·07	NS
8 : 0	2·03	2·46	0·20	2·95	3·06	0·12	NS
10 : 0	4·49	6·33	0·65	5·47	6·24	0·27	NS
10 : 1	0·61	0·78	0·16	1·32	1·31	0·09	NS
12 : 0	5·45	6·89	0·68	7·11	7·19	0·21	NS
14 : 0	7·17	7·15	0·38	7·48	7·26	0·14	NS
14 : 1	0·47	0·43	0·05	0·72a	0·57a	0·04	*
16 : 0	28·04a	24·55a	0·82	22·21a	21·29a	0·23	**
16 : 1 *cis*-7	0·64	0·66	0·07	0·52	0·46	0·04	NS
16 : 1 *cis*-9	5·71	4·77	0·36	6·22a	5·36a	0·23	*
18 : 0	2·64	2·12	0·20	0·92	1·01	0·07	NS
18 : 1 *cis*-9	23·42	19·30	1·45	16·26	16·69	0·46	NS
18 : 1 *cis*-11	1·57	1·44	0·11	0·83	0·88	0·04	NS
18 : 2 n-6	8·31	8·27	0·39	6·77	7·21	0·22	NS
18 : 3 n-3	9·14	14·47	1·27	20·85	20·97	0·42	NS
CLA	tr	tr	0·00	0·03	0·04	0·00	NS
20 : 4 n-6	0·32	0·41	0·03	0·35A	0·44A	0·03	**
SFA	49·81	49·48	1·20	46·13	46·06	0·58	NS
MCSFA	11·97	15·67	1·47	15·52	16·49	0·55	NS
LCSFA	37·84a	33·81a	0·84	30·61	29·57	0·28	**
UFA	50·19	50·52	1·20	53·87	53·94	0·58	NS
MUFA	32·42	27·37	1·63	25·87	25·28	0·55	NS
PUFA	17·77a	23·15a	1·21	28·00	28·66	0·50	*
FA n-6	8·63	8·68	0·39	7·15	7·69	0·23	NS
FA n-3	9·14	14·47	1·27	20·85	20·97	0·42	NS
UFA : SFA	1·02	1·05	0·05	1·19	1·19	0·03	NS
MUFA : SFA	0·67	0·57	0·04	0·57	0·56	0·02	NS
PUFA : SFA	0·36	0·48	0·03	0·62	0·63	0·02	NS
PUFA : UFA	0·36	0·46	0·03	0·52	0·53	0·01	NS
FA n-6 : n-3	1·23a	0·77a	0·12	0·37	0·37	0·02	*
18 : 2/18 : 3 ratio	1·19a	0·74a	0·12	0·35	0·35	0·02	*
AI	1·24	1·19	0·04	1·10	1·07	0·02	NS
TI	0·77	0·54	0·02	0·37	0·36	0·01	NS

† For abbreviation key, see Table 1
‡ A1, †† A2 = Mares age (††A1 ⩽ 10 years, n = 5; †† A2 > 10 years, n = 7)
§ SED as for Table 1

increased. This resulted in an increase in the ratio of unsaturated fatty acids (including MUFA) to saturated fatty acids.

Effect of the number of foalings on fatty acid profile in colostrum and milk

Colostrum from mares that had foaled fewer than 5 times, contained statistically significantly less 8 : 0 and more 18 : 1 *cis*-9 than colostrum from mares with a higher number of foalings (Table 2). In colostrum from mares with fewer than 5 foalings, the ratio of saturated acids to PUFA was smaller than in colostrum from mares with a higher number of foalings. In addition, calculated AI was also lower. The number of foalings had a statistically significant effect on proportions of 10 : 0, 12 : 0, 14 : 0, 16 : 1 *cis*-7 and 20 : 4. Milk from mares that had given birth to fewer than 5 foals contained statistically significantly less middle-chain saturated fatty acids, such as 10 : 0, 12 : 0 and 14 : 0 and more unsaturated fatty acids than milk produced by mares with more foalings. As for colostrum, also in milk the calculated AI value was lower.

Effect of mares' age on fatty acid profile in colostrum and milk

Age of the examined mares of up to 10 and over 10 years had a statistically significant influence on the proportion of the 16 : 0 in colostrum and 14 : 1, 16 : 0, 16 : 1 *c*9 and 20 : 4 acids in milk (Table 3). In colostrum collected from mares up to 10 years of age, the proportion of 16 : 0 was higher and that of PUFA lower than in colostrum collected

https://doi.org/10.1017/S0022029908003336 Published online by Cambridge University Press

from older mares. Colostrum of younger mares was characterized by a higher ratio of n-6 to n-3 acids, primarily 18 : 2 to 18 : 3, compared with colostrum produced by older animals. Milk obtained from mares younger than 10 years contained higher proportions of 14 : 1, 16 : 0 and 16 : 1c9 and less 20 : 4 than milk from the second age group.

Discussion

There is limited published information on fat content in mares' colostrum. As with other animal species, colostrum contains more fat than milk does. The present results are similar to those in literature, although the absolute fat content in colostrum collected from mares of the Konik breed differs from that of other breeds (Smoczyński & Tomczyński, 1982; Csapó et al. 1995; Salimei et al. 2002; Pikul & Wójtowski, 2008).

Results of fat content analysis during the entire lactation period are sometimes difficult to interpret owing to changes in feeding during the course of lactation, which interfere with the effect of lactation stage. However, it may be assumed that fat content decreases until the 3rd month of lactation, then it remains stable or decreases slightly until the 6th month (Brzeski & Kulisa, 1979; Hoffman et al. 1998; Mariani et al. 2001; Pikul & Wójtowski, 2008). Milk fat content is highly variable during this period, but the general trend based on pooling of literature data is an inverse function of lactation stage (Doreau & Martuzzi, 2006).

In our investigations, the observed changes in milk fat content of the Konik breed mares during the entire lactation period were similar to those in the literature, although the total fat content in milk of mares of this breed was considerably higher than that reported by Tomczyński et al. (1999). This may be attributed to different methods of fat acquisition from the milk of mares, as well as changes in feeding regimes for mares, introduced in recent years. Similar differences in fat content were reported in milk of Wielkopolska breed mares (Tomczyński et al. 1999; Pikul & Wójtowski, 2008).

Genetic effects have seldom been studied. Csapó et al. (1995) found no difference in fat composition between Breton, Boulonnais, Haflinger and Hungarian Draught mares. However, differences were reported by Pelizzola et al. (2006) who compared milk obtained from Haflinger, Quarter horse, Sella and Salto, and Rapied Heavy Draft breed mares. The Quarter horse breed, as compared with the other investigated breeds, exhibited distinctive characteristics such as a high main constituent content and a high percentage of essential linoleic and linolenic fatty acids. Differences in the basic composition of fatty acids in milk collected from mares of Arab, Małopolska and Huculska breeds were also reported by Kulisa (1977). In both of the latter studies, the authors failed to show any influence of lactation period on the composition of milk fatty acids, similarly to Hoffman et al. (1998), who investigated the composition of fatty acids in milk of Thoroughbred mares. Differences in the ratio of 18 : 2 to 18 : 3 acids as well as linoleic acid (LA)/alpha-linolenic (ALA) to saturated/unsaturated fatty acids in milk of Thoroughbred and Haflinger mares were reported by Orlandi et al. (2002). Those researchers found a significant increase in the proportion of 18 : 3 with the lengthening of the lactation period in the case of Thoroughbred mares. In their subsequent investigations conducted on milk of Haflinger mares, Orlandi et al. (2003) showed that fatty acids found in highest amounts during lactation were 16 : 0, 18 : 1 and 18 : 3. The content of 18 : 2 was significantly lower on the 105th day of lactation in comparison with milk obtained on the 30th day of lactation. A statistically significant effect of lactation period on changes in the proportions of 18 : 2 and 18 : 3 in milk from mares of the Wielkopolska breed were reported by Pikul & Wójtowski (2008). As lactation progressed, the proportion of 18 : 2 decreased, while that of 18 : 3 increased. In our experiments on milk of Konik mares no statistically significant effect of lactation period on changes in the contents of 18 : 2 and 18 : 3 was found. At the same time, it should be emphasized that the ratio of 18 : 2/18 : 3 in the fat of milk from Konik mares was over 6-times lower than in the milk of Wielkopolska breed mares (Pikul & Wójtowski 2008). This is the result of a considerably higher content of 18 : 3 and a lower content of 18 : 2 in milk of Konik mares in comparison with the milk of Wielkopolska breed mares. The most likely cause of this was the different feeding regime adopted for those mares. The above results are similar to those obtained by Csapó et al. (1995) and Hoffman et al. (1998) but differ from data presented by Orlandi et al. (2002, 2003).

The present results indicate that saturated fatty acids in milk of Konik mares constitute almost 50% of fatty acids, with such long-chain acids as 16 : 0 and 14 : 0 and middle-chain 10 : 0 and 12 : 0 acids predominating. Mares' milk, similarly to human milk, is characterized by a lower proportion of saturated fatty acids than cows' milk. In the total amount of fatty acids, unsaturated fatty acids constituted approximately 54%, of which 25% was MUFA, among which 18 : 1 *cis*-9 constituted 16%. The percentage of MUFA in mare's milk was lower than in human milk and similar to that in cows' milk. PUFA in mares' milk made up over 28% of total fatty acids. The 18 : 3 (about 21%) and 18 : 2 (up to 7%) fatty acids were dominant in this group. LA and ALA fatty acids are required in human nutrition as they cannot be produced by the human body and may be extremely important for formula-fed infants. One of the main arguments used when recommending mares' milk is its high content of polyunsaturated fatty acids of the n-3 family. However, owing to its high price, the consumption of significant amounts of ALA from mares' milk is limited (Doreau & Martuzzi, 2006).

Results for the ratio of n-6 to n-3 fatty acids in milk of Konik mares are quite interesting. The ratio for colostrum

https://doi.org/10.1017/S0022029908003336 Published online by Cambridge University Press

was determined as 1, while that for milk was 0·4 and the value of this ratio was influenced by neither lactation period nor the number of foalings. However, it changed with the season of milk production by mares. It was significantly higher in milk from mares in the period from March to May than in June to November. These changes can also be related to dietary variations. The ratio of fatty acids belonging to these two families is influenced by changes in the contents of 18 : 2 (n-6) and 18 : 3 (n-3) fatty acids. This ratio in our investigations was considerably lower than that reported by Orlandi et al. (2002, 2003) and this can be attributed to a much lower percentage share of the 18 : 3 n-3 acid.

Fatty acids can promote or prevent atherosclerosis and coronary thrombosis based on their effects on the serum cholesterol and low density lipoprotein-cholesterol concentrations (Ulbricht & Southgate, 1991). The equations proposed by Ulbricht & Southgate (1991) for the atherogenic (AI) and thrombogenic indices (TI) indicated that 12 : 0, 14 : 0 and 16 : 0 are atherogenic and that 14 : 0, 16 : 0 and 18 : 0 are thrombogenic. The n-3, n-6 and MUFA are anti-atherogenic and antithrombogenic. High and equilibrate essential fatty acid content together with low AI (1·08) and TI (0·37) observed in our study, indicate the immune modulatory properties of mares' milk. AI values recorded in our study are higher and those of TI lower than in milk of donkeys, for which their respective values are 0·89 and 0·73 (Chiofalo et al. 2005) and also distinctly lower than in cows' milk (cows were fed a basal diet containing 44% forage and 56% concentrate mix), for which these values amount to 2·51 and 1·86, respectively (Allred et al. 2006). Values of these indices do not change significantly during the course of lactation.

Conclusions

Significant differences were demonstrated between colostrum and milk of mares of the Konik breed in terms of fat content as well as the composition of selected fatty acids. A significant effect was demonstrated for lactation stage and number of foalings on fat content as well as fatty acid composition. Milk from Konik mares is characterized by a high content of PUFA, primarily 18 : 3 (n-3) and 18 : 2 (n-6), low ratios of n-6 to n-3 fatty acids as well as low values of AI and TI. Mares of the Konik breed can be used to produce milk containing fatty acids having nutritional and extra-nutritional properties. Moreover, milk of mares of the Konik breed consists of 10·6% total solids, including 1·9% total protein (1·0% casein and 0·7 whey proteins), 1·5% fat, 6·8% lactose and 0·4 ash. Their size, low environmental requirements and character traits, facilitating their utilization in horse-riding lessons for children and teenagers, as well as hippotherapy for children with developmental deficits, together with their potential milk-producing purpose, will probably promote enhanced interest of breeders in increasing the population of Konik horses in Poland.

This research was supported by the Polish Ministry of Education and Science (Grant No. 2 PO6Z 070 27).

References

Allred SL, Dhiman TR, Brennand CP, Khanal RC, McMahon DJ & Luchini ND 2006 Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. *Journal of Dairy Science* **89** 234–248

Brzeski E & Kulisa M 1979 The N-acetylneuraminic acid and fat level in the milk of Arabian mares. *Roczniki Naukowe Zootechniki* **6** 29–35

Chiofalo B, Azzara V, Picollo D, Liotta L & Chiofalo L 2005 Variation of fatty acids in ass's milk during lactation. *Large Animals Review* **1** 39–44

Csapó J, Stefler J, Martin TG, Makary S & Csapó-Kiss Z 1995 Composition of mares' colostrum and milk. Fat content, fatty acid composition and vitamin content. *International Dairy Journal* **5** 393–402

Doreau, M & Martuzzi F 2006 Fat content and composition of mare's milk. In *Nutrition and Feeding of the Broodmare*, pp. 77–87 (Eds N Miraglia & W Martin-Rosset) EAAP publication, no 120. Wageningen Academy Publishers

Ellinger S, Linscheid KP, Jahnecke S, Goerlich R & Enbergs H 2002 The effect of mares' milk consumption on functional elements of phagocytosis of human neutrophil granulocytes from healthy volunteers. *Food and Agricultural Immunology* **14** 191–200

Folch J, Lees M & Sloane-Stanley GM 1957 A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226** 497–509

Fökel C, Schubert R, Kaatz M, Schmidt I, Hipler U, Vogelsang H and Jahreis G 2008 Oral treatment with mares' milk in patients with atrophic dermatitis. *European Journal of Clinical Nutrition* (in press)

Frąckowiak H & Komosa M 2006 The dynamics of hoof growth of the primitive Konik horses (*Equus caballus gmelini* Ant.) in an annual cycle. *Biological Rhythm Research* **37** 223–232

Hoffman RM, Kronfeld DS, Herbein JH, Swecker WS, Cooper WL, Harris PA 1998 Dietary carbohydrates and fat influence milk composition and fatty acid profile of mares' milk. *Journal of Nutrition* **128** 2708S–2711S

Kahle C 2001 A study on the effectiveness of using mare milk in the state of gastric intestine diseases. Diploma thesis., Friedrich-Schiller-Universität Jena, Institut für Ernährungswissenschaften

Kny G 1998 Untersuchungen zur Qualität von frischer und gefriergetrockneter Stutenmilch. Dissertation, Veterinärmedizinische Fakultät der Universität Leipzig, 1–103

Kramer JCK, Fellner V, Dugan MER, Sauer FD, Mossoba MM & Yurawecz MP 1997 Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* **32** 1219–1228

Kulisa M 1977 A study on the quality of raw and freeze-dry mare's milk. *Roczniki Naukowe Zootechniki* **4** 79–89

Mariani P, Summer A, Martuzzi F, Formaggioni P, Sabbioni A & Catalano AL 2001 Physicochemical properties, gross composition, energy value and nitrogen fractions of the Haflinger nursing mare milk throughout 6 lactation months. *Animal Research* **50** 415–425

Orlandi M, Goracci J & Curadi MC 2002 Essential fatty acids (EFA) in haflinger and thoroughbreed mare's milk. *Annali della Facoltá di Medicina veterinaria* **5** 319–325

Orlandi M, Goracci J & Curadi MC 2003 Fat composition of mares' milk with reference to human nutrition. *Annali della Facoltá di Medicina veterinaria* **56** 97–105

Pelizzola V, Contarini G, Povolo M & Giangiacomo R 2006 Chemical-physical characteristic and fatty acid composition of mare's milk. *Milchwissenschaft* **61** 33–36

Pikul J & Wójtowski J 2008 Fat and cholesterol content and fatty acid composition of mares' colostrum and milk during five lactations months. *Livestock Science* **113** 285–290

Salimei E, Varisco G & Rosi F 2002 Major constituents, leptin, and non-protein nitrogen compounds in mares' colostrum and milk. *Reproduction, Nutrition, Development* **42** 65–72

SAS® 1996 SAS User's Guide. Relase 6.12, Statistics. SAS Inst. Cary NC, USA

Smoczyński S & Tomczyński R 1982 The contents of certain free amino acids and fatty acids in the milk of Arabian, Małopolski and Huculski mare. *Zeszyty Naukowe Akademii Rolniczo-Technicznej w Olsztynie Technologia Żywności* **17** 77–84

Tomczyński R, Roman K & Szybińska E 1999 A comparative analysis of the chemical composition of milk from mares of the Wielkopolska and Konik Polski breeds. *Natural Sciences* **2** 87–96

Ulbricht TLV & Southgate DAT 1991 Coronary heart disease: seven dietary factors. *Lancet* **338** 985–992

Vetulani T 1925 A study on Konik horses from the area of Biłgoraj. Uniwersytet Poznański. *Roczniki Nauk Rolniczych i Leśnych* **14** 1–52

https://doi.org/10.1017/S0022029908003336 Published online by Cambridge University Press