Assessing heterogeneity of the composition of mare's milk in Flanders

De beoordeling van de samenstelling van paardenmelk die in Vlaanderen wordt verkocht

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ABSTRACT

In this study, the effect of farm, time, season and health was evaluated on the composition of mare's milk sold in Flanders. The content of the analyzed components (i.e. fat, fatty acids, protein, lactoferrin, lysozyme, lactose and urea) differed significantly ($p < 0.0001$) between farms, at a given moment in time. Within each farm, large month-to-month variations for most milk components ($p < 0.01$ to 0.0001) were observed. The variation over time between different farms was smaller. These findings indicate that the composition of the mare's milk consumer portions varies substantially between the different farms and also over time within each farm. Season, nutrition, udder health and worm burden are believed to contribute significantly to this variation.

SAMENVATTING

In deze studie werd de invloed van het bedrijf, het seizoen en de gezondheid van de dieren op de samenstelling van consumptiemelk afkomstig van merries beoordeeld. Ook de fluctuaties in de tijd werden bekeken. Uit de resultaten blijkt dat er op een gegeven tijdstip een significante variatie ($p < 0{,}0001$) bestaat in vet, vetzuren, eiwit, lactoferrine, lysozyme, lactose en ureum tussen bedrijven onderling. Op bedrijfsniveau werden er grote significante fluctuaties in de tijd vastgesteld voor de meeste melkcomponenten ($p < 0{,}01$ tot 0,0001). De fluctuaties tussen de verschillende bedrijven waren beduidend kleiner dan die binnen de bedrijven. Er kan besloten worden dat de samenstelling van paardenmelk weinig homogeen is, zowel tussen de bedrijven op een vast tijdstip als binnen de bedrijven in de loop van het jaar. Er zijn significante aanwijzingen dat seizoen, voeding, uiergezondheid en het niveau van wormbesmetting de samenstelling van deze melk kunnen beïnvloeden.

INTRODUCTION

Little is known about the variation of the composition of mare's milk in time or space (different farms). Mare's milk is commonly used in the diet of certain Asian and Russian populations. Recently, the interest in mare's milk has increased in Western European countries due to the possible health promoting characteristics of this product (Stoyanova et al., 1988; Jahreis et al., 1999; Sarwar et al., 2001; Benkerroum, 2008; Salamon et al., 2009).

The composition of mare's milk and its comparison to human and bovine milk have been reported in the literature. Mare's milk has a lower fat content, a higher lysozyme content than and a comparable lactoferrin content to human and cow's milk (Malacarne et al., 2002). The total amount of unsaturated fatty acids in mare's milk is highly comparable

to human milk and higher than the amount in cow's milk. The polyunsaturated fatty acids (PUFA) linoleic acid of the omega-6 group and alpha-linolenic acid of the omega-3 group are present in considerably larger proportions in mare's milk than in human or cow's milk. Medium chain fatty acids (MCFA) consist of 8 to 12 carbons and are considered to be antibacterial (Skrivanová et al., 2009; Sprong et al., 2001). The proportion of these MCFA, especially capric acid (C10:0) and lauric acid (C12:0), is approximately two times higher in mare's milk than in human and cow's milk. Combined with the lower amount of saturated fatty acids (SFA), the presence of these MCFA could be beneficial to human health (Malacarne et al., 2002). Additionally, the relatively high proportion of the whey protein fractions lactoferrin and lysozyme, which are considered antimicrobial active factors, might also play a role in the health claim of mare's milk.

The content of mare's milk is variable over the period of lactation (Mariani et al., 2001; Summer et al., 2004; Pikul and Wójtowski, 2008; Salamon et al. 2009). Experiments show that the content of fat and protein decreases (Mariani et al., 2001; Summer et al., 2004; Pikul and Wójtowski, 2008), and that lactose increases (Mariani et al., 2001) during lactation. Salamon et al. (2009) observed that the linoleic acid and linolenic acid concentrations decrease, while the antibacterial fatty acids capric and lauric acid increase over lactation time. Pikul and Wójtowski (2008) also found a decrease of linoleic acid, but linolenic acid increased significantly during month four and five of lactation. The diet fed to the mare is considered to be a major determinant of the equine milk composition. The composition of milk differs clearly between mares that are fed a diet rich in forage and mares that receive a diet rich in concentrates (Doreau et al., 1992; Hoffman et al., 1998). Most studies report that breed does not affect milk composition (Neseni, 1958; Kulisa, 1977; Doreau, 1991; Pikul and Wójtowski, 2008; Salamon et al., 2009), except for Pietrzak-Fiecko et al. (2009) who reported that the fatty acid composition of mare's milk is breed specific. Gibbs et al. (1982) found a significant decrease of protein content with higher parity. In Murgese mares, machine-milking significantly increases the fat content in milk compared to hand-milking (Caroprese et al., 2007). A study of asinine milk has revealed daily rhythmicity in lipid, protein and lactose content (Piccione et al., 2008). Fat and lactose have been found to peak during the night, while protein content peak during the day.

The aim of this study was to evaluate the variability of the composition of mare's milk sold at farms in Flanders (Belgium) and to explain part of this variability. The study consisted of two parts. In the first part, the variation between and within ten different farms at a given time was evaluated. In a second study, the variation over time between and within four different farms was assessed, and several contributors to the variations were identified.

To be consistently health promoting, variations in milk composition should be minimal.

MATERIALS AND METHODS

Collection of milk samples

Consumer portions are a mixture of milk from all lactating mares of a single milking time or milking day. The milk is collected in a tank by machine-milking, and is cooled, stored and frozen in 250 mL consumer portions. In the first study, five consumer portions were bought at ten equine farms in East and West Flanders in November 2008 during a one-week period. Out of these ten farms, four were chosen for a monthly (first week of the month; one consumer portion) collection during 20 months (May 2009 - December 2010; second study). At some of these four farms, there were periods of non-milking.

All samples were immediately transported to the laboratory using a cooling device, and stored at -20°C until further analysis. At the time of analysis, the frozen samples were thawed in a water bath at 25°C.

Farm management information

Information about the diet composition, the amount of concentrates given to each mare, about breed and lactation stage was obtained by verbal communication with the farmers.

Collection of feces samples

At the four farms of the longitudinal study, the eggs per gram feces (EPG) of strongyles were determined monthly during 20 months to evaluate the worm burden at farm level. Rectal feces samples were collected from maximum five mares (in average 3.23) per farm. Fecal samples were mixed, and analyzed according to the McMaster technique (Ward et al., 1997).

Analysis of gross milk composition

The fat, protein, urea and lactose determinations were performed in a laboratory at the University College Ghent. The five consumer portions bought in November 2008 on the ten different farms (n = 50) were analyzed at the same time. The samples (one at a time and per farm) gathered on the four farms between May 2009 - December 2010 (n = 66) were analyzed twice. Milk fat was determined by the IDF Gerber method No.105:1981 (IDF, 1969). Verification of the fast Gerber method was done in the first study according to the Röse-Gottlieb AOAC method No. 905.02 (AOAC, 1996). The total nitrogen (N) amount in the different samples was verified using the Kjeldahl method. The total protein content was calculated as total N amount x 6.38 according to AOAC method No. 991.20 (AOAC, 1996). An enzyme kit (Megazyme, catalogue No. K-URAMR) was used according to the manufacturer's instructions to determine the urea content of the samples spectrophotometrically. Another enzymatic test kit (Megazyme, catalogue No. K-LACGAR) was

used to determine the lactose content of the samples.

Fatty acids were determined in the form of fatty acid methyl esters, obtained by extraction (cf. Röse-Gottlieb) and methylation. They were quantified using gas chromatography (GC). This analysis includes saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA), omega 3 polyunsaturated fatty acids (n-3 PUFA) omega 6 polyunsaturated fatty acids (n-6 PUFA) and some other fatty acids not included in the previous categories (Vlaeminck et al., 2005). The lysozyme and lactoferrin determinations using SDS-PAGE (Wambacq, 2005) were done in the same laboratory. All of the analyses in Lanupro were done on two consumer portions of the ten different farms and in duplicate on each of the consumer portions bought at the four selected farms.

Somatic cell count analysis

The somatic cell count (SSC) was determined using a fluoro-opto electronic method (Fossomatic 5000 cell counter; Foss Electric, Hillerød, Denmark) by the Milk Control Center (MCC, Lier, Belgium) (Vangroenweghe et al., 2005).

Statistical analysis

SAS Version 9.2 learning edition 4.1 was used to explore and analyze the data. A paired t-test was used to search for significant differences between the Gerber method and the Röse-Gottlieb reference method. As the different components in the first study were analyzed on two (fatty acids, lysozyme and lactoferrin) or five (all of the other components) samples per farm, the variance within (= due to measurement method and different consumer portions examined) and between the ten farms was evaluated using a linear mixed model with farm as random effect. The possible influence of the amount of provided concentrates was analyzed using a linear mixed model with farm as random effect and concentrates as continuous fixed effect.

The analysis of heterogeneity within and between farms over time was based on a linear mixed model with farm as random effect. Additionally, season and EPG were added as covariates to the analysis, with season as categorical and EPG as continuous fixed effect. Pearson correlation coefficients between the different milk components were derived.

RESULTS

The management information of the ten farms involved in this study is shown in Table 1. At all of the ten farms, roughage was provided ad libitum. The average somatic cell counts (SCC) of farm 1 and 2 were high compared to the normally low SCC in mare's milk (Dankow et al., 2006), but this was mainly due to one high SCC in both farms (farm 1: 4, 671,000 in November 2010; farm 2: 1,248,000 in November 2009). The overall average SCC of the four farms equaled 122,734 cells/ml (n = 66).

The composition of mare's milk is summarized in Table 2. The results by the quick Gerber method and Röse-Gottlieb method for fat determination did not differ significantly ($p < 0.05$). Therefore, only the quick Gerber method results are shown.

The fatty acid composition is summarized in Table 3.

Except for lysozyme (74.8%), most of the variability in the results of the first study (95.7% to 99.8%) was found to be due to differences between the farms, indicating no variation due to the analysis method or

Table 1. Farm information.

Farm	Breed (n1)	Lactation stage	Concentrates (kg/day)	Milking period	Milkings/ day	Average EPG over 20 months (n2)	Average SCC over 20 months (n2)
1	New Forest /Friesian* (6)	variable	5	Continuous	4	7.7 (19)	97 447 (19)
2	Tinker* (10)	variable	3	August-January	0-3	532 (11)	444 045 (11)
3	Mixed breeds	variable	1	Irregular	Irregular		
4	Warmblood	end	6	May-December	3-4		
5	Shire* (8)	variable	1.5	July-April	1	19.6 (16)	24 000 (16)
6	Haflinger* (10)	variable	1	Continuous	3-4	1039 (20)	49 025 (20)
7	Haflinger	variable	4	Irregular	Irregular		
8	New Forest	variable	1	Irregular	Irregular		
9	Mixed breeds	variable	3	Irregular	Irregular		
10	Haflinger	variable	4	Irregular	Irregular		

* Farms followed over a 20-month period (May 2009 – December 2010); EPG: eggs per gram feces; SCC: Somatic Cell Count. (n1): number of mares milked on a daily basis; (n2): number of samples that were collected and analyzed; Irregular: mares are milked depending on the commercial needs; if not milked, foals stay with their mothers to sustain milk production.

Table 2. Summary statistics of mare's milk composition on 10 farms in November 2008.

	n Samples	Min	Max	Mean	Median	Variance	Std Dev?
Fat (g/100 g)	50	0.40	1.70	0.98	1.05	0.12	0.35
Protein (g/100 g)	50	1.48	1.97	1.76	1.77	0.01	0.12
Lysozyme (g/L)	20	0.63	1.03	0.79	0.80	0.01	0.09
Lactoferrin (g/L)	20	0.32	0.73	0.5	0.43	0.02	0.15
Urea (g/L)	50	0.21	0.42	0.33	0.33	0.00	0.06
Lactose (g/100 g)	50	6.44	7.16	6.83	6.83	0.05	0.23

Table 3. Fatty acids composition of mare's milk on 10 farms in November 2008 (g/100g FA).

	Min	Max	Mean
SFA	37.46	52.72	43.05
SCFA	0.80	5.34	1.75
MCFA	7.51	15.07	10.81
LCFA	23.05	37.45	30.49
MUFA	18.87	33.46	28.47
n-6 PUFA	4.66	19.35	11.24
C18:2	4.39	18.73	10.8
other n-6 PUFA	0.25	0.61	0.44
n-3 PUFA	8.10	31.99	14.43
C18:3	7.62	31.23	13.87
other n-3 PUFA	0.41	0.76	0.56
Other	0.40	1.12	0.62
other SFA	0.32	0.86	0.48
CLA	0.08	0.37	0.14

FA: fatty acids; SFA: saturated fatty acids; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids;LCFA: long-chain fatty acids; MUFA: mono unsaturated fatty acids; n-6 PUFA: omega 6 polyunsaturated fatty acids; n-3 PUFA: omega 3 polyunsaturated fatty acids; CLA: conjugated linoleic acid

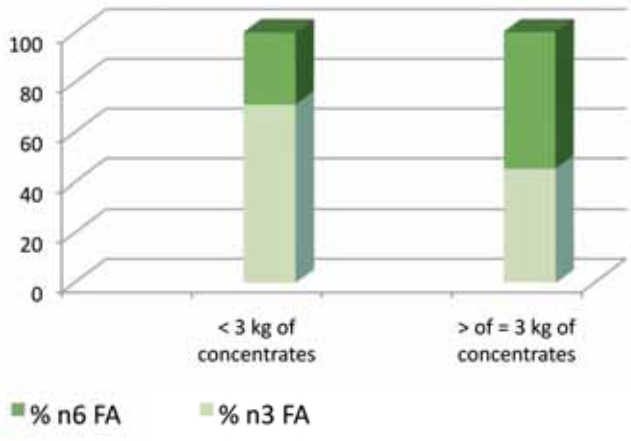

Figure 1. Effect of variable amount of concentrates on linoleic (increase) and linolenic (decrease) acids in mare's milk.

the different consumer portions. Significant variations ($p < 0.0001$) between farms were seen for all analyzed components.

The amount of concentrates in the diet was not significantly associated with the amount of fat, protein, lysozyme, lactoferrin, urea and lactose. Linoleic acid appeared to be substantially higher ($p < 0.01$) in milk from farms where mares received more concentrates. Figure 1 shows the relationship between the daily amount of concentrates and n-6 and n-3 content in the milk.

Table 4 presents the individual and combined farm means, the minima and maxima of the different milk components during the 20-month-sampling period. Figure 2 shows the variation of the protein content over time. Similar patterns (not shown) were found for fat, n-3 and n-6.

The variability of different components caused by between-farm and intra-farm factors over a period of 20 months is shown in Table 5.

The statistical analysis of seasonal and EPG influences on milk composition revealed a significant effect of season ($p < 0.05$) for fat, n-6 to n-3 ratio and urea. The fat content appeared to be higher in milk produced during autumn and winter, while the ratio of omega 6 to omega 3 fatty acids was higher during winter and spring. The values for urea were highest in autumn. The n-6 fatty acids were the only component on which the EPG had a significant ($p < 0.05$) influence.

The most striking ($p < 0.0001$) correlations were found between urea and n-3 ($r = 0.71$), urea and protein ($r = 0.46$), protein and lactose ($r = 0.53$) and SFA with urea ($r = -0.35$), n-3 ($r = -0.52$) and MUFA ($r = -0.47$). The somatic cell count seemed to affect the lactose content negatively, but only at a low level ($r = -0.25$ with $p < 0.05$). When SCC increased, there was a tendency ($p = 0.06$) for protein and SFA to decrease.

DISCUSSION

The aim of this study was to assess the variation of the composition of mare's milk sold at farms in Flanders (Belgium) both over space and time and to explain part of this variability. The first observation was the large variations in milking procedures and management at the different farms.

The total fat content showed extreme variations between farms at a fixed time with a very low fat content found in three of the farms (≤ 0.60%). Only Mariani et al. (2001) mentioned a similar low fat content (0.44 g/100 g at 180 days of lactation), most

Table 4. Mare's milk composition at four different farms over a 20-month period.

	Farm 1	Farm 2	Farm 3	Farm 4	Combined
Fat (g/100 g)	0.70 (0.3-1.5)	1.16 (0.9-1.9)	0.74 (0.3-1.2)	0.73 (0.4-1.1)	0.86 (0.3-1.9)
FA (g/100 g FA)					
SFA	49.37 (32.0-58.6)	47.05 (37.4-57.3)	45.14 (35.3-54.3)	41.95 (36.1-56.4)	46.37 (32.0-58.6)
MUFA	25.74 (19.9-33.5)	27.18 (24.8-33.9)	24.22 (12.9-34.3)	28.58 (20.7-35.6)	26.37 (12.9-35.6)
n-6 PUFA	10.59 (6.4-13.2)	5.18 (3.1-6.8)	9.56 (4.3-13.4)	9.71 (6.0-14.8)	8.52 (3.1-14.8)
n-3 PUFA	9.16 (4.5-18.4)	15.67 (9.2-22.0)	12.66 (6.0-18.4)	15.00 (8.0-23.4)	13.00 (4.5-23.4)
Protein (g/100 g)	1.76 (1.5-2.1)	1.85 (1.7-2.0)	1.92 (1.7-2.2)	1.72 (1.6-1.8)	1.82 (1.5-2.2)
Lysozyme (g/L)	0.74 (0.5-1.2)	0.81 (0.6-1.2)	0.82 (0.5-1.3)	0.77 (0.5-1.1)	0.79 (0.5-1.3)
Lactoferrin (g/L)	0.30 (0.2-0.6)	0.30 (0.2-0.5)	0.28 (0.1-0.8)	0.40 (0.2-1.0)	0.31 (0.1-1.0)
Urea (g/L)	0.24 (0.2-0.4)	0.32 (0.2-0.4)	0.32 (0.2-0.5)	0.36 (0.2-0.5)	0.30 (0.2-0.5)
Lactose (g/100 g)	6.91 (6.5-7.5)	6.97 (6.7-7.5)	6.77 (5.5-7.2)	6.72 (6.4-7.0)	6.87 (5.5-7.5)

FA: fatty acids; SFA: saturated fatty acids; MUFA: mono unsaturated fatty acids; n-6 PUFA: omega 6 polyunsaturated fatty acids; n-3 PUFA: omega 3 polyunsaturated fatty acids

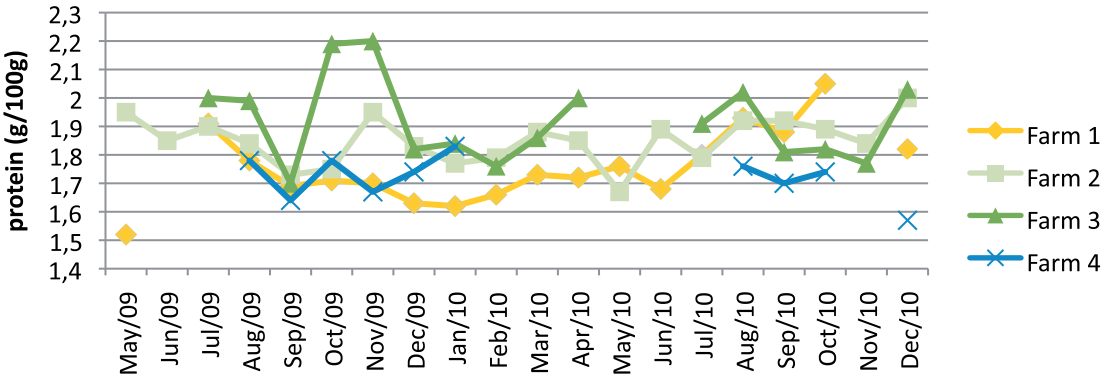

Figure 2. Monthly protein percentage in mare's milk of four farms.

likely because hand-milking causes a lower fat content (Caroprese et al., 2007). In the present study, no hand-milking was performed. Doreau et al. (1992) found a higher fat content in milk from mares that were fed with a forage-rich diet. In all of the ten farms of the present study, the mares were provided with a diet consisting of roughage ad libitum and a variable amount of concentrates. However, the amount of concentrates did not affect the fat content of the milk in this study. Over time, the fat content ranged from 0.26 to 1.91 g/100 g (Table 4). Depending on the farm, large fluctuations in fat content were observed during the 20-month-observation period. The highest fat content of the 20-month period was found in milk of farm 2. This might be due to a diet rich in hay given to the mares at this farm, which confirms the results of Doreau et al. (1992). Moreover, in autumn and winter, the fat content was significantly higher than in the other two seasons, even though a low fat content is to be expected at the end of lactation in those periods (Mariani et al., 2001; Pikul and Wójtowski, 2008). This is most likely due to a diet with relatively more roughage (Doreau et al., 1992) given in those seasons and to the presence of mares at different lactation stages.

It was found that the mean protein content in all samples was less than the crude protein fraction mentioned by Malacarne et al. (2002). Nevertheless, this result is comparable to values of milk from mares after two months of lactation as has been reported by Mariani et al. (2001). Although in this study, the protein content showed significant differences between farms, the farm with end-stage mares represented a rather high protein content per 100 g milk compared to most of the other farms. This might be explained by the fact that at the end of lactation, less milk is produced with a relatively higher protein content. The higher protein concentrations in milk from mares fed a diet rich in forage, as reported by Doreau et al. (1992), could not be proven statistically in the present study. The mean protein percentages of the four different farms during the 20-month study were relatively close: the variability between farms was only 29%, so the largest variations over time were present within the farms. The mean lysozyme content was similar to the value reported by Jauregui-Adell (1974). Malacarne et al. (2002) found a slightly lower value, and reported a higher lactoferrin amount than found in the present study. Although the average concentrations of whey proteins lysozyme and lactoferrin at the four farms

Table 5. Variability of milk components between and within four farms over a 20-month period.

Component	Between farms (%)	Within farms (%)
Fat	37%	63% [c]
n-6	58%	42% [c]
n-3	37%	63% [c]
Protein	29%	71% [b]
Lysozyme	0%	100% [a]
Lactoferrin	0%	100% [a]
Urea	27%	73% [d]
Lactose	8%	92% [a]

a: <0.0001; b: <0.001; c: <0.01; d: <0.05

were very similar, the minimum and maximum values widely varied within the farms over time (Table 4).

Salimei et al. (2002) reported comparable results for the mean urea content. Large fluctuations were observed within the farms of the present study. There was a significant seasonal influence, with the highest amounts of urea in spring, summer and autumn, decreasing in winter. In winter, mares are housed inside, and fed a more balanced diet. Most likely, a more balanced diet limits the loss of N and the formation of urea. In general, as the dietary N intake increases, the plasma urea concentrations, urea pool size, urea entry rate and urea excretion rate also increase (Prior et al., 1974).

The mean lactose content was in agreement with values found in the literature (Mariani et al., 2001; Malacarne et al., 2002). A high lactose content (7.11 g/100 g) was found in the milk of two farms in November 2008. A minimum content of 5.47 and a maximum content up to 7.5 g/100 g were found in the second study. However, a very low milk lactose value of 5.47 g/100 g was observed in milk of farm 3 in July 2010. This low value has not been reported in the literature so far. The lactose content in the study by Mariani et al. (2001) was never lower than 6.36 g/100 g (at four days post parturition).

There are remarkable differences between the fatty acid content found in the present study (Table 3) and those reported in the literature. The mean SFA in this study was 10% lower than the results reported by Malacarne et al. (2002), but comparable to the values found by Pikul and Wójtowski (2008). Both studies reported slightly lower SCFA amounts and higher amounts of MCFA. The mean LCFA content in the present study is in agreement with the amount mentioned by Pikul and Wójtowski (2008), but lower than that reported by Malacarne et al. (2002). Unsaturated fatty acids account for more than 50% of the total fatty acid content. Experiments performed by Pikul and Wójtowski (2008) suggested approximately the same ratio of MUFA and PUFA as mentioned in Table 3, but Malacarne et al. (2002) reported less PUFA. The present study showed that the average amount of n-6 PUFA, which consists mainly of linoleic acid, was low in comparison to the values reported by Pikul and Wójtowski (2008). However, the results of Malacarne et al. (2002) are much more comparable to the result of this study. In contrast with the amount of n-6, the amount of n-3 PUFA and the results for linolenic acid were very high compared to the literature values (Malacarne et al., 2002; Pikul and Wójtowski, 2008). Pikul and Wójtowski (2008) suggest a significant effect of lactation stage, and have found that the n-6 PUFA content decreases from approximately 20% of total fatty acids in the colostrum to 15.7% in milk of the fifth month after foaling. n-3 PUFA was found to increase during lactation from approximately 5.8% in colostrum to 7.6% after five months of lactation. However, at the end of lactation, the milk of the mares showed rather opposite values, as the amount of n-6 was the highest of all of the farms, and n-3 was one of the lowest values. The trend of a higher milk concentration of linoleic acid and a lower concentration of linolenic acid is in agreement with the results of Hoffman et al. (1998) and Doreau et al. (1992) who stated that there is no hydrogenation before the absorption of dietary fatty acids in the horse, which results in a correlation of the long chain fatty acids found in the milk and the composition of the provided nutrition. Simopoulos (2004) mentioned an optimal balance of n-6 to n-3 fatty acids of 1/1 to 5/1 in the human diet to obtain beneficial health effects on different diseases. Because an increasing amount of concentrates results in increasing linoleic acid and decreasing linolenic acid in milk, a more favorable n-6/n-3 ratio could be achieved. In the present study, n-6/n-3 ranged from 0.25 to 1.99 with a mean of 0.96. Other studies reported ratios of 0.33 to 3.5; large differences seem to be present in mare's milk (Hoffman et al., 1998; Malacarne et al., 2002; Pikul and Wójtowski, 2008; Salamon et al., 2009). In the present study, it was found that over time, large ranges could be seen between minimum and maximum values for the different groups of fatty acids (Table 4). In contrast to n-6, where 58% of the variability could be explained by between-farm differences, the different values for n-3 were mostly due to within-farm factors. Milk of farms providing different amounts of concentrates seemed to contain fatty acids with various ratios of n-6 and n-3 PUFA. The lowest mean n-6 PUFA and the highest mean n-3 PUFA over time were found in milk from the farm giving only concentrates (farm 6). As the precise composition of the concentrates was unknown, the component responsible for differences seen in the milk of mares that received more concentrates than mares that were given less concentrates, was also unknown. The farm that provided the highest amount of concentrates (farm 1) showed opposite values with the highest mean n-6 fraction and the lowest mean n-3 fraction. Besides concentrates to roughage ratio, the precise diet composition and lactation stage may also influence the composition of fatty acids (Doreau et al., 1992; Hoffman et al.,

1998; Mariani et al., 2001). It has been shown that season has no significant effect on neither n-6 nor n-3 content. However, in the present study, the n-6/n-3 ratio was significantly influenced by season. In spring and winter, this ratio seems to be more favorable towards human nutrition than the ratio in the other months (Simopoulos, 2004).

Monitoring the health of lactating mares seems to be important. In the present study, the n-3 fatty acids showed a significant decrease when EPG increased ($p < 0.05$). However, no large impact was expected of low to normal worm infestations. Somatic cell counts > 100,000 cells/ml were found, which reflects udder disease in horses (Böhm et al., 2009), Nevertheless, the farm keepers did not mention mastitis as a problem, nor did they wonder about the potential causes. Inadequate milking hygiene and milking mechanisms could be the reasons for this finding. However, the reasons why mastitis and occasionally high SCC's are not an issue in horse milking farms may be the lower milk yield, more milking sessions per day and the fact that foals are kept with their mothers overnight. High SCC's have been measured and controlled several times in other, unpublished studies. Furthermore, negative influences of increased SCC's on some milk components were seen in the present study. Monitoring udder health on horse milking farms is therefore advisable.

CONCLUSION

There is a large between-farm variation of mare's milk composition at a fixed point in time. Over time, there is a larger within-farm than an inter-farm variation concerning the composition of mare's milk. Differences in nutrition, worm burden and udder health cause significant intra-farm variations.

REFERENCES

A.O.A.C. (1996). Official Methods of Analysis. *Association of Official Agricultural Chemists.* Washington, D.C., Chapter 33.

Benkerroum N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. *African Journal of Biotechnology, 7*, 4856-4867.

Böhm K. H.,Klug E., Jacobs, B. J. (2009). Mastitis in the mare - a long-term study on the incidence, clinical symptoms, diagnostics, microbiology, therapy and economic importance, as well as recommendations for veterinary practice. *Praktischer Tierarzt 90*, 842-849.

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 90*, 2773-2777.

Dankow R., Wojtowski J., Pikul J. (2006). Effect of lactation on the hygiene quality and some milk physicochemical traits of the Wielkopolska mares. *Archiv für Tierzucht 49* (special issue), 201-206.

Doreau M. (1991). Le lait de jument. *INRA Productions Animales 4*, 297-302.

Doreau M., Boulot S., Bauchart D., Barlet J.-P., Rosset W. (1992). Voluntary intake, milk production and plasma metabolites in nursing mares fed two different diets. *Journal of Nutrition 122*, 992-999.

Gibbs P.G., Potter G.D., Blake R.W., McMullan W.C. (1982). Milk production of quarter horse mares during 150 days of lactation. *Journal of Animal Science 54*, 496-499.

Hoffman R.M., Kronfeld D.S., Herbein J.H., Swecker W.S., Cooper W.L., Harris P.A. (1998). Dietary carbohydrates and fat influence milk composition and fatty acid profile of mare's milk. *Journal of nutrition 128*, 27088-27118.

International Dairy Federation (IDF) (1969). International Standard FIL-IDF 1A.

Jahreis G., Fritsche J., Möckel P., Schöne F., Möller U., Steinhart H. (1999). The potential anticarcinogenic conjugated linoleic acid, cis-9,trans-11 C18:2: in milk of different species: cow, goat, ewe, sow, mare, woman. *Nutrition Research 19*, 1541-1549.

Jauregui-Adell J. (1974). Heat stability and reactivation of mare milk lysozyme. *Journal of Dairy Science 58*, 835-838.

Kulisa M. (1977). The composition of mare's milk in three horse breeds with reference to N-acetylneuraminic acid. Acta Agraria et Silvestria. *Series Zootechnica 27*, 25-37.

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 12*, 869-877.

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

Neseni N., Flade E., Heidler G., Steger H. (1958). The yield and composition of mare's milk throughout lactation. *Archiv für Tierzucht 1*, 91-129.

Piccione G., Fazio F., Caola G., Refinetti R. (2008). Daily rhythmicity in nutrient content of asinine milk. *Livestock Science 116*, 323-327.

Pietrzak-Fiecko R., Tomczynski R., Świstowska A., Borejszo Z., Kokoszko E., Smoczynska K. (2009). Effect of mare's breed on the fatty acid composition of milk fat. *Czech Journal of Animal Science 54*, 403-407.

Pikul J., Wójtowski J. (2008). Fat and cholesterol content and fatty acid composition of mares' colostrums and milk during five lactation months. *Livestock Science 113*, 285-290.

Prior, R.L., Hintz, H.F., Lowe, J.E., Visek, W.J. (1974). Urea recycling and metabolism of ponies. *Journal of Animal Science* 38, 565-571.

Salamon R.V., Salamon Sz., Csapó-Kiss Zs., Csapó J. (2009). Composition of mare's colostrums and milk I. Fat content, fatty acid composition and vitamin contents. *Alimentaria 2*, 119-131.

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

Sarwar, A., Enbergs, H., Klug, E. (2001). Influences of parity, age and mineral and trace element mixture on lysozyme activity in mare's milk during early lactation period. *Veterinarski Archiv 71*, 139-147.

Simopoulos, A.P. (2004). Omega-6/omega-3 essential fatty acid ratio and chronic diseases. *Food Reviews International 20*, 77-90.

Skrivanová, E., Molatová, Z., Skrivanová, V., Marounek,

M. (2009). Inhibitory activity of rabbit milk and medium-chain fatty acids against enteropathogenic Escherichia coli O128. *Veterinary Microbiology 135*, 358-362.
Sprong, R.C., Hulstein, M.F.E., Van der Meer, R. (2001). Bactericidal activities of milk lipids. *Antimicrobial Agents and Chemotherapy 45*, 1298-1301.
Stoyanova, L.G., Abramova, L.A., Ladodo, K.S. (1988). Freeze-dried mare's milk and its potential use in infant and dietetic food products. *Voprosy Pitania 2*, 64-67.
Summer, A., Sabbioni, A., Formaggioni, P., Mariani, P. (2004). Trend in ash and mineral element content of milk from Haflinger nursing mares throughout six lactation months. *Livestock Production Science, 88*, 55-62.
Ullrey, D.E., Struthers, R.D., Hendricks, D.G., Brent, B.E. (1966). Composition of mare's milk. *Journal of Animal Science 25*, 217-222.
Vangroenweghe, F., Duchateau, L., Boutet, P., Lekeux, P., Rainard, P., Paape, M.J., Burvenich, C. (2005). Effect of carprofen treatment following experimentally induced escherichia coli mastitis in primiparous cows. *Journal of Dairy Science 88*, 2361-2376.
Vlaeminck, B., Dufour, C., van Vuuren, A.M., Cabrita, A.M.R., Dewhurst, R.J., Demeyer, D., Fievez, V. (2005). Use of odd and branched-chain fatty acids in rumen contents and milk as a potential microbial marker. *Journal of Dairy Science 88*, 1031-1042.
Wambacq E. (2005). *Antimicrobial Components in Mare's Milk*. Master's dissertation, Ghent, Ghent University, 114.
Ward, M.P, Lyndal-Murphy, M., Baldock F.C. (1997). Evaluation of a composite method for counting helminth eggs in cattle faeces. *Veterinary Parasitology* 73, 186-187.

Mededeling

6de European Equine Health & Nutrition Congress

"Feeding for Gastrointestinal Health"

Op **1 en 2 maart 2013** vindt in Gent (BE) de zesde editie van het European Equine Health & Nutrition Congress plaats. Tijdens dit congres gaan gerenommeerde nationale en internationale sprekers in op de relatie tussen voeding en maagdarmgezondheid van paarden. De eerste dag richt zich op het voorkomen van voeding gerelateerde aandoeningen. Dag twee focust op de voeding van paarden met maagdarmproblemen. Naast lezingen wordt een workshop programma aangeboden. Het 6de European Equine Health & Nutrition Congres wordt georganiseerd in samenwerking met verschillende onderzoeksinstellingen en universitaire klinieken in België (Universiteit Gent, Université de Liège) en Nederland (Universiteit Utrecht, Wageningen University & Research Centre).

Workshop programma (vrijdagochtend, 1 maart 2013, ca. 8.30u)[1]

- Grazing management & Deworming strategies – *D. van Doorn & E. Claerebout*
- Dental pathologies with GI consequences & dietary solutions – *M. Hesta & L. Vlaminck*
- Practical quality assessments of feedstuffs and water for horses – *A. Zeyner & M. van den Top*
- Clinical workshop: "Colic in the horse" – *H. Amory & G. van Loon*

Demonstration of the updated FRASC program – *P. Bollen*

Congresprogramma[1]

Vrijdag, 1 maart (vanaf 11.15u):

Nutrition and the prevention of gastrointestinal problems in healthy horses

- Ingestive behavior of horses "wild horses versus the feeding of the performance horse" – *M. Clauss*
- Nutritional strategies gastrointestinal health "the basics" – *A. Jansson*
- "How may feed technology and feed conservtion methods help or affect gastrointestinal health?" – *A. Zeyner*
- "Hygienic quality of feed & Mycotoxins" – *J. Fink*
- "Dietary strategies for optimizing gastrointestinal health: an update on pre- and probiotics" – *V. Julliand & A. Zeyner*
- "Dietary management for reducing the risk of gastrointestinal disorders (colic)" – *C. Proudman*

Zaterdag, 2 maart (vanaf 8.45u):

Feeding horses with gastrointestinal problems

- "Normal function, digestion, physiology and motility of the gastrointestinal tract of horses" – *A.M. Merritt, V. Julliand & P. Deprez*
- Pathology of gastrointestinal diseases – R. Ducatelle
- The normal versus disturbed microflora in horses – *F. van Immerseel*
- "Overview of structural and specific feed related gastrointestinal diseases" – *C. Proudman*
- Feed allergens: What do we know? – *H. van der Kolk, M. Hesta a.o.*
- How to feed a horse with gastric ulcers? – *A.M. Merritt*
- Equine Clinical Nutrition I (Adults and foals): Focus on small intestinal problems, dysphagia and anorectic horses – *T. Mair*
- Equine Clinical Nutrition II: Focus on large intestinal problems – *T. Mair*

[1] Het programma kan door de organisatie gewijzigd worden wegens praktische redenen of de beschikbaarheid van de sprekers.

Meer informatie over het programma en de registratieis te lezen op de congres website: http://www.equine-congress.com.