Effects of Acute Changes in the Energy and Protein Intake Levels over the Short-term on the Maternal Milk Amino Acid Concentrations in Lactating Mares

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ABSTRACT : This study was designed to test the effects of changes in energy and protein intake levels on the maternal milk amino acid concentrations over the short-term in lactating mares. Three lactating mares were enrolled for the study 7 weeks after parturition. A low-energy and low-protein diet (LEP) was administered during the first week of the study, followed by administration of a high-energy and high-protein diet (HEP), again for a week (day 1 to day 7), and milk was sampled thrice daily at intervals of 8 h during the study period. The mean amino acid concentrations in the maternal milk, except for those of proline, serine and valine, were significantly higher in the HEP feeding period than during the LEP feeding period ($p<0.05$). The sum of the concentrations of all the amino acids (TAA) in the maternal milk samples during the HEP and LEP feeding periods was 1,644.9±26.9 and 1,542.3±36.0 mg/100 g, respectively, the difference between the two was not significant. When the ratio of each amino acid concentration to the TAA in the maternal milk was analyzed, there were significant differences between the HEP and LEP feeding periods for all amino acids, except glycine, serine, alanine and histidine. It was found that the concentrations of glutamic acid+glutamine, serine, threonine, arginine and valine were significantly higher ($p<0.05$) on day 1 than on day 7 during the LEP feeding period, and there were no such differences during the HEP feeding period. In regard to the effects of changes in the energy and protein intake levels in lactating mares, no changes in milk amino acid concentrations were found following administration of HEP for a week, whereas 7 days of administration of LEP was associated with a decrease in the amino acid concentrations. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 855-860)***

Key Words : Amino Acids, Milk, Lactating, Mare

INTRODUCTION

Maternal milk is an important source of nutrients for foals during growth and development. Its importance increases especially during the period when the foals do not have adequate access to grass (Gibbs et al., 1982; Oftedal et al., 1983; Doreau et al., 1986). In humans and milking cows, the composition of maternal milk is known to be affected by the nutritional composition of the ingested diet (Powers et al., 1995; Preissinger et al., 1998; Wang et al., 2004). In this context, our attention was drawn to a report that the maternal milk amino acid concentrations were elevated following ingestion of high-quality protein by the mares (Glade et al., 1990).

It has been reported that amino acids are particularly important for the growth of foals (Staniar et al., 2001), that the amino acid composition of the maternal milk protein in pigs affects the absorption of protein in young pigs, and that there is an ideal amino acid composition for optimal growth and development (Newport et al., 1985). The amino acid composition of the ingested protein affected the growth rate of calves in one study (Khan et al., 2002). The amino acid concentrations, and the composition of maternal milk are thus very nutritionally important for the suckling foal. To allow appropriate nutritional management of horses during the lactating period and foals during the suckling period, it is essential to clarify the relationship between the nutritional composition of the diet given to horses feeding their foals, and the maternal milk composition.

The composition of a lactating mare's milk is affected by several factors, including the stage of lactation, individual differences, and the parity of the mare (Doreau et al., 1990; Doreau et al., 1991). The present study was undertaken to examine the effects of changes in the energy and protein intake levels by lactating mares on the maternal milk amino acid concentrations over the short-term.

MATERIALS AND METHODS

Test horses and period of study

Three lactating thoroughbred mares (mean body weight, 566 kg; mean age, 9 years) were examined 7 weeks after parturition. The study period was divided into two phases: the high-energy and high-protein feeding period (HEP), and the low-energy and low-protein feeding period (LEP), lasted for a week (day 1 to day 7). The two dietary feeding periods were switched in the same horse (Figure 1). Table 1 shows the amounts of the diet and the nutrients fed to each horse during the two dietary phases. The amounts of

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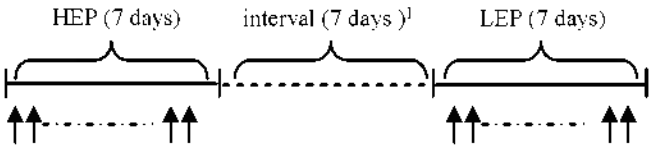

Figure 1. Test protocol outline. The study period was divided into two phases: the high-energy and high-protein feeding period (HEP), and the low-energy and low-protein feeding period (LEP), lasted for a week (day 1 to day 7). The two dietary feeding periods were switched in the same horse. 1 An interval period feeding medium-energy and medium-protein for diminishing previous feeding effect.

digestible energy and crude protein were 120.2% and 160.0% of the daily requirement for a lactating mare (NRC 1989; Japanese Feeding Standard for Horse, 1998) during the HEP feeding period, and 93.1% and 87.0% of the daily requirement during the LEP feeding period, respectively. Medium-energy and medium-protein feeding period (ordinal), which were described in a previous study and one-week interval period between diet treatments, are shown in Table 1. The daily pasture intake during grazing was estimated using the double-indicator method, using chromium oxide and lignin in a previous study (Van Soest, 1967; AOAC, 1999; Asai et al., 1999; Takagi et al., 2002). Because the results of a previous study (Matsui et al., 2003) suggested that the maternal milk amino acid concentrations might vary depending on the time of sampling of the milk, milk samples were collected thrice a day at intervals of 8 hours during each phase of the study. About 50 ml of milk was sampled each time by hand, and the samples were stored frozen at -80°C until analysis. The milk samples were dried using a freeze drier (FDU-830, Tokyo Rikakikai Co., Tokyo). The amounts of the total solids in the milk were calculated from the differences between the pre-drying and post-drying weights. Prior to the amino acid analysis, the freeze-dried milk samples were subjected to hydrolysis in 6 N HCl for 24 h at 110°C. The HCl was removed by drying the sample under reduced pressure in the freeze-drier. The hydrolyzed samples were diluted with 0.02 N HCl, and the glutamic acid+glutamine, proline, isoleucine, lysine,

Table 1. The daily amounts of feed and nutrients ingested by lactating mares during the high-energy and high-protein feeding period (HEP) and the low-energy and low-protein feeding period (LEP)

		HEP		LEP		Interval [1]	
Oats	kg/day	2.7		1.8		2.7	
Pelleted diet	kg/day	3.2		1.8		3.2	
Alfalfa hay	kg/day	1.0		1.0		1.0	
Soybean meal	kg/day	1.5					
Timothy hay	kg/day	5.7		5.7		5.7	
Dry matter of pasture [2]	kg/day	4.9		5.0		5.0	
Mineral supplement	kg/day	0.1		0.1		0.1	
Digestible energy intake	Mcal/day	38.2		29.6		33.5	
The ratio to the requirement [3]	%	120.2		93.1		105.3	
Crude protein intake	g/day	2,231		1,213		1,493	
The ratio to the requirement [3]	%	160.0		87.0		106.8	
Glutamic acid+glutamine	mg/day (TAA [4] %)	324.7	(16.24)	147.8	(13.88)	189.5	(14.81)
Proline	mg/day (TAA %)	267.9	(13.40)	161.7	(15.18)	189.4	(14.80)
Methionine	mg/day (TAA %)	28.3	(1.42)	16.2	(1.52)	20.3	(1.59)
Isoleucine	mg/day (TAA %)	87.5	(4.38)	45.6	(4.28)	53.9	(4.21)
Lysine	mg/day (TAA %)	113.2	(5.66)	58.3	(5.47)	68.1	(5.32)
Glycine	mg/day (TAA %)	99.8	(4.99)	56.4	(5.30)	67.7	(5.29)
Serine	mg/day (TAA %)	78.8	(3.94)	41.3	(3.88)	50.2	(3.92)
Threonine	mg/day (TAA %)	77.5	(3.88)	43.9	(4.12)	51.7	(4.04)
Alanine	mg/day (TAA %)	111.9	(5.60)	66.0	(6.20)	78.6	(6.14)
Aspartic acid+asparagine	mg/day (TAA %)	210.8	(10.55)	113.2	(10.63)	131.1	(10.25)
Phenylalanine	mg/day (TAA %)	100	(5.00)	54.7	(5.14)	65.0	(5.08)
Arginine	mg/day (TAA %)	119.2	(5.96)	56.2	(5.28)	68.7	(5.37)
Leucine	mg/day (TAA %)	155.7	(7.79)	83.6	(7.85)	100.6	(7.86)
Histidine	mg/day (TAA %)	48.9	(2.45)	24.3	(2.28)	29.8	(2.33)
Tyrosine	mg/day (TAA %)	62.7	(3.14)	33.1	(3.11)	40.0	(3.13)
Valine	mg/day (TAA %)	111.9	(5.60)	62.8	(5.90)	74.8	(5.85)
TAA [4]	mg/day	1,998.8		1,065.1		1,279.4	

[1] Interval period (7 days) feeding medium-energy and medium-protein for diminishing previous feeding effect.
[2] The daily pasture intake during grazing was estimated in a previous study by the double-indicator method using chromium oxide and lignin.
[3] The percentage of intake relative to the requirement to National Research Council (1989).
[4] The sum of the amino acid concentrations measured in the present experiment.

Table 2. The mean concentrations of solids, fats, and amino acids (AA) during the high-energy and high-protein (HEP) feeding period and low-energy and low-protein (LEP) intake feeding period, and those changes on different days during each feeding period

		Overall [2]	P-value			Day on feed [1]						
			Diet	Days	Diet·days	1	2	3	4	5	6	7
%												
Solid	HEP	11.17	NS	NS	NS	11.16	11.43	11.31	11.01	11.04	11.18	10.99
	LEP	11.07				11.09	11.16	10.94	10.94	10.91	11.09	11.61
SEM		0.06				0.14	0.13	0.10	0.14	0.16	0.20	0.18
Fat	HEP	1.79	NS	NS	NS	1.75	2.01	1.78	1.74	1.89	1.75	1.57
	LEP	1.92				1.90	1.93	1.72	1.96	1.81	2.07	2.30
SEM		0.04				0.07	0.13	0.09	0.12	0.14	0.17	0.17
mg/100 g												
Glutamic acid+glutamine	HEP	337.76	*	NS	NS	338.45	329.64	334.89	335.44	323.84	336.43	344.31
	LEP	312.11				346.8[a]	334.16[a]	330.11[a]	302.96[ab]	301.49[ab]	321.87[a]	264.58[b]
SEM		4.32				11.95	8.54	12.37	14.01	6.92	8.59$	16.09$
Proline	HEP	207.31	NS	NS	NS	186.87	177.42	188.6	183.78	205.83	224.35	241.03
	LEP	208.20				231.71	228.99	230.53	201.33	207.53	224.84	171.03
SEM		6.18				17.63	18.18	19.76	17.31	11.42	12.51	18.05$
Methionine	HEP	30.57	***	NS	NS	31.62	31.44	31.39	29.26	28.75	29.85	31.28
	LEP	28.18				31.01	29.74	28.99	26.65	27.26	29.16	24.74
SEM		0.42				1.16	0.89	1.23	1.11	0.63	0.93	1.56
Isoleucine	HEP	79.27	*	NS	NS	80.44	77.9	79.15	77.35	76.68	79.38	80.27
	LEP	73.17				81.05	78.33	77.04	71.53	70.6	74.26	62.16
SEM		1.05				2.78	1.87	3.09	3.43	1.74	2.28	3.79
Lysine	HEP	121.60	*	NS	NS	122.16	119.29	121.33	120.43	117.36	121.05	123.03
	LEP	112.54				124.57	120.71	118.78	109.37	109.02	114.99	95.23
SEM		1.56				4.27	2.99	4.43	5.08	2.49	3.19	5.82
Glycine	HEP	28.03	***	NS	NS	28.33	27.85	28.25	27.56	27.62	28.13	27.9
	LEP	25.86				28.41	27.87	27.18	25.02	25.17	25.41	22.16
SEM		0.34				0.9	0.57	0.94	1.1	0.55	0.8	1.32
Serine	HEP	67.81	*	NS	*	64.9	68.25	68.77	70.3	61.06	67	71.3
	LEP	63.12				69.78[ab]	71.99[a]	67.91[ab]	58.17[bc]	59.73[abc]	66.3[ab]	49.44[c]
SEM		1.18				2.79	3.05	3.28	3.57	2.05	2.79$	4.05$
Threonine	HEP	58.29	*	NS	*	57.61	58.42	58.76	58.77	54.6	57.48	59.96
	LEP	53.93				59.78[a]	59.6[a]	57.09[ab]	51.54[bc]	51.58[bc]	55.04[ab]	44.33[c]
SEM		0.78				1.91	1.72	2.17	2.5	1.01	1.7$	2.97$
Alanine	HEP	55.68	**	NS	NS	56.16	54.28	55.83	54.1	54.28	55.72	56.73
	LEP	52.08				57.63	56.11	54.85	50.52	50.38	52.77	44.13
SEM		0.71				1.97	1.48	1.99	2.23	0.96	1.54	2.65$
Aspartic acid+asparagine	HEP	146.35	*	NS	NS	147.36	144.55	145.88	145.15	142.17	145.3	147.37
	LEP	134.77				148.63	144.62	142.34	131.78	130.6	135.92	114.32
SEM		1.83				4.93	3.39	5.17	5.93	2.74	3.88	6.97
Phenylalanine	HEP	69.81	*	NS	NS	70.14	68.32	69.52	68.74	67.22	69.66	71.13
	LEP	64.54				71.34	69.09	68.32	62.78	62.45	66.13	54.72
SEM		0.89				2.43	1.75	2.56	2.91	1.4	1.82	3.35
Arginine	HEP	90.94	*	NS	NS	91.66	89.22	90.41	89.67	86.74	90.48	93.14
	LEP	84.45				94.01[a]	91.15[a]	89.08[a]	82[ab]	81.25[ab]	86.87[a]	70.69[b]
SEM		1.20				3.19	2.45	3.41	3.80	1.82	2.57$	4.51$
Leucine	HEP	153.77	*	NS	NS	154.31	149.75	153.87	151.68	147.35	153.87	157.06
	LEP	142.78				156.91	153.03	151.3	139.27	137.86	146.89	120.56
SEM		2.02				5.24	4.05	5.81	6.6	3.18	4.32	7.51
Histidine	HEP	41.02	*	NS	NS	41.14	40.06	40.75	40.91	39.2	40.96	41.71
	LEP	38.15				42.26	40.87	40.37	36.9	36.8	39.36	32.32
SEM		0.54				1.43	1.02	1.56	1.82	0.96	1.11	1.98
Tyrosine	HEP	58.64	*	NS	*	58.76	59.43	57.06	58.78	56.07	57.26	60.34
	LEP	53.39				59.9	58.47	56.31	51.08	51.54	54.66	43.74
SEM		0.77				2.14	1.46	2.09	2.33	1.1	1.56	3.21
Valine	HEP	98.06	NS	NS	NS	99.83	95.36	96.97	95.03	94.81	98.28	100.47
	LEP	95.04				106.73[a]	102.3[ab]	102.03[ab]	90.98[bc]	90.49[bc]	95.72[ab]	80.39[c]
SEM		1.32				3.99	2.28	4.07	4.17	2.05	2.52	4.43$
Total of amino acids (TAA)	HEP	1,664.90	NS	NS	NS	1,629.74	1,591.16	1,621.44	1,606.96	1,583.58	1,655.20	1,707.03
	LEP	1,542.3				1,710.54[a]	1,667.03[a]	1,642.23[a]	1,491.88[ab]	1,493.74[ab]	1,590.2[a]	1,294.55[b]
SEM		22.8				62.69	51.91	68.81	71.75	32.31	44.48$	83.68$

*** p<0.001, ** p<0.01 * p<0.05 from difference between HEP and LEP.

[a,b,c] Values with different characters in the same rows are significantly different. (p<0.05). $. Significant different between HEP and LEP in the same columns for the amino acid of the row (p<0.05)

[1] The day from the beginning of study feed ingeste [2] Averaged values during each feeding period (7 days)

glycine, serine, threonine, alanine, aspartic acid+asparagine, arginine, leucine, histidine, tyrosine and valine concentrations of the samples were analyzed using a high-performance amino acid analyzer (L-8800, Hitachi High-Technologies Co., Tokyo) (Le Boucher et al., 1997). After another session of hydrolysis, the methionine and phenylalanine concentrations were also analyzed using a high-performance amino acid analyzer (Penke et al., 1974). The milk lipid level was analyzed with a Gerber butyrometer (H-155, Kokusan Co., Tokyo) by the Gerber method. The concentrations of these milk components were compared between milk samples obtained during the HEP and LEP feeding periods.

Statistical analysis

The differences in the parameters were examined by repeated-measures ANOVA using the JMP software (SAS Institute Inc, 2002). The linear model included the effect of diet, the effect of repeated measurements over different days, the effect of different sampling times during the same day, and interactions among these factors. Since there were no significant effects of the sampling times and the interactions among the factors, the data were pooled and the effects of diet and the day of measurement were re-analyzed. When the effect of the interaction between diet and the day of measurement was significant, the effect of the day of measurement within the same dietary phase and the effect of diet within the same day were analyzed. Significant differences were analyzed by the paired t-test.

RESULTS

There were no significant differences in the average concentrations of the total solids or fat in the milk samples obtained from the mares during the HEP and LEP feeding phases (Table 2). However, the concentrations of all the amino acids, except those of proline, serine and valine, were significantly higher during the HEP than during the LEP feeding phase ($p<0.05$). The sum of the amino acid concentrations measured in the milk samples in the present experiment (TAA) was not significantly different between the HEP and LEP feeding phases. However, significant differences due to interactions between the diet and the days of measurement were observed for glutamic acid+glutamine, proline, serine, threonine, arginine, valine and TAA ($p<0.05$). Changes in the concentrations of the amino acids during the one-week study period for each diet (day 1 to 7) are shown in Table 2. The concentrations of glutamic acid+glutamine, serine, threonine, arginine, valine and TAA were significantly lower ($p<0.05$) on day 7 than on day 1 during the LEP feeding period. Furthermore, the concentrations of glutamic acid+glutamine, serine, threonine, alanine and arginine on day 7 were significantly lower ($p<0.05$) in the LEP feeding phase than in the HEP feeding phase. To examine the changes in the amino acid composition of the milk protein, the percent concentration of each amino acid relative to the TAA was analyzed (Table 3). While the percent concentrations of glutamic acid+glutamine, methionine, isoleucine, lysine, glycine, threonine, alanine, aspartic acid+asparagine, phenylalanine, arginine and leucine were significantly higher during the HEP than during the LEP feeding period ($p<0.05$), the percent levels of proline and valine were significantly higher during the LEP feeding period than during the HEP feeding period ($p<0.05$). On the other hand, it was found that the effects of interactions between the diet and the day of measurement were significant for glutamic acid+glutamine, proline, methionine, lysine, aspartic acid+asparagine, phenylalanine, arginine, leucine and histidine ($p<0.05$).

DISCUSSION

In the present study, administration of a high-energy and high-protein diet was not associated with any significant increase in the amino acid concentrations in maternal milk. In contrast to our results, Gibbs et al. (1982) reported that the protein content of milk increased following administration of a high-protein diet and Bovera et al. (2002) reported that protein concentration of milk was

Table 3. The mean ratios of the amino acid concentrations to the sum of the total amino acid concentration measured in the present experiment (TAA) through during the high-energy and high-protein (HEP) intake phase, and low-energy and low-protein intake phase

		Diet		Pooled SEM	P value		
		HEP	LEP		Diet	Days	Diet×days
Glutamic acid+glutamine/TAA[1]	%	20.59	20.27	0.060	**	NS	*
Proline/TAA	%	12.33	13.33	0.269	**	*	*
Methionine/TAA	%	1.86	1.84	0.012	**	NS	**
Isoleucine/TAA	%	4.83	4.76	0.022	*	NS	NS
Lysine/TAA	%	7.41	7.31	0.025	**	NS	*
Glycine/TAA	%	1.72	1.69	0.014	*	NS	NS
Serine/TAA	%	4.16	4.07	0.051	**	*	NS
Threonine/TAA	%	3.56	3.50	0.019	NS	NS	NS
Alanine/TAA	%	3.39	3.39	0.015	NS	NS	NS
Aspartic acid+asparagine/TAA	%	8.93	8.77	0.039	**	NS	*
Phenylalanine/TAA	%	4.25	4.19	0.012	**	NS	**
Arginine/TAA	%	5.54	5.48	0.015	**	**	*
Leucine/TAA	%	9.37	9.27	0.027	**	NS	**
Histidine/TAA	%	2.50	2.48	0.009	*	NS	*
Tyrosine/TAA	%	3.58	3.46	0.018	***	**	NS
Valine/TAA	%	5.97	6.19	0.040	*	NS	NS

*** $p<0.001$, ** $p<0.01$, * $p<0.05$ from difference between HEP and LEP.

[1] The sum of amino acids concentrations that were measured in the present experiment.

increased in buffalo fed an excessive energy and protein diet. During the HEP feeding period, it was not clear that the absence of any significant change in the milk amino acid concentrations was because of the absence of effects of both high energy and high protein levels, or was because of these levels counteracting each other's influence. On the other hand, administration of a low-energy and low-protein diet was associated with reduced concentrations of various amino acids. In this connection, Sutton et al. (1977) reported that the colostrum of pregnant dairy cows ingesting 85% of the NRC requirement of energy contained smaller amounts of protein than that of animals ingesting 100% of the energy requirement. In this study, differences in the milk amino acid concentrations between the LEP and HEP phases began to be noted on day 5 or 6 of the respective study phases, and the difference became statistically significant on day 7 ($p<0.05$). This result suggests that low-energy and low-protein intake levels by the horses begin to be reflected in the amino acid composition of the milk about 6 days later. Dietary protein restriction in rats was associated with reduced milk protein concentration on days 2, 4 and 12 of lactation as compared to the corresponding concentrations in rats ingesting a high-protein diet (Pine et al., 1994). Thus it was found that the restricted intake of energy and protein was associated with reduced concentrations of amino acids in mare's milk, and the increased amino acid concentrations were not associated with high energy and protein intake by the mares, at least over the short term.

The mean levels of each of amino acids relative to TAA over the 7-day study period differed significantly between the two dietary phases. It was suggested that this disparity was because of changing in the percent level of amino acids concentrations relative to the TAA during the first half of the HEP feeding period. Proline was the amino acid contained in the second largest amount among the milk amino acids, therefore, the percent levels relative to the TAA of the other amino acids which are present in smaller amounts were relatively increased during the HEP feeding period than during the LEP feeding period during the first half of treatment. This smaller percent level of proline relative to the TAA in the feed of the HEP than those of the LEP might be the cause of the decreased proline concentrations in the maternal milk. The quality (in terms of the amino acid content) of the milk protein in the diet was found to influence the amino acid content in cow's milk (Baker et al., 1995).

Based on these results, we may say that a decrease in the dietary intake level of energy and protein by lactating mares was associated with a decrease in the amino acid concentrations of the maternal milk, and that these effects appear 6 or more days after the commencement of such a diet.

We hypothesized that an increase in the dietary energy and protein intake by the lactating mares may be associated with an increase in the amino acid concentrations in the maternal milk. We also wanted to verify whether improvement of the nutritive value of maternal milk was possible when it was low because of advanced age. However, we could not confirm that ingestion of a high-protein and high-energy diet leads to high amino acid concentrations in the maternal milk. On the other hand, a decrease in the dietary protein intake by a lactating mare to below the daily requirement was associated with a reduction in the concentrations of amino acids in milk, which are of great nutritive importance in developing foals. Therefore, the importance of administration of appropriate amounts of protein to lactating horses cannot be overemphasized.

The nutritional composition of mare's milk might be influenced by lactating stage, parity, age and individual difference and so on, as well as diet (Doreau et al., 1989). Our objective was to determine whether ingested energy and protein had an influence on amino acid concentrations of milk, but other influences should have to be eliminated as far as possible. So, ingested energy and protein levels were switched for brief times on the same individual during the same lactation stage and the milk sampled at short intervals in order to be observed in detail. It was thought that there was no influence of lactation stage in this trial from the report that most of the changes of milk nutrient components were not seen from postpartum seven weeks to the ninth weeks (Oftedal et al., 1983). Degradation of concentrations of amino acids in milk was observed in LEP, however the period of study of dietary energy and protein level in this trial might be too short to decided the degree of influence of ingested nutritional level on amino acids concentrations in milk. Therefore, this study was a preliminary trial to propose appropriate energy and protein level to be ingested to lactating mare in order to produce milk containing appropriate amino acids.

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