

Chapter 6. Dietary Carbohydrates and Fat Influence Composition and Fatty Acid Profile of Mares' Milk

ABSTRACT

Equine milk composition reflects the mare's nutritional status and affects the foal. Our objective was to compare milk concentrations of protein, immunoglobulin G, fat, fatty acids, lactose, and solids-not-fat in milk of mares fed pasture and contrasting concentrates. Throughout gestation and lactation, grazing mares were supplemented with a corn and molasses concentrate (SS), or a corn oil and fiber supplement (FF). The concentrates were isocaloric and isonitrogenous, with mineral contents balanced with pasture to meet or exceed current recommendations. Milk was sampled at 6–12 h and 24–48 h after parturition, and at 1, 2, 4, and 6 mo of lactation. The fatty acid profile of milk fat was analyzed by gas chromatography, immunoglobulin G concentration by radial immunodiffusion. In colostrum sampled at 6 to 12 h after foaling, there was higher protein, immunoglobulin G, and solids, lower lactose, and a tendency for higher fat in the milk of FF mares, as compared with SS mares. The fatty acid profile indicated higher linoleic, trans-vaccenic and homo- γ -linolenic acids, but lower linolenic, palmitoleic and margaroleic acids in the FF milk. The presence of trans-vaccenic acid in milk indicates hydrogenation prior to absorption, hence suggests fermentation in the small intestine. Dietary linoleic acid in FF mares' milk may reduce the risk of gastric ulcers in foals. Increased immunoglobulin G content of the FF mares' milk may

enhance passive immunity. Supplementation of corn oil and fiber in the pregnant mare's diet may improve health of foals.

Key Words: Mare, Milk, Fatty Acid, Immunoglobulin, Foal Health

Introduction

Milk production and composition, including fatty acids, amino acids and vitamins may change in response to effects of lactational stage, age, parity, and nutrition (Doreau et al. 1992, Csapó et al. 1995). Milk composition of mares fed a diet rich in forage (95% hay, 5% concentrates), compared with milk from mares fed a diet rich in concentrate (50% forage, 50% concentrate), had higher milk fat and protein, higher linolenic acid, and lower linoleic acid concentrations (Doreau et al. 1992). Little or no hydrogenation of unsaturated fatty acids by intestinal microorganisms is expected before absorption in hind gut fermenting animals, therefore milk long chain fatty acid composition should be directly related to fatty acid content of dietary fats in these species.

In contrast, bovine milk contains primarily saturated fatty acids due to extensive hydrogenation of dietary fatty acids by ruminal microbes prior to absorption (Sutton and Morant 1989). The extent of hydrogenation is influenced by the roughage content of the ration. Low roughage diets tended to reduce the proportions of saturated fatty acids and increased proportions of oleic, linoleic and linolenic acids in bovine milk (Sutton and Morant 1989).

Our objective was to compare concentrations of protein, immunoglobulin G, fat, fatty acids, lactose, and solids-not-fat in milk of mares fed pasture plus concentrates rich in either starch and sugar or fat and fiber, which have been found previously to influence growth (Hoffman et al., 1996).

Materials and Methods

Twenty Thoroughbred mares were maintained on mixed bluegrass and clover pastures at the Virginia Tech MARE Center. Mares were paired by age, breeding date and sire, and were then assigned randomly to two groups. Throughout gestation and lactation, ten mares were fed a corn and molasses concentrate rich in starch and sugar (SS), and ten were fed a corn oil and fiber (beet pulp, soy hulls, oat straw) supplement (FF). The concentrates were balanced to be isocaloric and isonitrogenous, with mineral contents balanced to complement the pastures and meet or exceed current recommendations (NRC 1989). Milk was sampled at 6 to 12 h and 24 to 48 h after parturition, and at 1, 2, 4, and 6 mo of lactation. One sample was analyzed by the Virginia Tech DHIA laboratory for milk protein, fat, lactose and solids. Milk fat was removed by centrifugation of a second sample, and the fatty acid profile, including 4:0, 6:0, 10:0, 12:0, 14:0, 14:1, 15:0, 15:1, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1cis, 18:1trans, 18:2(*n*-6), 18:3(*n*-3), 20:0, 20:3(*n*-6), was analyzed as described by Sukhija and Palmquist (1988) using gas chromatography (Hewett Packard 5890A Gas Chromatograph). Samples of colostrum were diluted with distilled deionized water (1:5 vol/vol, colostrum:water), and immunoglobulin G concentration determined using radial

immunodiffusion (VMRD, Inc., Pullman, WA). Data were summarized as least squares means and standard errors. Analysis of variance with repeated measures was used to evaluate effects of diets, sampling times and their interaction (SAS/STAT[®], SAS Institute, Cary, NC).

Results

The dietary composition of the supplements is summarized in Table 6.1, and milk composition is summarized in Table 6.2. In colostrum sampled at 6–12 h after foaling, there was higher protein ($P = 0.015$) and solids ($P = 0.013$), lower lactose ($P = 0.035$), and a tendency for higher fat ($P = 0.24$) in the milk of FF mares, as compared with the SS mares (Figure 6.1). Radial immunodiffusion indicated a 4.2 fold increase in the immunoglobulin G concentration of the FF mares' milk (Figure 6.2). Over time in both groups, milk fat and lactose increased, and milk protein decreased. Milk fatty acid composition is summarized in Tables 6.3 and 6.4. The fatty acid profile indicated higher linoleic ($P = 0.0001$, Figure 6.3), trans-vaccenic ($P = 0.005$), and homo- γ -linolenic ($P = 0.006$) acids, and lower α -linolenic ($P = 0.016$), palmitoleic ($P = 0.087$), and margaroleic ($P = 0.009$) acids in the FF mares' milk. Over time for both groups (Table 6.3), there was a decrease in decanoic (SS, $r = -.910$, $P = .007$; FF, $r = -.911$, $P = .083$), margaric (SS, $r = -.979$, $P = .0002$; FF, $r = -.950$, $P = .002$), and stearic acids (SS, $r = -.921$, $P = .005$; FF, $r = .891$, $P = .011$) and an increase in myristoleic (SS, $r =$

.897, $P = .010$; FF, $r = .911$, $P = .007$) and palmitoleic acids (SS, $r = .889$, $P = .012$; FF, $r = .893$, $P = .011$).

Discussion

The supplementation of corn oil in the FF mares' diet progressively increased milk contents of fat and linoleic acid. Previous reports indicated an increase in milk linoleic acid in fat-supplemented mares (Zeyner et al. 1996), whereas milk fat concentration decreased in fat supplemented cattle (Sutton and Morant 1989). Research with humans indicated a role of linoleic acid in the prevention of gastric ulcers. Linoleic acid is a precursor of prostaglandin E, and stimulation by linoleic acid increased gastroduodenal prostaglandin formation (Grant et al. 1988). Prostaglandins are involved in the prevention of gastric ulcers by enhancing mucosal protective factors. Foals typically have a high incidence of gastric ulcers, and prevention is important from both clinical and production standpoints (Murray et al., 1990). The increased linoleic acid content in the FF mares' milk may reduce the risk of gastric ulcers in foals. In addition, linoleic acid is easily converted to arachidonic acid, which may be oxygenated to form a variety of eicosanoid compounds. Eicosanoids function through G protein-linked receptors to elicit cellular effects on many systems, including immune function, blood pressure, muscle (Smith 1989).

The increase in trans-vaccenic acid in the FF mares' milk agrees with previous data from fat-supplemented mares (Zeyner et al. 1996). The presence of trans-fatty

acids in mares' milk indicates fatty acid hydrogenation by intestinal microorganisms prior to absorption.

The failure of passive transfer of colostral immunoglobulins in the newborn foal increases the risk of neonatal septicemias and bacterial infections (McClure 1993). Serum immunoglobulin concentration in foals has been directly related to colostral immunoglobulin concentration (LeBlanc et al. 1986). In addition, vitamin E has been implicated in the stimulation of serum antibody synthesis, particularly immunoglobulin G antibodies (Hidiroglou et al. 1992). The high vitamin E content of the corn oil may have stimulated antibody synthesis in the FF supplemented mares. It should be noted that colostral immunoglobulin concentrations in the mare decrease rapidly within the first 24 h after parturition, and the wide window of sampling time in this study (6 to 12 h after foaling) may have confounded the results. As found here, the increased immunoglobulin G concentration of the FF mares' milk would enhance passive immunity.

Implications

Use of corn oil and fiber as an energy sources of concentrate fed to mares supplementary to pasture influenced milk composition in ways likely to improve foal health. Enhanced linoleic acid content of mares' milk may reduce the risk of gastric ulcers in foals. Increased immunoglobulin G content of colostrum from mares fed corn oil may enhance passive immunity.

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Table 6.1. Nutrient profile supplements and pasture fed to mares during gestation and lactation summarized on a dry matter basis as means \pm SE for supplements and as a 90% confidence interval for pasture.

Nutrient	SS (n = 10)	FF (n = 15)	Pastures (n = 38)
DM, %	90.0 \pm 0.36 ^a	91.7 \pm 0.30 ^b	15.7 – 36.5
DE, MJ/kg	14.7 \pm 0.2 ^a	10.8 \pm 0.1 ^b	9.2 – 14.1
CP, %	15.0 \pm 0.6	14.6 \pm 0.5	16.5 – 29.5
ADF, %	9.1 \pm 0.8 ^a	28.3 \pm 0.64 ^b	20.3 – 35.6
NDF, %	15.3 \pm 1.2 ^a	41.2 \pm 1.0 ^b	36.5 – 60.0
Fat, %	2.4 \pm 0.7 ^a	10.4 \pm 0.6 ^b	2.7 – 5.6
NSC, %	62.4 \pm 0.8 ^a	26.5 \pm 0.7 ^b	6.1 – 23.9
Ash, %	4.9 \pm 0.3 ^a	7.2 \pm 0.3 ^b	7.35 – 12.82
Ca, %	0.77 \pm 0.08 ^c	1.01 \pm 0.07 ^d	0.36 – 1.04
P, %	0.45 \pm 0.03	0.48 \pm 0.03	0.26 – 0.46
Mg, %	0.17 \pm 0.01 ^a	0.23 \pm 0.01 ^b	0.18 – 0.27
K, %	0.98 \pm 0.04 ^a	1.30 \pm 0.04 ^b	1.93 – 3.72
Na, %	.20 \pm .02	0.20 \pm 0.01	0 – 0.035
S, %	0.17 \pm 0.01 ^a	0.20 \pm 0.01 ^b	0.16 – 0.39
Fe, mg/kg	191 \pm 31 ^a	485 \pm 26 ^b	0 – 1845
Zn, mg/kg	93.7 \pm 7.1	101 \pm 6.0	16.8 – 60.0
Cu, mg/kg	41.4 \pm 5.2 ^c	65.2 \pm 4.4 ^d	0 – 63.2
Mn, mg/kg	35.6 \pm 3.0 ^a	59.2 \pm 2.5 ^b	51.8 – 124.6
Mo, mg/kg	1.59 \pm 0.12 ^c	1.98 \pm .10 ^d	0.8 – 3.1
Se, mg/kg	0.6 ^e	0.6 ^e	< 0.08
I, mg/kg	0.6 ^e	0.6 ^e	< 0.08

^{a,b}Values with subscripts c,d are different (P < 0.001).

^{c,d}Values with subscripts e,f are different (P < 0.05)

^e Calculated using NRC (1989) tables.

Table 6.2. Contents of milk fat, protein, lactose and solids of mares in response to fat and fiber supplementation. Data (percentages) are summarized as means and SEM.

	24-48 h		1 mo		2 mo		4 mo		6 mo		SEM
	SS	FF	SS	FF	SS	FF	SS	FF	SS	FF	
Fat	2.07	2.15	2.08	2.22	2.22	2.27	2.61	2.61	1.80	2.69	0.16
Protein	3.11	3.54	2.23	2.21	1.82	1.76	1.80	1.77	1.67	1.65	0.15
Lactose	5.95	5.76	6.52	6.52	6.78	6.75	6.72	6.69	6.99	6.80	0.07
Solids not Fat	9.81	9.23	9.50	9.47	9.34	9.26	9.27	9.21	9.43	9.20	0.20

Table 6.3. Composition of fatty acids in mares' milk not influenced ($P > .10$) by fat and fiber supplementation. Values represent each fatty acid as a percentage of total milk fatty acids, means and SEM. Analysis of variance with repeated measures (SAS, 1988) was used to evaluate differences in response to diet.

FA		6–12 h	24–48 h	1 mo	2 mo	4 mo	6 mo	SEM
C6:0	SS	.05	.12	.25	.25	.21	.17	.02
	FF	0	.07	.25	.28	.17	.15	
C8:0	SS	2.23	2.26	2.63	2.59	2.23	2.22	.14
	FF	2.45	1.85	2.64	2.40	1.82	1.92	
C10:0	SS	6.33	5.22	5.87	5.01	4.08	3.72	.30
	FF	7.50	4.45	5.53	4.34	3.29	3.47	
C12:0	SS	6.65	5.84	6.51	5.79	5.05	5.49	.31
	FF	8.45	4.88	6.19	4.69	4.07	4.55	
C14:0	SS	6.23	6.24	6.37	5.71	5.35	5.95	.24
	FF	7.43	5.57	6.08	4.79	4.62	4.93	
C14:1	SS	.30	.50	.49	.58	.68	1.60	.07
	FF	.24	.32	.52	.46	.56	.72	
C15:0	SS	.26	.27	.23	.23	.28	.35	.02
	FF	.19	.27	.24	.28	.30	.21	
C15:1	SS	0	.02	.02	.05	0	0	.01
	FF	0	.02	.05	.11	0	0	
C16:0	SS	20.22	22.24	19.61	19.31	19.21	19.27	.37
	FF	20.40	22.66	19.31	17.62	18.72	17.72	
C17:0	SS	.27	.26	.22	.17	.15	.04	.02
	FF	.22	.28	.20	.18	.15	.03	
C18:0	SS	2.41	2.03	1.70	1.29	1.04	.85	.06
	FF	2.41	2.22	1.53	1.16	1.03	.80	
C18:1c	SS	17.55	23.05	19.40	19.13	23.43	21.53	.66
	FF	22.35	23.74	17.71	18.59	23.28	21.51	
C20:0	SS	.02	0	0	.15	0	0	.01
	FF	0	0	.02	.05	0	0	

Table 6.4. Composition of fatty acids in mares' milk influenced ($P < .10$) by fat and fiber supplementation. Values represent each fatty acid as a percentage of total milk fatty acids, means and SEM. Analysis of variance with repeated measures (SAS, 1988) was used to evaluate differences in response to diet noted by P .

FA		6–12 h	24–48 h	1 mo	2 mo	4 mo	6 mo	SEM	P
C16:1	SS	3.28	5.10	4.72	5.26	6.18	6.94	.18	.087
	FF	2.94	4.23	4.68	4.77	5.55	6.07		
C17:1	SS	.37	.52	.51	.52	.63	.56	.03	.009
	FF	.09	.38	.49	.50	.61	.49		
C18:1t	SS	.05	.04	.03	.16	.10	.11	.05	.005
	FF	.97	.57	.08	.16	.28	.31		
C18:2 (n-6)	SS	10.35	10.86	8.34	7.87	11.21	7.36	.49	.0001
	FF	18.22	16.68	11.77	13.37	19.58	17.07		
C18:3 (n-3)	SS	20.57	13.49	20.87	22.91	18.36	22.07	.94	.016
	FF	4.97	10.25	20.58	23.91	14.57	18.50		
C20:3 (n-6)	SS	.50	.39	.33	.48	.34	.35	.05	.006
	FF	.81	.60	.54	.57	.51	.38		