Temporal folate status during lactation in mares and growth in foals

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Objective—To identify changes in folate status of mares and foals during lactation and growth, respectively.

Animals—20 Thoroughbred mares and foals.

Procedures—Pregnant mares, and following foaling the same mares with their foals, were maintained on mixed grass-legume pasture and fed either a traditional dietary supplement rich in sugar and starch (SS) or a dietary supplement high in fat and fiber (FF). Blood samples were collected monthly from mares and foals up to 6 months after foaling. Total folate concentration in feed and forage was determined. Analyses of plasma folate, RBC folate, plasma homocysteine (HCY), and milk folate concentrations were performed.

Results—Mare plasma folate concentrations declined moderately during 6 months of lactation. Mare RBC folate concentrations initially increased after foaling up to 3 months but declined toward the end of the study. Plasma HCY concentration was higher for mares fed the SS supplement, compared with mares fed the FF supplement from foaling to 6 months of lactation. Milk folate concentrations decreased during the first 3 months and then increased. Foal plasma folate initially declined but then increased. Stable concentrations of RBC folate were observed in foals. Plasma HCY concentrations in foals were unaffected by growth during the last 5 months. References range values for plasma folate, RBC folate, milk folate, and plasma HCY concentrations in healthy lactational mares and young growing foals were determined.

Conclusions and Clinical Relevance—Folate status was not impaired in lactating mares and growing foals under the conditions in our study. It appears that folate supplementation is not necessary. (*Am J Vet Res* 2005;66:1214–1221)

Folate is a B vitamin required during lactation and growth because of its function as a 1-carbon donor in DNA, RNA, and protein biosynthesis.[1] Impaired folate status has been shown to cause megaloblastic anemia in pregnant and lactating women,[2] congenital birth defects in infants,[3,4] and hyperhomocysteinemia, an independent risk factor for heart disease in adults.[5] In horses, low serum folate concentrations have been observed in mares maintained on pasture in their first month of lactation[6] and in mares receiving antifolate drugs orally during pregnancy, which resulted in clinical disorders in foals.[7,8] In addition, a significant decline in plasma folate and increase in plasma **homocysteine (HCY)** occurred in geldings receiving antifolate drugs orally despite coadministration of natural and synthetic folate sources.[a]

Daily oral supplementation of synthetic folic acid has been shown to alleviate signs of folate deficiency during pregnancy and lactation in humans.[9,10] No such studies have been conducted in lactating mares and young growing foals, and it remains unclear whether folate supplementation is necessary. Therefore, the main objectives of this study were to assess the effects of lactation and growth on broodmares and foals, measure indicators of folate status in lactating mares and growing foals, and evaluate the use of plasma HCY as an indicator of folate status in horses. This study was conducted in coordination along with a companion study examining the effects of a high-fat and -fiber dietary supplement on growth in Thoroughbred mares and foals.[11] Because the dietary supplements were fed to provide similar amounts of nutrients, it was not an objective to test for differences in folate status between the dietary groups.

Materials and Methods

Animal and diets—Twenty Thoroughbred mares (mean ± SE, 609.9 ± 16.2 kg; 12.9 ± 1.1 years old) from the Virginia Tech Middleburg Agricultural Research and Extension Center were used. Mares were bred during May and June to 1 of 4 Thoroughbred stallions. Following pregnancy detection, mares were paired by age and expected foaling date and randomly assigned to 1 of 2 groups. Both groups were maintained on similar but separate mixed orchard grass-bluegrass and white clover pasture and were offered either a dietary supplement rich in **sugar and starch (SS)**, similar in nutrient composition to commercially available products, or a dietary supplement higher in **fat and fiber (FF)**. Ingredient and nutrient compositions of the supplements are provided (**Appendix 1**).[b] Synthetic folic acid was excluded from the vitamin premix so that the folate sources were only those contained inherently in the forage and supplement. Mixed orchard grass-legume hay was also fed during the winter months when pasture was scarce. Mares were group fed their

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supplements twice daily and fed amounts to meet half of the daily digestible energy requirements based on the National Research Council's recommendations,[12] with the remaining requirement met by pasture and hay consumption. Foaling occurred between April and May. Within 1 week of foaling, mares and foals were placed back on pasture with the other mares and foals from their experimental group.

Blood and milk sample collection—Samples of blood and milk were collected to assess folate status by measuring RBC, plasma, and milk folate concentrations and plasma HCY concentrations. Sample collection was conducted from 8:00 AM to 11:00 AM every month beginning at foaling and ending at weaning when foals were approximately 6 months of age. Whole blood (10 mL) was collected from the jugular vein into EDTA collection tubes.[c] Milk (13 mL) was collected manually into plastic bags,[d] filtered through a 4 × 4-inch gauze pad to remove foreign particles, and transferred to a light-protective polypropylene vial with sodium ascorbate[e] added to achieve a final concentration of 1.0% sodium ascorbate (wt/vol). For processing of RBCs, 100 µL of whole blood was removed from blood collection tubes and transferred to 2-mL polypropylene tubes[f] containing 1 mL of 0.3% (wt/vol) sodium ascorbate dissolved in deionized water. The remaining whole blood sample was then centrifuged at 2,000 × *g* for 5 minutes. Plasma was transferred into 2-mL polypropylene vials, and sodium ascorbate was added to a final concentration of 0.3% (wt/vol) sodium ascorbate. All samples were kept out of direct light to prevent photooxidation of folate and were stored at –80°C until analyzed.

Supplement and forage sample collection—Pasture samples (1 to 2 kg wet weight) were obtained monthly by clipping forage by use of hand-held electric clippers that had a 10.2-cm-wide edge. Samples were collected at random stops in each pasture housing the horses. Hay samples (0.8 kg wet weight) were obtained by core sample collection.[13] Samples of each supplement (0.5 kg wet weight) were taken by random grab samples from 5 bags. Samples of pasture, hay, and supplement were weighed, dried in a forced-air oven, and then weighed again for **dry matter (DM)** determination. Forage and supplement samples were then ground through a 0.5-mm screen mill[g] prior to analysis of total folate concentration as determined by microbial assay.[h]

Plasma, RBC, and milk folate concentrations—After validation for use in horses, samples of plasma, RBC, and milk were analyzed for total folate concentration by **radioimmunoassay (RIA)**.[i] Validation of the RIA for horse plasma was conducted by assessing recovery of known amounts of added 5-methyltetrahydrofolate[j] to plasma and by dilution parallelism (1:1, 1:2, 1:4, and 1:6; **Appendix 2**).[i] All plasma samples were diluted 1:4 prior to analysis and run in duplicate. For the determination of RBC folate concentration, whole blood was diluted 1:11 in a 1% (wt/vol) sodium ascorbate solution immediately following sample collection as per kit directions. However, analysis of RBC folate at that dilution yielded concentrations of folate that were between 2 and 6 times lower than published concentrations in horses.[14] Further testing of the commercial kit revealed that the highest yield of folate was obtained by diluting the samples 1:50 with distilled water prior to analysis. The amount of sodium hydroxide-potassium cyanide provided in the commercial assay was increased in the unknown samples from 50 to 125 µL/sample with additional sodium hydroxide-potassium cyanide for a final dilution of 1:51.25. The latter step was found to increase the yield of folate, most likely by increasing the denaturation of intracellular folate-binding proteins in the RBC. Plasma concentration of folate was subtracted from the concentration of whole-blood folate for each horse during each month to estimate RBC folate concentration.

Highest concentrations of milk folate were obtained by use of a 1:30 dilution of milk prior to analysis. Use of trienzyme treatment with α-amylase, protease, and rat serum folate conjugase, which has been shown to be successful at increasing extraction of folate from foods[15] and human milk,[16,17] was assessed in the present study. To remove endogenous folate from pooled rat serum, 5.0 g of activated acid-washed charcoal[e] was mixed with 50 mL of pooled rat serum for 2 hours, and centrifuged at 50,000 × *g* for 2 hours and then the mixture was filtered through a 0.45-µm-diameter filter[k] to remove remaining charcoal. Milk samples (1:30 dilution; 0.3 mL) were incubated with 0.1 mL of folate conjugase for 3 hours; 0.2 mL of α-amylase[e] (10 mg/mL) for 1, 4, or 8 hours; or 0.1 mL of protease[e] (4 mg/mL) for 4, 8, or 12 hours. Total milk folate concentration was increased only after incubation of milk with 0.2 mL of α-amylase (10 mg/mL) for 1 and 4 hours (**Figure 1**). Following these preliminary tests, folate concentration in experimental milk was assessed by use of an RIA with 0.5 mL of milk (1:30 dilution) that was mixed with 0.3 mL of α-amylase and incubated at 37°C for 2 hours prior to analysis.

Plasma HCY concentration—Plasma HCY concentration was determined with a precolumn derivatization procedure followed by reverse-phase separation and fluorescence detection by use of **high-pressure liquid chromatography (HPLC)**. The precolumn derivatization and HPLC separation materials were purchased as a kit,[l] and methods provided in the kit were followed. The HPLC system[m] was equipped with a thermostat-controlled autosampler, quaternary pump with degasser, fluorescence detector, and reverse-phase analytic column (70 × 3.2 mm [internal diameter]) fitted with a microguard.[l] Briefly, 50 µL of plasma, 100 µL of internal standard, 50 µL of reduction reagent ($NaBH_4$), and 100 µL of derivatization reagent (ammonium 7-fluorobenzo-2-oxa1,3-diazole-4-sulphonate solution) were added together, mixed thoroughly, and incubated for 5 minutes at 50°C. After cooling the mixture at 4°C for 5 minutes, the proteins were precipitated by adding 100 µL of precipitation reagent (trichloroacetic acid), mixing thoroughly, and centrifuging the proteins into a pellet at 10,000 × *g* for 5 minutes. The supernatant was transferred to screw-cap vials and placed in the autosampler. Twenty microliters of plasma was injected onto the heated (45°C) column at a flow rate of 0.7 mL/min with the fluorescence detector at a wavelength of 385 nm for excitation and 515 nm for emission. The plasma concentration of HCY was quantified from its retention time relative to known standards, whereas peak areas were converted to plasma concentration of HCY by reference to the internal standard.

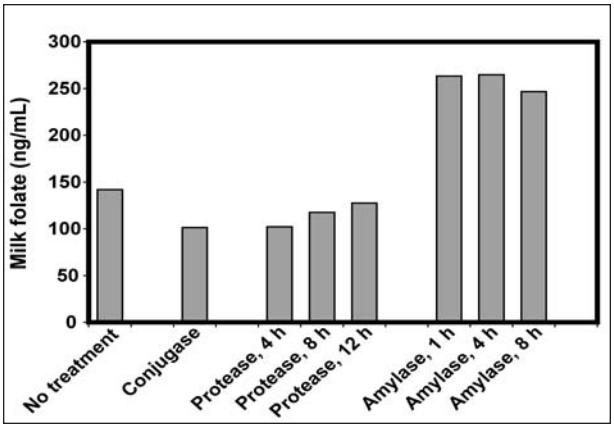

Figure 1—Total folate concentration in mare's milk with no treatment or after incubation with rat serum conjugase for 3 hours; protease for 4, 8, or 12 hours; or α-amylase for 1, 4, and 8 hours.

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Table 1—Mean body weight of mares and foals and average daily gain (ADG) of foals fed either sugar and starch (SS) or fiber and fat (FF) supplements.

Group	Foaling	Month 1	2	3	4	5	6	SE
Mare								
SS(kg)	598.5[a]	616.9[b]	624.1[b]	617.7[b]	620.7[b]	624.2[b]	633.1[c]	16.2
FF (kg)	580.9[a]	592.0[b]	597.8[b]	602.9[b]	604.3[b]	611.2[b]	621.9[c]	16.2
Foal								
SS (kg)	58.6[a]	100.6[b]	142.2[c]	177.3[d]	217.0[e]	249.6[f]	279.0[g]	3.9
FF (kg)	60.8[a]	99.2[b]	140.7[c]	182.4[d]	219.5[e]	252.3[f]	275.6[g]	3.9
ADG								
SS (kg)	NA	1.8[a]	1.3[b]	1.2[b,c]	1.2[b,c]	1.1[c]	0.9[d]	0.04
FF (kg)	NA	1.6[a]	1.4[b]	1.3[b,c]	1.2[b,c]	1.0[c]	0.7[d]	0.04

[a-g] Within a row, means with unlike superscript differ significantly ($P < 0.05$).
NA = Not Applicable.

Statistical analyses—A software program[n] was used for data tabulation and statistical analyses. Diet (SS or FF supplements), time (month of lactation or growth), and their interaction were evaluated by use of an ANOVA with repeated measures with a mixed procedure and repeated statement. The Tukey test was used to determine differences between months for the variables investigated. Relationships among dependent variables were evaluated by use of regression analyses.[o] Data are presented as means ± SE, except for reference values that are 95% confidence intervals. Values of $P < 0.05$ were considered significant.

Results

Body weight and average daily gain—Mean body weight of mares and mean body weight and **average daily gain (ADG)** of foals during the 6-month experimental period were measured **(Table 1)**. No differences in body weight of mares and body weight and ADG of foals were observed between dietary groups. Body weight of mares was significantly affected by month of lactation with the lowest body weight observed at foaling and highest body weight observed at 6 months of lactation. Likewise, body weight of foals was significantly affected by month of growth with the highest ADG of foals occurring at 1 month followed by a gradual decline in ADG of foals up to 6 months of age.

Folate in feeds—Total folate concentrations in SS, FF, and orchard grass-alfalfa hay were 1.6 ± 0.4 mg/kg, 1.9 ± 0.9 mg/kg, and 1.4 ± 0.2 mg/kg of DM, respectively. Total folate concentration in the bluegrass-white clover mixed pasture changed with time **(Figure 2)**. Pasture folate concentration peaked in April (4.48 ± 0.73 mg/kg of DM) and October (4.88 ± 0.66 mg/kg of DM), with the lowest values occurring in March (1.65 ± 0.2 mg/kg of DM) and June (2.2 ± 0.4 mg/kg of DM). Total folate intake per day for mares **(Figure 3)** was calculated from folate concentrations in feed and forage, body weight (Table 1), and estimated intake on a body weight basis. Daily folate intake of foals was not shown because forage intake could not be accurately estimated; therefore, its relative contribution is unknown.

Mare folate status—A significant main effect caused by month of lactation was found on plasma folate concentrations in mares **(Figure 4; Table 2)**. Plasma folate concentrations in all mares were significantly highest at foaling with the lowest values observed at 3 months of lactation in mares fed the FF supplement and at 4 months of lactation in mares fed the SS supplement. Plasma folate concentrations in mares fed the SS supplement during the last 3 months

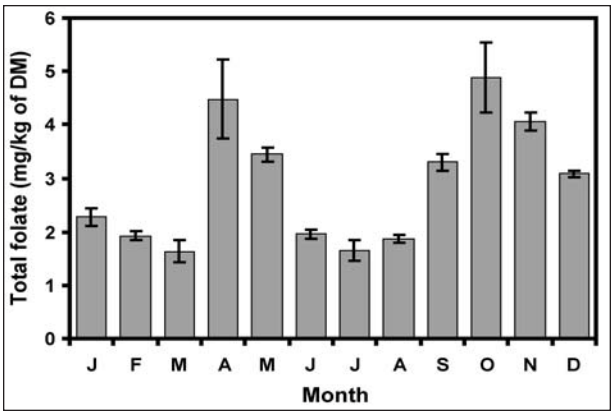

Figure 2—Mean ± SE monthly (January [J] through December [D]) total folate concentrations (mg/kg of dry matter [DM]) in bluegrass-white clover mixed pasture as determined by microbial assay.

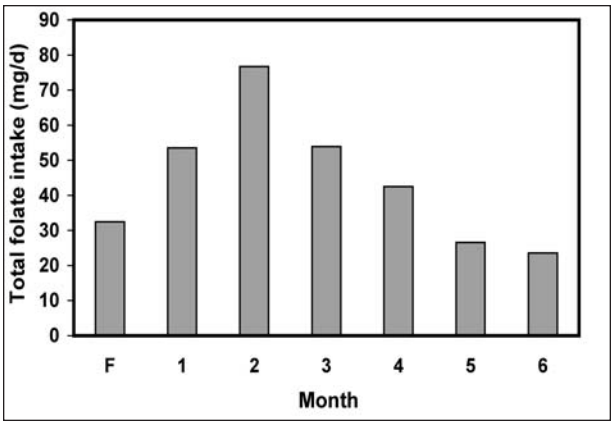

Figure 3—Calculated total folate intake (mg/d) of mares from foaling (F) through 6 months of lactation.

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Table 2—Reference ranges for indices of folate status for lactational mares and growing foals.

Item	Mare		Foal	
	Mean	95% CI	Mean	95% CI
Plasma folate (ng/mL)	18.2	17.6–18.8	16.2	15.0–17.4
RBC folate (ng/mL)	711.2	694.6–727.8	742.7	727.6–757.8
Milk folate (ng/mL)	182.9	178.1–187.6	NA	NA
Plasma HCY (μmol/L)	5.7	5.4–6.0	5.7	5.1–6.4

CI = Confidence interval. HCY = Homocysteine. NA = Not Applicable.

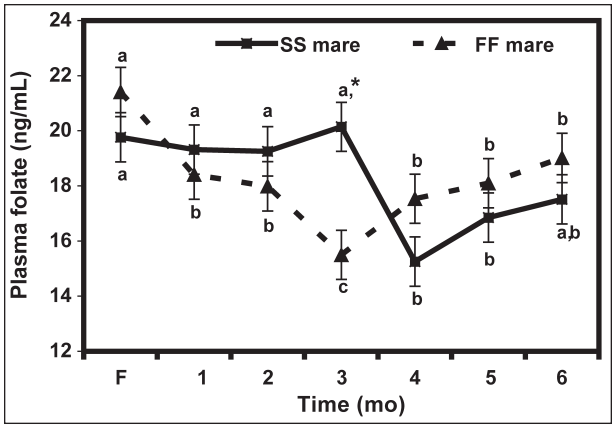

Figure 4—Mean ± SE plasma folate concentrations in mares fed sugar and starch (SS) or fiber and fat (FF) supplements from F through 6 months of lactation. [a-c]Means with unlike superscripts differ significantly ($P < 0.05$) within treatment groups. *Significantly ($P < 0.05$) higher plasma folate concentrations in mares fed the SS supplement, compared with mares fed the FF supplement.

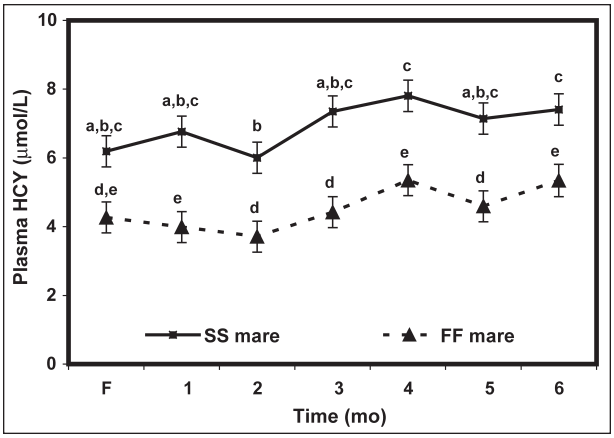

Figure 6—Mean ± SE plasma homocysteine (HCY) concentrations in mares fed SS or FF supplements from F through 6 months of lactation. Mares fed SS had significantly ($P < 0.05$) higher concentrations of plasma HCY, compared with mares fed the FF supplement. [a-e]Means with unlike superscripts differ significantly ($P < 0.05$) within and between treatment groups.

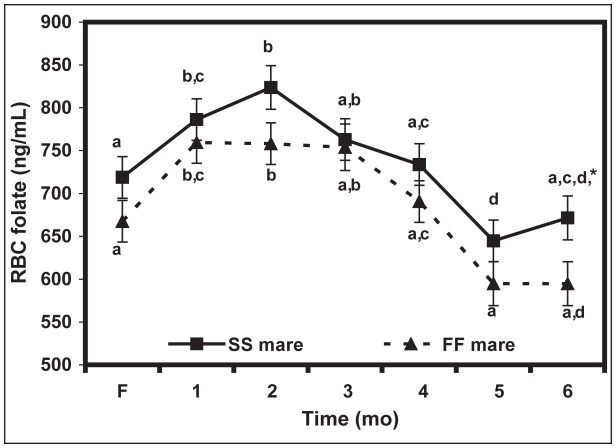

Figure 5—Mean ± SE RBC folate concentrations in mares fed SS or FF supplements from F through 6 months of lactation. *Significantly ($P < 0.05$) higher RBC folate concentration in mares fed the SS supplement, compared with mares fed the FF supplement. *See* Figure 4 for remainder of key.

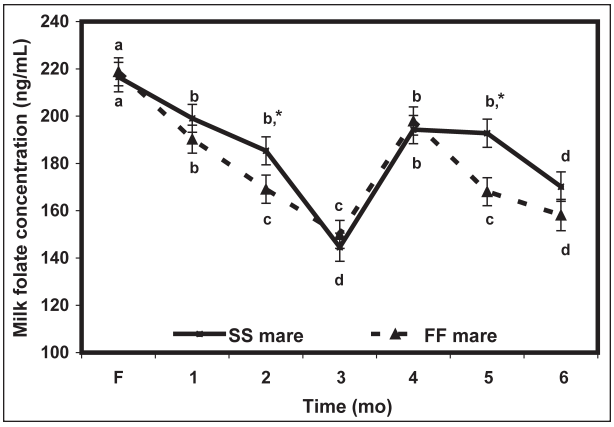

Figure 7—Mean ± SE total milk folate concentrations in mares fed SS or FF supplements from F through 6 months of lactation. *Milk folate concentrations significantly ($P < 0.05$) higher in mares fed the SS supplement, compared with mares fed the FF supplement. *See* Figure 4 for remainder of key.

of lactation were significantly lower than during the first 3 months of lactation. No significant ($r = 0.50$; $P = 0.24$) correlation was found between monthly mare plasma folate concentrations and pasture folate concentrations. No main effect of diet on mare plasma folate concentrations was found during lactation.

Month of lactation influenced RBC folate concentrations in both groups of mares (**Figure 5**). Red blood cell folate concentrations significantly increased after foaling until 3 months of lactation in both groups of mares. After 3 months of lactation, RBC folate concentrations significantly declined, resulting in lowest concentrations observed between 5 and 6 months in mares fed the FF or SS supplements. No significant correlation ($r = 0.17$; $P = 0.70$) was found in mares between monthly plasma folate and RBC folate concentrations. A significant ($r = –0.62$; $P = 0.13$) correlation was not

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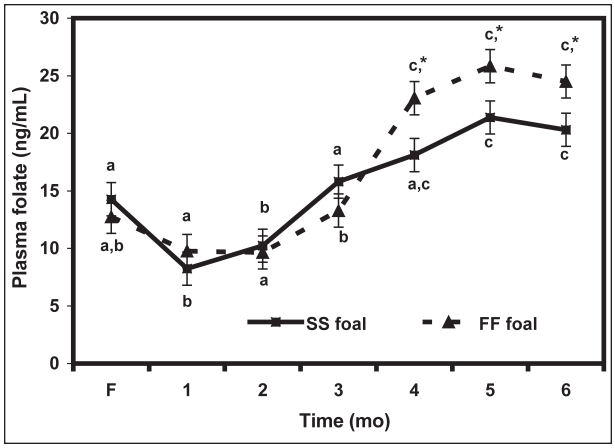

Figure 8—Mean ± SE plasma folate concentration in foals of mares fed SS or FF supplements from F to 6 months of age. *Plasma folate concentrations significantly ($P < 0.05$) higher in foals of mares fed the FF supplement, compared with foals of mares fed the SS supplement. *See* Figure 4 for remainder of key.

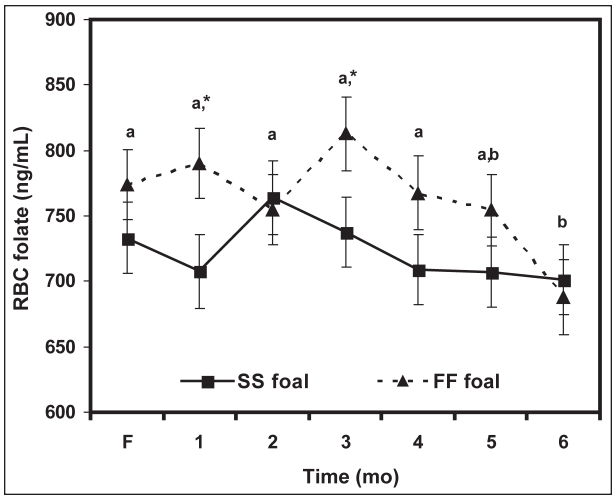

Figure 9—Mean ± SE RBC folate concentrations in foals of mares fed SS or FF supplements from F to 6 months of age. *Plasma HCY concentration significantly ($P < 0.05$) higher in foals of mares fed the FF supplement, compared with foals of mares fed the SS supplement. *See* Figure 4 for remainder of key.

found between monthly mare RBC folate concentration and pasture folate concentration. A significant main effect of diet on mare RBC folate concentration was found, indicating that mares fed the SS supplement had higher RBC folate concentrations, compared with mares fed the FF supplement.

Plasma HCY concentrations increased significantly from 6.2 ± 0.56 µmol/L at foaling to 7.4 ± 0.60 µmol/L after 6 months of lactation in mares fed the SS supplement, whereas plasma HCY concentrations in mares fed the FF supplement remained stable after foaling (**Figure 6**). No significant correlation was observed in mares between monthly plasma HCY concentration and plasma folate concentration ($r = –0.71$; $P = 0.07$) or RBC folate concentration ($r = –0.61$; $P = 0.14$). Plasma HCY concentrations were significantly higher throughout lactation in mares fed the FF supplement, compared with mares fed the SS supplement.

A significant main effect of month of lactation on

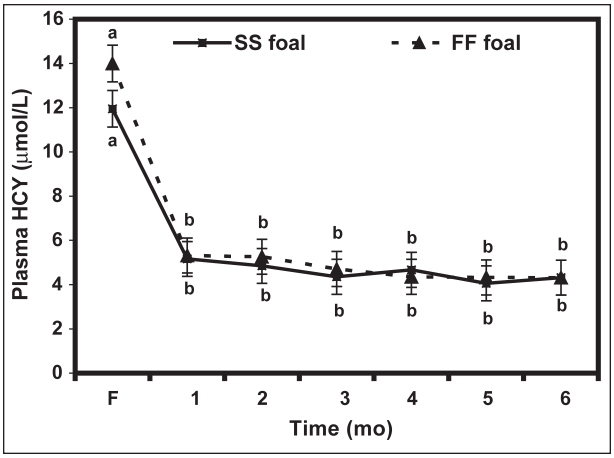

Figure 10—Mean ± SE plasma HCY concentrations in foals of mares fed SS or FF supplements from F to 6 months of age. *See* Figure 4 for remainder of key.

milk folate concentrations was found in mares fed the FF or SS supplements (**Figure 7**). A higher milk folate concentration was found in mares fed the SS supplement, compared with mares fed the FF supplement, but this difference was not significant ($P = 0.076$). Milk folate concentration for all mares was significantly higher at foaling than at any other time (217.8 ± 12.4 ng/mL). In both groups of mares, milk folate concentrations significantly declined during the initial 3 months of lactation followed by a significant increase at 4 months of lactation and then another significant decline by 6 months of lactation. No significant relationship was observed between monthly milk folate concentration and mare plasma folate ($r = 0.54$; $P = 0.20$), mare RBC folate ($r = –0.05$; $P = 0.89$), or pasture folate ($r = 0.35$; $P = 0.43$) concentrations.

Foal folate status—Plasma folate concentrations were significantly higher in foals of mares fed the FF supplement, compared with foals of mares fed the SS supplement, at 4, 5, and 6 months of age (**Figure 8**; Table 2). A significant rapid decline in plasma folate concentration was found from birth to 1 month of age in foals of mares fed the FF or SS supplements. A significant gradual increase in plasma folate concentration was found between 4 to 6 months of age in foals of mares fed the FF or SS supplements. No significant correlations were observed between monthly concentration of foal plasma folate and pasture folate ($r = 0.22$; $P = 0.62$) or milk folate ($r = –0.18$; $P = 0.69$) concentrations. No main effect of diet on foal plasma folate concentrations was found.

No significant differences in monthly concentrations of RBC folate in foals of mares fed the SS supplement during the experimental period were found (**Figure 9**). However, foals of mares fed the FF supplement experienced a significant decline in RBC folate concentration at 6 months of age. No significant correlations were observed between monthly concentration of foal RBC folate and foal plasma folate ($r = –0.72$; $P = 0.06$), pasture folate ($r = –0.63$; $P = 0.12$), or milk folate ($r = 0.02$; $P = 0.96$) concentrations. At 1 and 3 months of age, significantly higher concentrations of RBC folate were observed in foals of mares fed the FF sup-

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plement, compared with foals of mares fed the SS supplement.

Plasma folate concentrations of all foals were significantly highest at birth (13.35 ± 1.9 Ng/mL), compared with all other months. Plasma folate concentrations remained stable in all foals from 2 to 6 months with a mean concentration of 4.7 ± 0.09 Ng/mL. No significant correlation was observed between monthly concentration of foal plasma HCY concentration and foal plasma (r = –0.30; P = 0.50) or foal RBC folate (r = 0.45; P = 0.59) concentrations. No differences were observed in foal plasma HCY concentrations between foals of mares fed the FF or SS supplements during the 6-month experimental period.

Discussion

To our knowledge, this is the first published study that monitored temporal folate status of lactating mares and young growing foals. Assessment of folate status of mares and foals was primarily determined on the basis of RBC folate and plasma folate concentrations as direct measures of folate status with consideration of plasma HCY concentration as an indirect indicator of folate status. Red blood cell folate is less sensitive to dietary folate intake and short-term fluctuations in folate status, compared with plasma folate. Therefore, RBC folate concentrations are more reflective of the body stores of folate at the time of RBC synthesis and should be used as a measure of folate status.[1,18]

Homocysteine is a nonessential sulfur containing amino acid whose metabolism is catalyzed by folate-, B_{12}-, and B_6-dependent enzymes.[5] Therefore, a deficiency in 1 or more of these B vitamins may negatively impact HCY metabolism. Homocysteine metabolism involves the conversion of HCY to methionine via methionine synthase, an enzyme that requires 5-methyltetrahydrofolate as a methyl donor and vitamin B_{12} as a cofactor. When concentrations of 5-methyltetrahydrofolate are limited, intracellular HCY becomes concentrated and leaks out into the plasma, resulting in high plasma concentrations of HCY or hyperhomocysteinemia. Plasma HCY concentration is negatively correlated with dietary folate intake and plasma folate concentration in humans, allowing its plasma concentration to be used as an indicator of folate status.[5,19]

The decline in folate concentration in plasma and milk in mares shortly after foaling, which was accompanied by a decline in pasture folate concentration, indicates a decrease in maternal folate reserves occurred during early lactation. Although a decline in mare RBC folate concentration was not observed during early lactation, it is likely that the decline in RBC folate concentration in mares during late lactation reflected a lowered folate status caused by the progress of lactation. This can be attributed to the fact that if the folate status of mares declined during early lactation, less folate would have been available during erythropoiesis. Because of the lowered folate status of mares, we expected to see a reciprocal increase in plasma HCY concentration. However, plasma HCY concentration in mares did not increase significantly over the experimental period, indicating that folate status was most likely not compromised sufficiently to impair HCY metabolism, causing hyperhomocysteinemia. Although a decline in folate status occurred in mares during early lactation, it was not sufficient to cause hyperhomocysteinemia associated with a folate deficiency that would ultimately require supplementation. In the absence of a parallel control group of nonpregnant mares, it is not possible to attribute the changes in folate status in mares during the lactational period as a result of only lactation because time and season may have also been factors.

Previous studies[6,14,20] of folate status in reproducing mares have evaluated plasma or RBC folate concentrations at 1 or more specific points in the reproductive cycle rather than over an extended period. Lactating mares generally had higher concentrations of plasma folate and RBC folate concentrations than in previous reports of pregnant mares[14,20] and grazing mares in their first month of lactation.[6,14] The higher concentrations of plasma and RBC folate concentrations of mares observed in our study may have been partially caused by a higher quality pasture diet accompanied by a customized balanced pasture supplement and improved analytical methods. The grass-legume pasture and pasture supplements fed to mares throughout pregnancy and lactation inherently contained folate at a concentration of 1.6 to 1.9 mg/kg, which is relatively high, compared with other feedstuff.[12] Because folate is labile under boiling conditions, we did not use an RIA that did not require a boiling step (as that of older RIA kits).[14]

Milk folate concentrations averaged 182.9 ng/mL during lactation, which is considerably higher than the concentration of 1.4 ng/mL observed previously.[22] Incubation with α-amylase and the use of the no-boil RIA kit to evaluate milk folate concentrations resulted in a 54% increase in the apparent milk folate concentrations over our untreated samples. Adding protease or rat serum conjugase did not significantly increase the recovery of milk folate of mares, as has been shown in human milk.[15]

A rapid decline was found in plasma folate concentrations in foals immediately following foaling, which coincided with a period of high ADG and decline in pasture folate concentration. Like pasture folate concentrations, plasma folate concentrations in foals increased during the last 3 months of the study to concentrations higher than those observed at foaling. Unlike plasma folate, concentration of RBC folate remained relatively stable in foals over the 6-month observational period. In addition, plasma HCY concentrations were stable during the experimental period. The abnormally high concentrations of plasma HCY observed at foaling were more likely a product of hemolysis of the fragile foal RBCs rather than a product of impaired folate status. The fact that RBC folate and plasma HCY concentrations remained stable in foals indicates that the concentration of folate provided in the milk of mares and the consumption of forage and feed by the foal in the later stages of growth were sufficient to maintain adequate folate stores in foals during the early growth period. In addition, the lowered folate status of mares observed during early lactation did not significantly impact the folate status of foals during that period.

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The main effects of diet on folate status were noted in the plasma HCY concentrations of mares. Mares fed the SS supplement had higher concentrations of RBC folate, compared with mares fed the FF supplement, and mares fed the SS supplement also had higher concentrations of plasma HCY. This finding is difficult to explain because it would be expected that the higher folate status observed in the mares fed the SS supplement would translate to lower plasma HCY concentrations. It is likely that the differences in plasma HCY concentrations were less the result of changes in folate status and more related to B_{12} or B_6 status because those vitamins also affect HCY metabolism. The added dietary fiber in mares fed the FF supplement may have resulted in an increased microbial synthesis, which may have contributed to a greater utilization of microbially derived B vitamins. If this were the case, a higher B_{12} or B_6 status in mares fed the FF supplement would result in lowered plasma HCY concentrations. More research is needed in this area to clearly identify whether a high supplement increases microbial synthesis of B vitamins.

Mares and foals maintained on quality grass-legume pasture and fed dietary supplements similar to that used in our study do not appear to require additional folate supplementation to maintain folate status during lactation and growth. The reference values for the indices of folate status established in our study may be useful when assessing folate status in horses.

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b. DHI Forage Testing Laboratory, Ithaca, NY.
c. Vaccutainer, Becton Dickinson & Co, Franklin Lakes, NJ.
d. Whirl-Pak bags, Nasco, Fort Atkinson, Wis.
e. Sigma Chemical Co, St Louis, Mo.
f. Sarstedt, Newton, NC.
g. Wiley Mill Model 4, Thomas Scientific, Swedesboro, NJ.
h. Ralston Analytical Laboratories, St Louis, Mo.
i. Folate dualcount solid phase no-boil assay, Diagnostic Products Corp, Los Angeles, Calif.
j. Schirks Laboratories, Jona, Switzerland.
k. Millipore Corp, Bedford, Mass.
l. Bio-Rad Laboratories, Hercules, Calif.
m. 1100 series LC 3D ChemStation, Agilent Technologies, Wilmington, Del.
n. SAS for Windows, SAS Institute Inc, Cary, NC.
o. SlideWrite for Windows, Advanced Graphics Software, Encinitas, Calif.

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Appendices appear on the next page

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Appendix1—Ingredient and nutrient composition of sugar and starch (SS) and fiber and fat (FF) dietary supplements.*

Item	SS (n = 6)	FF (6)
Ingredient (%)	—	
Dent yellow grain corn	60.0	1.5
Soybean meal 48%	15.5	2.0
Oat straw	7.0	7.0
Alfalfa	NA	13.5
Soybean hulls	4.0	4.0
Beet pulp	NA	10.0
Cereal by-product	NA	56.0
Cane molasses	10.0	5.0
Dicalcium phosphate	1.5	NA
Limestone	1.0	NA
Mineral premix†	0.5	0.5
Vitamin premix‡	0.5	0.5
Mean ± SE nutrient composition		
DM (%)	95.8 ± 0.4	97.9 ± 0.69
DE (Mcal/kg)	3.7 ± 0.05	3.0 ± 0.00
CP (%)	15.8 ± 1.1	13.9 ± 0.22
ADF (%)	7.4 ± 0.95	22.3 ± 0.90
NDF (%)	15.8 ± 1.4	31.4 ± 0.68
Fat (%)	2.8 ± 0.18	10.2 ± 1.0
NSC (%)	59.4 ± 3.1	34.1 ± 1.9
Ash (%)	6.2 ± 0.75	11.7 ± 0.14
Ca (%)	0.86 ± 0.14	2.3 ± 0.03
P (%)	0.63 ± 0.08	0.93 ± 0.08
Mg (%)	0.20 ± 0.02	0.57 ± 0.05
K (%)	1.0 ± 0.08	1.2 ± 0.01
Na (%)	0.21 ± 0.03	0.27 ± 0.02
S (%)	0.20 ± 0.01	0.19 ± 0.01
Cl (%)	0.86 ± 0.40	0.50 ± 0.03

*Analyzed[b] on a dry-matter (DM) basis. †The mineral premix provided the following per ton of supplement: NaCl, 3,774 g; Zn, 422 g; Fe, 208 g; Cu, 89.5 g; Mn, 50.3 g; Se, 1.095 g; and KI, 0.415 g. ‡The vitamin premix provided the following per ton of a supplement: vitamin A, 1,380,080 IU; vitamin D3, 258,000 IU; vitamin E, 26,455 IU; riboflavin, 701 mg; niacin, 3,009 mg; thiamin, 1,400 mg; biotin, 42 mg; and β-carotene, 3,527 mg.

DE = Digestible energy. CP = Crude protein. ADF = Acid detergent fiber. NDF = Neutral detergent fiber. NSC = Nonstructural carbohydrates.

NA = Not Applicable.

Appendix 2—Recovery of plasma folate after spiking and dilution parallelism of radioimmunoassay.[h]

Procedure	Observed	n	Expected	Observed/ expected (%)
Dilution factor				
1:1	8.93	5	NA	NA
1:2	5.03	5	4.47	112
1:4	2.55	5	2.23	114
1:6	1.60	5	1.49	107
5-mTHF added (ng/mL)				
0	8.93	5	NA	NA
1.5	5.85	5	5.97	98
3	7.29	5	7.47	98
6	9.58	5	10.47	91
12	13.02	5	16.47	79

5-mTHF = 5-methyltetrahydrofolate. NA = Not Applicable.

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