SHORT COMMUNICATION

Response of serum concentrations of folates to dietary supplements of folic acid given to ewes during gestation

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Girard, C. L., Castonguay, F. and Matte, J. J. 1999. **Response of serum concentrations of folates to dietary supplements of folic acid given to ewes during gestation.** Can. J. Anim. Sci. **79**: 387–389. Serum folates were determined during the 24 h following the ingestion of 0, 130, 260 or 520 mg of folic acid at 12, 61 and 131 d of gestation in 38 ewes. The response of serum folates increased with the dose ingested ($P < 0.0001$) but decreased as gestation progressed ($P < 0.005$).

Key words: Ewe, dietary supplements, folic acid, vitamin B_{12}

Girard, C. L., Castonguay, F. et Matte, J. J. 1999. **Effets d'un supplément alimentaire d'acide folique sur les concentrations sériques de folates chez la brebis gestante.** Can. J. Anim. Sci. **79**: 387–389. Les concentrations sériques de folates pendant les 24 h suivant l'ingestion de 0, 130, 260 ou 520 mg d'acide folique ont été mesurées chez 38 brebis primipares à 12, 61 et 131 jours de gestation. Les folates sériques ont augmenté avec la dose ingérée ($P < 0.0001$) mais la réponse a diminué au cours de la gestation ($P < 0.005$).

Mots clés: Brebis, suppléments alimentaires, acide folique, vitamine B_{12}

In sheep, serum concentrations of folates decrease between mating and 60 d of gestation and increase before lambing time (Girard et al. 1996), following a pattern similar to that observed in sows (Matte and Girard 1999). Moreover, the decrease in serum concentrations of folates observed between mating and 60 d of gestation is greater in prolific breeds (Girard et al. 1996). These results suggest that the demand for folic acid might be increased during gestation in ewes.

The objective of the present experiment was to determine if dietary supplements of folic acid given to ewes at different stages of gestation would induce an increase in serum concentrations of folates.

Experimental Procedures

Primiparous ½ Suffolk – ½ Romanov ewes from the herd of the Agriculture and Agri-Food Canada Sheep Research Farm at La Pocatière were used. Ewes were cared for according to the guidelines of the Conseil canadien de protection des animaux (1993). At approximately 10 mo of age, estrus was synchronized by insertion of a vaginal sponge (Véramix, Upjohn, Orangeville, ON). At sponge removal (14 d later), each ewe received an i.m. injection of 400 IU of pregnant mare's serum gonadotrophin (Équinex, Laboratoires Ayerst, St-Laurent, QC) to stimulate follicular development. Fifty-five hours after sponge removal, ewes were inseminated with fresh semen from Hampshire sires from the Centre d'insémination ovine du Québec, La Pocatière, QC. Approximately 55 d after insemination, gestation was confirmed by echography. Only data from 38 pregnant ewes undergoing complete gestation are reported in the present paper.

The ewes were housed continuously indoors in pens of 8 to 10 animals each. During the experimental period, ewes had free access to a high-moisture grass silage (approximately 25% DM and 16% CP), fresh water and minerals. From 4 wk prior mating to 3 wk after mating, the ewes were also fed 400 g d^{-1} of a commercial concentrate (15% CP). Six weeks before lambing, this commercial concentrate was offered again, starting at 200 g d^{-1} per ewe. The quantity of concentrate was increased gradually to reach 600 g d^{-1} at lambing. Fresh feed was offered daily at the same hour. One week before blood sampling, the ewes were placed in individual stalls where they were kept until 24 h after the test-meal.

Tests were performed at 12, 61 and 131 d of gestation. These times were chosen according to Girard et al. (1996). In the morning of these days, one blood sample (T_0) was taken from each ewe before feeding the test meal to determine serum and red blood cell concentrations of folates, packed cell volume and serum concentration of vitamin B_{12}. Ewes were assigned to four different feeding treatments, 0, 130, 260 or 520 mg of folic acid (10% pteroylmonoglutamic acid and 90% wheat middlings, Hoffman-LaRoche, Cambridge, ON). Doses were calculated using a mean body

Abbreviation: **AUC**, area under the curve; **CP**, crude protein; **DM**, dry matter weight of 60 kg for ewes, according to data obtained on cattle (Girard et al. 1992). Supplementary folic acid was fed mixed with 100 g of the commercial concentrate. Each ewe received the same dose of folic acid at all three stages of gestation. Blood samples were taken 1, 2, 4, 6, 8 and 24 h after the test-meal for determination of serum concentrations of folates.

Blood samples were collected by jugular venipuncture with a Vacutainer® tube. Whole blood was collected in heparinized tubes. Packed cell volume was determined in duplicate on fresh blood for calculation of red cell concentrations of folates. Whole blood was then transferred into polypropylene tubes, and stored at –20°C until assayed. Blood collected for serum was collected in plain Vacutainer® tubes and allowed to clot in darkness for 24 h at 4°C. Serum was separated by centrifugation at 1854 × *g* for 10 min, transferred to polypropylene tubes, and stored at –20°C until assayed.

Procedures were previously described for determination of concentrations of folates in serum (Girard et al. 1996) and red cells (Lévesque et al. 1993) and for serum concentrations of vitamin B_{12} (Girard and Matte 1988). Serum and red cell folates, as well as serum vitamin B_{12}, were measured in duplicates in two different assays using commercial radioassay kits for human serum (Quantaphase Folate and Quantaphase vitamin B_{12}, Bio-Rad Laboratories (Canada) Ltd., Mississauga, ON) and validated for sheep. The interassay coefficients of variation were 3.36% (n = 798), 4.12% (n = 114), 4.31% (n = 114) for serum and red cell folates and serum vitamin B_{12}, respectively.

The number of lambs born was analyzed according to the following model :

$$Y_i = m + S_i + e_i,$$

where Y_i indicates the dependent variable, the number of lambs. The overall mean is m, S_i is the effect of the supplement of folic acid and e_i is the residual error.

Serum and red cell concentrations of folates and serum concentrations of vitamin B_{12} during gestation were analyzed using the General Linear Model procedure of the SAS Institute, Inc.(1985) according to the following model:

$$Y_i = m + T_i + e_i,$$

where Y_i indicates the dependent variable, folates or vitamin B_{12}. The overall mean is m, T_i is the effect of time of gestation and e_i is the residual error. The analysis of repeated measurements was done according to Rowell and Walters (1976). Residual effect of a meal supplemented with folic acid at one stage of gestation on the dependent variables at the following stage of gestation was tested at 61 and 131 d of gestation using the model described previously for analysis of the number of lambs born. There was no residual effect on the studied variables at 61 and 131 d of gestation (P > 0.5).

Responses of serum concentrations of folates to the ingestion of dietary supplements of folic acid were measured by the height of the post-ingestion peak and the area under the curve calculated using the trapezoidal summation method (Abramovitz and Slegun 1972). After a logarithmic transformation to obtain homogeneity of variance, data were analyzed according to the following model:

$$Y_{jk} = m + S_j + T_k + ST_{jk} + e_{jk}$$

where Y_{jk} indicates the dependent variable, S_j is the effect of dietary supplements of folic acid, T_k is the effect of stage of gestation and e_{jk} is the residual error. Linear, quadratic and cubic effects of supplements were calculated when the level of significance reached 95%. The analysis of repeated measurements was done according to Rowell and Walters (1976).

Results and Discussion

The number of lambs born per ewe was similar among the four doses of folic acid (mean = 1.8, SE = 0.2; P > 0.5). This was expected because only one meal supplemented with folic acid fed at three different times (12, 61 and 131 d of gestation) would be unlikely to alleviate the increased demand for folic acid during gestation.

The highest value of serum concentrations of folates was observed at 12 d of gestation (quadratic effect of stage of gestation, P = 0.001) (Table 1) whereas red blood cell concentrations of folates decreased linearly throughout gestation (linear effect, P = 0.0001). Changes in serum concentrations of folates during gestation were similar to those previously observed in ewes (Girard et al. 1996) and sows (Matte and Girard 1999). They could reflect an increased demand for folates by the embryos during this period of intensive and rapid cell division and tissue growth, likely at the expense of other folate pools as shown by the decrease of the incorporation of folates into red blood cells.

Serum concentrations of vitamin B_{12} increased progressively during gestation (quadratic effect, P = 0.03) (Table 1). These concentrations are greater than those reported for sheep (Ramos et al. 1994) but most studies published on sheep were conducted with a sub-optimal supply of cobalt. In the present experiment, calculated average daily intake of cobalt supplied by the mineral supplement ranged from 70 to 100 mg per ewe. Consequently, it would be unlikely that tissue utilization of folates was impaired by a lack of vitamin B_{12} (Bässler 1997).

Areas under the curve for serum concentrations of folates during the 24 h following the ingestion of different amounts of folic acid were used to estimate the absorption of dietary folic acid. The AUC increased with the dose of folic acid ingested (cubic effect, P = 0.0001) (Table 2) as was observed with ruminant calves (Girard et al. 1992). However, AUC decreased linearly as gestation progressed (linear, P = 0.005, and quadratic, P = 0.13, effects of time of gestation) (Table 2). There was no significant interaction between dose of folic acid and time of gestation (P = 0.27). The peak height in serum concentrations of folates increased with the dose of folic acid (cubic effect, P = 0.0001) but it was similar for all stages of gestation (P > 0.4). Consequently, the decreased response in serum concentrations of folates as gestation progressed is probably due to a

Table 1. Serum and red blood cell concentrations of folates and serum concentrations of vitamin B_{12} (T_0) at three different stages of gestation of primiparous ewes (mean ± SE ; *n* = 38)

	Gestation (d)		
	12	61	131
Serum folates (ng mL^{-1})[z]	1.48 ± 0.19	0.90 ± 0.03	0.98 ± 0.03
Red cell folates (ng mL^{-1})[y]	11.17 ± 1.45	6.48 ± 0.92	4.48 ± 0.60
Serum vitamin B_{12} (ng mL^{-1})[x]	2.44 ± 0.12	3.30 ± 0.08	3.87 ± 0.10

[z]Quadratic effect of time of gestation, $P = 0.001$.
[y]Linear effect of time of gestation, $P = 0.0001$.
[x]Quadratic effect of time of gestation, $P = 0.03$.

Table 2. Areas under the curve and peak height of serum concentrations of folates after ingestion of four different doses of folic acid given to primiparous ewes at three different stages of gestation

	Gestation (d)		
Supplementary folic acid (mg)	12	61	131
		AUC (ng mL^{-1} h ± SE)[z]	
0 (*n* = 8)	21.8 ± 1.1	20.7 ± 1.2	20.9 ± 1.1
130 (*n* = 9)	411.6 ± 1.1	361.4 ± 1.2	278.7 ± 1.1
260 (*n* = 9)	1085.7 ± 1.1	699.2 ± 1.2	837.1 ± 1.1
520 (*n* = 10)	2230.5 ± 1.1	1844.6 ± 1.1	1844.6 ± 1.1
		Peak height (ng mL^{-} ± SE)[y]	
0 (*n* = 8)	1.3 ± 1.1	1.2 ± 1.2	1.0 ± 1.1
130 (*n* = 9)	57.4 ± 1.1	53.5 ± 1.2	48.9 ± 1.1
260 (*n* = 9)	95.6 ± 1.1	83.9 ± 1.2	99.5 ± 1.1
520(*n* = 10)	188.6 ± 1.1	167.3 ± 1.1	194.4 ± 1.1

[z]Cubic effect of folic acid, $P = 0.0001$; linear ($P = 0.005$) and quadratic ($P = 0.13$) effects of time of gestation.
[y]Cubic effect of folic acid, $P = 0.0001$.

more rapid disappearance of folates from serum after the peak, indicating an increased uptake of folates by the tissues. This observation is in accordance with the decrease in serum and red cell concentrations of folates throughout gestation. Consequences of these changes in folate status throughout gestation need to be investigated taking into account the beneficial effect of this vitamin on survival rate of embryos and litter size in other polytocous species.

In conclusion, results from the present experiment demonstrate that dietary supplements of folic acid could be successfully used to increase serum concentrations of folates at different stages of gestation in ewes. Serum concentrations of folates increased rapidly in response to a single meal supplemented with folic acid, even at the smallest dose studied. Long-term effects of a folic acid supplementation on its absorption and tissue utilization remain to be studied as well as its effects on reproductive and lactational performance of ewes.

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