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Influence of folic acid supplements on the carry-over of folates from the sow to the piglet

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This experiment aimed to investigate the influence of folic acid supplements on the carry-over of folates from the sow to the fetus during late gestation and to the suckling piglet. Two groups of sixteen German Landrace sows received, during gestation and lactation, a diet supplemented with either 0 or 10 mg folic acid/kg. Increased folic acid concentrations in the serum of sows were detected only at the end of gestation (day 100) and at the end of lactation (day 28). The supplementation with folic acid to the sows' diet improved the folic acid supply of the fetus compared with unsupplemented controls; values were respectively 92·6 *v.* 56·2 nmol folates/l serum in newborn piglets and 171·9 *v.* 76·3 μmol folates/g fresh liver in stillborn piglets ($P < 0·05$). Folate concentrations in colostrum and milk (day 28) were 3·6- and 5·0-times higher in supplemented than unsupplemented sows. This treatment effect was also reflected in the serum of piglets until weaning. Therefore, the folic acid supply for the suckling piglet is dependent mainly upon the carry-over of maternal folates via colostrum and milk.

Folic acid: Sow: Piglet

Folic acid is of great importance for the synthesis of DNA and is involved in several reactions of the amino acid metabolism (Bässler, 1997). Therefore, active proliferative cells such as those from the placenta, embryo, fetus or the newborn increase the folic acid requirements of the dam.

Matte *et al.* (1993) and Duquette *et al.* (1997) showed that folates can cross the placental barrier in swine. Indeed, folic acid supplements to gilts tended to increase the fetal folates on day 50 of gestation (Matte *et al.* 1993) and the folate content in uterine secretions on day 12 of gestation (Duquette *et al.* 1997). The uterine transfer of folates in multiparous sows during early gestation was less apparent (Matte *et al.* 1996). In addition, little is known about the folate supply status of newborn piglets before the first ingestion of colostrum and whether it is possible to increase this status by supplementing the sow with folic acid during gestation. Furthermore, little is known about the folate content of colostrum and on the effect of dietary supply of folic acid during gestation on folate transfer to colostrum. Therefore, the aim of the present experiment was to evaluate the effect of dietary supplements of folic acid on the carry-over of folates to the fetus during gestation and to the suckling piglet during lactation.

Material and methods

Animals and treatments

A total of thirty-two German Landrace sows were randomly assigned to two different treatments (twelve reproductive cycles of gilts and thirty of multiparous sows were used in the experiment). At mating the average body weight of gilts was 123 kg and that of multiparous sows was 171 kg. The first group received a basal diet during gestation and lactation (described in Table 1) according to the recommendations of the German Society of Nutrition Physiology (1987). The second group was fed the basal diet supplemented with 10 mg folic acid/kg feed. All sows were mated with Pietrain boars. Pregnancy was verified by ultrasonic examination at day 30 post-mating. During gestation, all sows were kept in a group housing with individual feeding. At about day 110 of gestation the animals were transferred into nursing cages in which they stayed during the 28 d lactation. All stables were without bedding material. Immediately after birth all piglets were weighed individually. On day 2 of life they received an intramuscular injection of 200 mg Fe^{3+} as Fe dextran.

Gestating sows were fed 2·3 kg gestating diet (Table 1)

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Table 1. Composition of the gestation and lactation basal diet and of the diet for suckling pigs (Mean values and standard deviations)

	Gestation diet		Lactation diet		Diet for suckling pigs	
	Mean	SD	Mean	SD	Mean	SD
Ingredient (g/kg)						
Barley	573·0		330·0		440·0	
Wheat	—		332·5		280·0	
Oats	200·0		100·0		—	
Wheat bran	140·0		—		—	
Soyabean meal	50·0		180·0		190·0	
Soyabean oil	10·0		20·0		10·0	
Dicalcium phosphate	—		3·5		—	
Minerals and vitamins*	27·0		30·0		—	
Minerals and vitamins†	—		—		20·0	
Lysine monochloride	—		2·0		—	
Fishmeal	—		—		60·0	
Sodium chloride	—		2·0		—	
Analysed nutrients‡						
DM (g/kg)	884·1	7·3	883·4	6·3	873·2	6·4
Organic matter (g/kg DM)	942·0	1·4	936·5	2·1	940·9	1·5
Crude protein (g/kg DM)	146·3	5·2	189·6	3·6	230·9	6·0
Diethyl ether (g/kg DM)	32·8	3·7	33·6	5·0	32·2	4·2
Crude fibre (g/kg DM)	74·9	3·4	52·3	4·4	48·3	4·1
N-free extracts (g/kg DM)	688·0	5·5	661·0	8·5	629·5	5·6
Starch (g/kg DM)	441·3	12·6	466·4	7·6	449·6	10·3
Sugar (g/kg DM)	31·7	1·6	38·4	2·9	39·0	2·3
Metabolisable energy (MJ/kg DM)§	13·14		14·61		14·89	
Folic acid						
Calculated (mg/kg)‖	0·71		0·48		NA	
Analysed (mg/kg)¶	0·62	0·07	0·69	0·09	NA	
Ca	9·39	1·08	11·44	0·85	9·41	0·83
P	7·11	0·15	6·31	0·27	8·03	0·20

NA, not analysed.

* Composition (per kg): Ca 240 g, P 60 g, Na 55 g, Mg 10 g, Fe 5500 mg, Zn 4000 mg, Mn 2500 mg, Cu 950 mg, I 40 mg, Se 13 mg, vitamin A 120 mg, cholecalciferol 1 mg, vitamin E 1200 mg, menadione 40 mg, thiamin 40 mg, riboflavin 125 mg, vitamin B_6 80 mg, vitamin B_{12} 600 μg, pantothenic acid 245 mg, nicotinic acid 500 mg, choline chloride 2400 mg.

† Composition (per kg): Ca 220 g, P 80 g, Na 60 g, Mg 5 g, Fe 5300 mg, Zn 5000 mg, Mn 2400 mg, Cu 1000 mg, I 80 mg, Co 16·5 mg, Se 16·5 mg, vitamin A 180 mg, cholecaliferol 1·5 mg, vitamin E 1350 mg, menadione 40 mg, thiamin 60 mg, riboflavin 150 mg, vitamin B_6 100 mg, vitamin B_{12} 1200 μg, folic acid 15 mg, nicotinic acid 600 mg, panthotenic acid 250 mg, choline chloride 5000 mg.

‡ For gestation diet, lactation diet and diet for suckling pigs *n* 11, *n* 21 and *n* 4 respectively.

§ Bacterial fermentable substance, corrected metabolisable energy (German Society of Nutrition Physiology, 1987).

‖ According to the National Research Council (1998).

¶ For gestation diet and lactation diet *n* 15 and *n* 9, respectively.

from weaning until day 84 of gestation and 2·3 kg lactation diet from day 85 to 110 of gestation (Table 1) two times per d 07.00 and 14.00 hours six d per week and once per d on Sundays. Just before the expected farrowing date (3 d) the daily amount of feed was gradually reduced to 1·5 or 1 kg. After farrowing the amount of lactation diet provided to the sows per d was increased during the first 5 d of lactation to a total of 2·3 kg plus 0·3 kg for every piglet. Fresh water was provided *ad libitum*. The piglets received a diet for suckling pigs (Table 1) *ad libitum* (maximum consumption 5–7 g/piglet per d in the 4th week of the suckling period).

Folic acid supplements

Two different types of supplemented feed were prepared which were always used within 6 weeks after preparation. For the supplementation given during the period between weaning and day 84 of gestation, folic acid was mixed into the basal gestation diet to produce a premix which contained 230 mg folic acid/kg premix (analytical value of 181 (SD 39·2; *n* 12). The premix (100 g) was given as a 'top-dressing' to 2·2 kg of gestation diet for a daily allowance of 2·3 kg of feed per sow. The lactation diet was supplemented with folic acid to provide a level of 10 mg/kg feed.

Blood sampling

During gestation and lactation, blood samples were taken from sows at 07.00 hours immediately before feeding on day 30, 60 and 100 of gestation and on day 28 of lactation (plus or minus 1 d). About 9 ml blood was drawn from the jugular vein into sterile blood collection tubes (Monovetten, Sarstedt, Berlin, Germany).

From six litters in each treatment, three newborn piglets were chosen for blood sampling immediately after birth and before the first ingestion of colostrum. Their body weight was as close as possible to the average of the litter. For every litter, a group of four piglets of an average body weight similar to that of the whole litter were chosen for blood collection on day 2, 14 and 28 d of age. For blood sampling, piglets were manually restrained and 3 ml blood was drawn from newborn and 2 d-old piglets and 5 ml from older ones by puncture of the *v. jugularis* or *v. cava cranialis*.

Blood samples of dams and piglets were allowed to clot

in the dark at room temperature for 45–60 min, centrifuged for 30 min at 4°C and 3000 ***g*** and transferred into polypropylene tubes for storage at −20°C.

Milk sampling

During parturition or within 12 h post partum, samples of colostrum (40–70 ml) were obtained from sows by hand milking after intramuscular injection of 30–40 IU oxytocin. A similar amount of colostrum was taken from every lactating mammary complex. Milk samples were taken by the same procedure on the 28th day of lactation.

Liver samples

The livers from a total of seven stillborn piglets were used for hepatic folate determination. Those piglets were close to the average birth weight and showed no macroscopic variation of anatomical abnormalities. Livers were removed immediately after birth and kept frozen at −20°C until analysis.

Measurements

Dietary folates were analysed in duplicate on three hydrolysates of the same sample with commercial radioassay kits using [^{125}I]-labelled pteroylglutamic acid (QuantaphaseII Folate®; BioRad, Munich, Germany) as described by Matte *et al.* (1990). Preparation of samples before the assay was done according to a method adapted from Cerna & Kas (1983). In a 50 ml conical tube, 0·1 g feed was mixed with 12 ml McIlvain buffer (284 g Na_2HPO/l, 500 mg ascorbic acid/l, add distilled water, adjust pH to 4·6 with 3·3 M-NaOH and made up to 1 litre with distilled water) and autoclaved for 10 min at 121°C. The pH was adjusted to 7·0 with 3·3 M NaOH and the volume made up 20 ml with distilled water. The solution was vortexed and then centrifuged at 3000 ***g*** for 10 min. The supernatant fraction was used for folates determination. The effect of chicken pancreas conjugase (transformation of polyglutamates to monoglutamates) on concentrations of folates was tested using the method described by Cerna & Kas (1983). No effect of conjugase was noted on concentrations of dietary folates, and subsequently all assays were run without pretreatment with conjugase. The results seem to confirm previous observations (Rothenberg *et al.* 1974) on the versatility of the radioassay technique for both polyglutamates and monoglutamates. Results of parallelism tests were satisfactory (CV $<$ 10 %) between 0 and 5 mg/kg and inter-assays CV 9·1 %. Recovery tests from a simulated mixing in laboratory gave a mean value of 94·2 %. Serum folates were analysed in duplicate with commercial radioassay kits using [^{125}I]-labelled pteroylglutamic acid (QuantaphaseII Folate®, BioRad) validated for swine serum (Tremblay *et al.* 1986). Prior to analysis, samples were diluted 1:7–1:16 with saline (9 g NaCl/l) depending upon the expected amount of serum folates. Results of parallelism tests were satisfactory (CV $<$ 10 %) and recovery tests gave a mean of 103 %. Milk samples were prepared before determination in duplicate by the commercial radioassay kits (QuantaphaseII Folate®, BioRad) according to Matte & Girard (1989). In a conical tube for high-speed centrifugation the samples were mixed 1:1 (samples from control sows) or 1:10 (supplemented sows) with phosphate buffer (8·71 g K_2HPO_4/l, 2 g ascorbic acid/l, add bidistilled water, adjust pH to 7·8 with NaOH). After being covered with Al foil, samples were put into boiling water for 10 min, cooled in ice-cold water, and centrifuged at 20 000 ***g*** for 20 min. The supernatant fraction was used for folate analysis. No effect of chicken pancreas conjugase on the concentration of folates determined could be observed. Consequently, no conjugase was used for the assays. Results of parallelism tests were satisfactory (CV $<$ 10 %). Recovery tests from a simulated mixing gave a mean value 103·5 % and the inter-assay CV was 4·6 %. Liver samples were analysed according to Dumoulin *et al.* (1991). In a 50 ml conical tube, 4 ml ice-cooled buffer (0·5 mM-citric acid monohydrate+1 mM-disodiumphosphate, pH 5·5) and 1 g frozen liver sample were mixed and homogenised. A portion of this mixture (300 µl) was again mixed with 4 ml ascorbic acid (10 g/l, pH 6·0) in another conical tube for high-speed centrifugation. Afterwards, the sample was incubated at 75°C for 30 min, cooled in ice-cold water and centrifuged at 10 000 ***g*** for 10 min. A portion of the supernatant (1 ml) was mixed with 200 µl chicken pancreas conjugase and 800 µl ascorbic acid (0·01 g/l) and incubated at 37°C for 5 h. A portion of this final solution (200 µl) was used for determination by QuantaphaseII Folate® (BioRad). Results of parallelism tests were satisfactory (CV $<$ 10 %). Recovery tests gave a mean of 105·9 % and the inter-assays CV was 3·9 %.

Statistical analysis

Student's *t* test was used to analyse the influence of the folic acid supplements on the concentration of folates in the several tissues. Data concerning the changes of folate concentrations during a special period were analysed for each single group using the General Linear Models procedure of SAS® (6.12 for Windows®; Statistical Analysis Systems Inc., Cary, NC, USA). GLM procedure was also used to evaluate the interaction between the number of parities on the serum folate concentration in sows.

Results

Serum folate status of sows during gestation and lactation

Serum folates in unsupplemented controls tended (P = 0·063) to decrease from day 30 to day 60 of gestation (Table 2). Subsequently the concentration of folates remained stable until day 100 of gestation and decreased slightly but not significantly from day 100 of gestation to the end of lactation. In the group supplemented with folic acid, there was no effect (P = 0·24) of the stage of gestation or lactation on the serum folate concentrations (Table 2). Serum concentration of folates were higher (P = 0·03) in treated sows than in controls on day 100 of gestation and day 28 of lactation (Table 2).

Table 2. Concentration of serum folates of sows during gestation and lactation according to the treatments

(Mean values and standard deviations)

Treatment		Control		10 mg folic acid/kg feed*	
	n	Mean	SD	Mean	SD
Day 30 of gestation	19	136^{a}	43·5	141^{a}	34·4
Day 60 of gestation	21	102^{ab}	38·3	112^{a}	29·2
Day 100 of gestation	24	104^{Aab}	37·4	140^{Ba}	38·5
Day 28 of lactation	25	92·9^{Ab}	31·5	133^{Ba}	49·4

^{A,B}Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
^{a,b}Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* Gestation and lactation diet of sows. For details of diets, see Table 1.

Folates in colostrum and milk of sows

The concentration of folates in colostrum are shown in Table 3. These concentrations were 3–4 times higher ($P < 0·05$) than the folate concentration in the milk at the end of lactation in both controls and supplemented sows respectively. Nevertheless, milk and colostrum folates were higher ($P < 0·05$) in supplemented sows than in controls.

The concentration of colostral : serum folates on day 100 of gestation was 1:1 and 2·5:1 in controls and treated sows respectively. On day 28 of lactation, the ratio of the concentration of milk to serum folates was 1:3 and 1·1:1 in controls and treated sows respectively.

Serum and liver folates of piglets

The serum concentration of folates in newborn piglets was higher in treated than in controls before the first ingestion of colostrum (Table 4). The treatment effects on folate concentration in colostrum are reflected on the concentration of serum folates of piglets at 2 days of age. At birth, the folate concentration in the serum of the piglets was only half of the concentration detected in the serum of their dams on day 100 of gestation. However, at 28 days of lactation, serum folate concentration in piglets was 1·5 times and 2·1 times higher than the concentration of their dams' serum in controls and treated sows, respectively. The concentration of liver folates was at least doubled (172 *v.* 76·3 nmol/kg; $P = 0·02$) in stillborn piglets from sows receiving supplements of folic acid during gestation (Table 5).

Table 3. Concentration of folates in colostrum and milk of sows (nmol/l)

(Mean values and standard deviations)

Treatment		Control		10 mg folic acid/kg feed*	
	n	Mean	SD	Mean	SD
Colostrum	6	101^{Aa}	55·3	362^{Ba}	131
Milk (day 28 of lactation)	15	30·4^{Ab}	29·2	150^{Bb}	68·4

^{A,B}Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
^{a,b}Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* Gestation and lactation diet of sows. For details of diets, see p. 180.

Table 4. Concentration of folates in the serum of piglets during the suckling period

(Mean values and standard deviations)

Treatment	*n*	Control		10 mg folic acid/kg feed*	
		Mean	SD	Mean	SD
At birth	18	56·2^{Aa}	14·7	92·6^{Ba}	25·1
Day 2 of life	70	175^{Ab}	87·4	365^{Bb}	134
Day 14 of life	76	151^{Ab}	82·9	328^{Bb}	124
Day 28 of life	70	141^{Ab}	65·2	281^{Bb}	81·3

^{A,B}Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
^{a,b}Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* Gestation and lactation diet of sows. For details of diets, see Table 1.

Discussion

Serum folate status of sows during gestation and lactation

In spite of some variations in absolute values, the pattern of decrease in the concentration of serum folates in unsupplemented sows from day 30 to day 60 of gestation was similar to profiles previously reported by Matte *et al.* (1984, 1992; 1993), Tremblay *et al.* (1986; 1989), Anzhi & Cooper (1989), Thaler *et al.* (1989), O'Connor & Picciano (1993), Harper *et al.* (1994), Natsuhori *et al.* (1996), Fuchs *et al.* (1996) and Giguère *et al.* (1999).

Although not significant, the present decrease was numerically important i.e. approximately 25 % (Table 2). A decrease of serum folate concentrations during pregnancy was reported in cows, sheep, rats and humans (Ek & Magnus, 1981; Thenen, 1991; Girard *et al.* 1996; Girard & Matte, 1989). As suggested by Matte *et al.* (1992), such a phenomenon could be due to the metabolic utilisation from the dam for maintenance needs and deposition (products of conception). Changes in renal excretion are unlikely to be involved; Matte & Girard (1999) did not observe any major changes in urinary renal excretion in gestating sows.

Although all studies mentioned above agree on a decrease of concentration from the beginning to the middle of gestation, discrepancies are reported for the following second half of gestation and the lactation. In agreement with the present results, Matte *et al.* (1984), Harper *et al.* (1994) and Natsuhori *et al.* (1996) observed an almost stable concentration until the end of pregnancy. In contrast, O'Connor & Picciano (1993) described a small decrease during this period and Matte *et al.* (1992) as well as Thaler *et al.* (1989) detected a significant increase of the serum folate concentration.

During lactation, Matte *et al.* (1992) reported a decrease from farrowing to the 7th day of lactation followed by an

Table 5. Liver folate concentration of stillborn piglets (nmol total folates/kg original matter)

Treatment	*n*	Mean	SD
Control	2	76·3^{b}	2·04
10 mg folic acid/kg feed*	5	172^{a}	33·3

^{a,b}Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* Gestation and lactation diet of sows. For details of diets, see Table 1.

increase until the end (day 28) of lactation in gilts. In multiparous sows, the concentration increased during the whole period in studies by Matte & Girard (1989), O'Connor & Picciano (1993) and Harper *et al.* (1994). The present results failed to show any major changes in concentrations from day 100 of gestation to day 28 of lactation. The lack of repeated blood sampling of the sow during lactation in the present experiment did not allow a reliable comparison with profiles of folates reported during lactation in previous studies.

As far as the response to treatments is concerned, an effect was observed only on day 100 of gestation and day 28 of lactation. Such results are different from those reported in early and mid gestation by Tremblay *et al.* (1986), Thaler *et al.* (1989), Matte *et al.* (1992, 1993), Lynch & Sheehy (1994), Harper *et al.* (1994) as well as Fuchs *et al.* (1996), who observed a linear influence of increasing folic acid supplements on the concentration of folates in the serum of sows. The parity does not seem to be involved in explaining the difference from previous results because there was no interaction of the number of parities on the serum concentration in the present study; experimental conditions with only thirty-two sows and basic reproduction performance of the sows (9·25 liveborn piglets and 8·15 weaned piglets per litter) might be a factor to consider.

Folates in colostrum and milk of sows

The concentration of folates in milk on day 28 of lactation was 2–5 times lower than the corresponding values in colostrum. According to O'Connor *et al.* (1989), most of the decrease in the concentration of milk folates occurred at the beginning of lactation (day 0–7) There was a further decrease in the concentration up to day 21 of lactation but it was less pronounced than in early lactation. Such a profile was in agreement with Matte & Girard (1989).

In agreement with the present results, O'Connor *et al.* (1989) reported, in unsupplemented sows, similar concentration of folates in colostrum (23·1 nmol/l) and in serum (24·9 nmol/l) on day 110 of gestation. However, the high colostral concentration as compared with the serum value (day 100 of gestation) in treated sows in the present experiment, suggests a preferential route for supplemental folates towards the colostrum pool. As previously demonstrated by Matte & Girard (1999) there is no saturation of serum folate at a level of 10 mg folic acid/kg feed which supports the theory of a preferential route towards colostrum. In milk, folate concentrations were usually lower than their serum concentrations as also reported by Matte & Girard (1989), O'Connor *et al.* (1989) and Matte *et al.* (1992). However, in treated sows, the effect seen on colostrum persisted, although less markedly, for milk on day 28 of lactation, a phenomenon also shown by Matte *et al.* (1992).

In sheep, Girard *et al.* (1996) reported much higher colostrum folate concentrations than in sows (variation between different sheep breeds from 222 to 374 nmol/l) and a similar difference of concentrations between colostrum and milk. Nevertheless, folate concentrations of sows' milk seem to be relatively low compared with those in human subjects (152–548 nmol/l; Tamura *et al.* 1980; Selhub *et al.* 1984; O'Connor *et al.* 1991), sheep (164–220 nmol/l; Girard *et al.* 1996) or cows (134 nmol/l; Girard & Matte, 1989).

Serum and liver folates of piglets

As observed by Natsuhori *et al.* (1996), serum folate concentrations in newborn piglets were much lower before the first intake of colostrum than later during the suckling period. At birth, serum folate concentrations of piglets were about half of those detected in their dams which seems to indicate that the folate transfer 'in utero' is not an active and substantial process. After a sharp increase (4 times) in the serum folate concentration from birth to day 2 of life the concentration seems to drop slightly during the following period of suckling. Such a difference corresponded, in fact, to the changes observed in dams' colostrum and milk. Those results are in agreement with O'Connor *et al.* (1989) and Natsuhori *et al.* (1996) but are slightly different from Matte *et al.* (1992) who reported an increase up to 14 d of age followed by a gradual decrease towards weaning, on day 28. Therefore, as suggested by the present change in the ratios between serum folate concentrations in dams and piglets and as reported by Matte *et al.* (1992), the folate status of the young piglets is more influenced by the postnatal supply of folates from colostrum and milk than by the pre-natal provision.

In agreement with the treatment effects on serum folates of piglets at birth, the results on liver folates indicate that the transfer of folic acid 'in utero', although marginal, can be increased by the supplements of folic acid. However, the present response to treatments in the liver appeared to be much more marked than what was observed earlier in gestation for the whole fetus (Matte *et al.* 1993) Since there are no data reported on the folate content of livers from newborn piglets until now, our results from a few stillborn piglets can be judged as a first indication for the dependence of the folate carry-over into fetal liver on the folic acid supply of the sow. It should be emphasized again that the amount of liver samples is very limited.

Conclusions

Folic acid supplements during gestation and lactation influence the carry-over of folates from the sow to the fetus and to the suckling piglet. During the last period of gestation, a significant part of the available folates seems to be directed towards the mammary gland as suggested by the huge amount of folates in the colostrum. Moreover, folic acid supplements induced an intensive transport of folates into colostrum and milk as suggested by the higher concentration of folates in the colostrum and milk as compared to the serum; such transport was not apparent in unsupplemented sows. The priority of folate transport into the mammary gland is critical for the piglet taking into account his relatively low folate status before the first colostrum intake. Therefore, the folate supply for the newborn piglet is dependent mainly upon the carry-over of folates via colostrum and milk.

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