EFFECTS OF INTRAMUSCULAR INJECTIONS OF FOLIC ACID DURING LACTATION ON FOLATES IN SERUM AND MILK AND PERFORMANCE OF SOWS AND PIGLETS

J. J. Matte and C. L. Girard

Research Station, Agriculture Canada
Lennoxville, Québec, Canada J1M 1Z3

ABSTRACT

In order to determine the effect of folic acid on serum and milk folates in lactating sows as well as on serum folates and growth rate of the piglets, sows (n = 25) received either saline or 15 mg folic acid i.m. each week from d 2 after parturition to weaning, 26 d later. Blood samples were drawn from all sows at 110 d of gestation and every week during lactation. Milk samples were taken at d 7 and 21 of lactation. Piglets were weighed and blood samples were collected weekly during lactation. Serum folates of sows increased during lactation. The rate of increase was more pronounced (P < .0002) after folic acid injections. Milk folates concentrations decreased (P < .0007) from d 7 to 21 of lactation but were higher (P < .0001) in treated sows (11.8 ± .7 ng/ml) than in control sows (7.9 ± .4 ng/ml). Serum folates of piglets in control litters increased from 55.0 ± 2.2 ng/ml at 2 d of age to a peak value of 86.3 ± 3.1 ng/ml 2 wk later, and then gradually decreased. In piglets from treated dams, the time response curve was similar to that of the controls, but values were about 15% higher (P < .01). The growth rate of piglets until 8 wk of age was not changed (P > .47) by folic acid injection of sows. More studies are needed to evaluate the practical importance of changes in folates status in establishing the folic acid requirements of lactating sows.

(Key Words: Sows, Piglets, Lactation, Folic Acid.)


Introduction

Folic acid plays a fundamental role in synthesis of DNA, RNA and protein (Chang and Kaiser; 1972, Herbert and Das, 1976). Recently, it was shown that pregnant sows need more folic acid than suggested by NRC in 1979 (Matte et al., 1984a,b; Tremblay et al., 1986). In fact, Tremblay (1988) reported that a level as high as 5 mg/kg diet increased embryo survival by about 15% during the first 30 d of gestation. However, Pharazyn and Aherne (1987) found no improvement in the lactation performance of sows when dietary folic acid was increased from .45 to .90 mg/kg during lactation.

The lability of folic acid to heat and light (Ek and Magnus, 1980; Ford et al., 1983) makes verification of the efficacy of folic acid supplementation essential. This must be done through feed analysis but also by measuring changes of folates status of the animals. Intramuscular injections constitute a research tool to study folic acid requirements while overcoming problems associated with dietary supplementation of this vitamin.

This work was undertaken to measure the changes in folates status of sows and piglets in terms of serum folates concentrations as well as milk folates after i.m. injections of folic acid during lactation. To the best of our knowledge, such data have never been reported before. Weight losses of sows and growth rate of piglets also were recorded.

1The authors acknowledge the assistance of Michelle Guillette, Chrystiane Plante, Marjolaine St-Louis, Richard Bilodeau, Marcel Morissette, Mariette Vanier, Marie Daoust and Richard Lancôt for technical support, Maryse Dumais for statistical analysis and Louise Boisvert for manuscript preparation. This research was subsidized in part by Hoffman-La Roche, Ltd., Basle, Switzerland. Contribution no. 228, Agriculture Canada.

Received December 28, 1987.
Accepted August 4, 1988.
TABLE 1. COMPOSITION OF THE DIETS

<table>
<thead>
<tr>
<th>Ingredient, %</th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats</td>
<td>20.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>15.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Corn</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Barley</td>
<td>27.8</td>
<td>15.4</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>4.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Wheat shorts</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Soybean meal</td>
<td>1.9</td>
<td>13.2</td>
</tr>
<tr>
<td>Meat meal</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Animal fat</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Salt</td>
<td>.3</td>
<td>.4</td>
</tr>
<tr>
<td>Trace minerals and vitamin premixa</td>
<td>.5</td>
<td>.5</td>
</tr>
</tbody>
</table>

**Calculated composition, %b**

<table>
<thead>
<tr>
<th></th>
<th>Gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible energy, Mcal/kg</td>
<td>3.2</td>
<td>3.3 (3.12)</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.0</td>
<td>16.0 (16.5)</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>5.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Ca</td>
<td>.9</td>
<td>.9</td>
</tr>
<tr>
<td>P</td>
<td>.8</td>
<td>.8</td>
</tr>
<tr>
<td>Na</td>
<td>.2</td>
<td>.2</td>
</tr>
<tr>
<td>Folates (mg/kg)</td>
<td>1.5</td>
<td>1.5 (1.1)</td>
</tr>
</tbody>
</table>

*a* Supplied per kg of diet: 40 mg Mn, 75 mg Fe, 25 mg Cu, .10 mg Co, 100 mg Zn, .6 mg I, 1 mg Se, 10,000 U.I. vitamin A, 1000 U.I. vitamin D, 15 U.I. vitamin E, 1 mg vitamin K, .02 mg vitamin B12, 1 mg thiamin, 3 mg riboflavin, 8 mg pantothenic acid, 10 mg niacin, .2 mg biotin, 250 mg choline, .5 mg folic acid.

*b* Values in parentheses represent the results of the chemical analysis. A conversion factor of .775 was used between gross energy and digestible energy.

**Materials and Methods**

**Animals.** During gestation 50 Yorkshire sows of second parity were fed a commercial diet as described in Table 1. Daily intake was restricted to 2.5 kg. One week before the expected time of parturition, they were transferred to farrowing cages and fed a lactation diet (Table 1). This diet was available ad libitum throughout lactation. The mean daily feed intake during lactation was similar (5.1 ± 2.2 kg) between treatments. Creep feed was available to the piglets from d 21 to weaning on d 28. The calculated and chemical (values in parentheses) compositions of this commercial diet were: 3.45 (3.16) Mcal/kg, 19.0 (19.5) % crude protein, 5.0% crude fat, 3% crude fiber, 1.0% Ca, .75% P, .2% Na and .6 (.68) mg/kg folic acid. On d 114 of gestation, parturition was induced with PGF2α analog.2 Whenever possible, litter size was adjusted to eight or nine piglets within 3 d after parturition. The dams were weighed at 110 d of gestation, at parturition and at weaning, and piglets were weighed every week during lactation and at 8 wk of age.

**Treatments and Sampling.** At parturition, sows were randomly assigned to two groups of 25 animals each. The first group received four i.m. injections of 15 mg folic acid, one each week from d 2 after parturition until weaning, 26 d later. The second group of sows received saline injections according to the same schedule. Blood samples were withdrawn from sows at 110 d of gestation and from dams and piglets prior to the injection and on d 2, 9, 16, 23 and 28 of lactation. The procedure used for blood collection and serum storage has been described previously (Matte et al., 1984a, 1986). A milk sample was taken on d 7 and 21 of lactation. Piglets were removed from the dams 1 h before milk sampling. Milk was withdrawn completely from all glands after i.v. administration of 10 IU of oxytocin. The volume collected ranged from 250 to 400 g, a value similar to the amount ingested by piglets (Mahan et al., 1971) during a suckling episode.

2 Planate, ICI Pharma, Ontario, Canada.
Serum Folates Determinations. Serum folates were analyzed in duplicate with commercial radioassay kits using $^{[125]}$IPGA as described by Tremblay et al. (1986). The interassay coefficient of variation was 3.5%, and recovery tests using PteGlu were 99.9%. Other validation tests of this assay have been presented previously (Matte et al., 1984a).

Milk Folates Determinations. Milk folates were analyzed using the same kits. Preparation of samples before assay followed the method of Hoppner and Lampi (1981); the use of chicken pancreas conjugase (transformation of poly- to monoglutamates) was omitted because it did not change the values measured. This result seemed to confirm previous observations (Rotenberg et al., 1974; Schreiber and Waxman, 1974) on the versatility of the radioassay technique for mono- and polyglutamates. However, it also might indicate that sow’s milk contains mainly monoglutamates. The recovery test and the interassay coefficient of variation were, respectively, 103.5% and 4.5%. Despite its low concentration of folates compared with human and cow’s milk, sow’s milk contains an excess of unsaturated binding protein (Ford et al., 1975). It is unlikely that these proteins interfere with the radioassay because the preparation of samples and the radioassay use a boiling treatment of 10 and 20 min, respectively. Such treatments should denature folate binding proteins (Ford et al., 1975). Recovery test results confirm this point.

The radioassay technique also overcomes the problem of turbidity interference reported for milk folates determination by the microbiological assay using Lactobacillus casei (Cooperman et al., 1982). Folates concentrations measured in the present experiment were within the range of values reported previously by Ford et al. (1975).

In view of these observations it was concluded that the commercial radioassay kits are reliable for determination of folates in sow’s milk.

Measurements in Diets. Dry matter, crude protein and gross energy in diets were measured according to AOAC (1984) methods. Levels of folates in diets were determined by microbiological assay with Lactobacillus casei.

Statistical Analysis. Data were analyzed as a completely random design using the General Linear Models procedure of SAS (1985). The following model was used: $Y_{ij} = \mu + F_i + T_j + FT_{ij} + \epsilon_{ij}$, where $Y_{ij}$ indicates one of the following dependent variables: weight of sow, weight of piglets, serum folates of sow or piglets and milk folates. The overall mean is $\mu$; $F_i$ is the effect of folic acid administration, and $T_j$ is the effect of stage of gestation and (or) lactation. Least squares means were compared using orthogonal contrasts when appropriate.

Results

Body weight and serum folates concentrations of sows from 110 d of gestation to d 28 of lactation are illustrated in Figures 1 and 2, respectively. There was no treatment ($P < .28$) or treatment x time effect ($P < .85$) on body weight of sows. As lactation progressed, serum folates concentrations of sows showed a different pattern according to treatments; an interaction of treatment x linear effect of time ($P < .0002$) was found between 2 and 28 d of lactation. The rate of increase of serum folates concentrations was more pronounced in sows injected with folic acid.

Milk folates concentrations on d 7 and 21 of lactation are presented in Figure 3. Folates concentrations in milk decreased ($P < .0007$) from d 7 to 21 of lactation. However, at both stages of lactation, milk folates were 33% higher ($P < .0001$) for sows receiving folic acid injections.

![Figure 1. Body weight losses of sows from 110 d of gestation to 28 d after parturition according to treatments during lactation (values are means ± SE of 25 sows per treatment).](image-url)
Figure 2. Serum folates changes in sows from 110 d of gestation to 28 d after parturition according to treatments during lactation (values are means ± SE of 25 sows per treatment).

Figure 3. Folates concentrations in milk sampled at 7 and 21 d after parturition according to treatments during lactation (values are means ± SE of 25 milk samples per stage of lactation per treatment).

Figure 4. Serum folates changes in piglets from d 2 to d 28 after birth according to treatments given to sows during lactation (values are means ± SE of 226 piglets in treated litters and 228 piglets in control litters).

Figure 5. Growth rate of piglets from birth to 8 wk of age according to treatments given to sows during lactation (values are means ± SE of 226 piglets in treated litters and 228 piglets in control litters).

Serum folates concentrations and growth of piglets are shown in Figures 4 and 5, respectively. Serum folates in piglets increased from d 2 to 16 of age and then gradually decreased; the effect of age was quartic (P < .01). Serum folates in piglets were consistently higher (P < .007) throughout lactation when folic acid was administered to dams. There was no effect of treatment (P < .47) or treatment x age (P < .68) on piglets' growth from birth to 8 wk of age.

Discussion

Similarity in body weight loss of sows during lactation with or without folic acid injections showed that folic acid did not prevent the mobilization of body reserves induced by lactation.

As noted previously by Matte et al. (1984a) with gestating sows, serum folates are likely to be good indicators of folates status during the reproductive cycle of these animals because of the uniformity of the feeding regimen within a herd. In our experiment, blood samples were taken at the same time of day, 0830 to 1000.

In women, it has been observed previously that plasma folates increased as lactation proceeds (Ek, 1983). Our results showed a similar trend for lactating sows. The interaction between treatment and stage of lactation indicated that the response of serum folates to folic acid administration was more pronounced toward the end of lactation. This delayed response to treat-
ment probably is due to rapid capture of folates by the mammary gland and the depletion of tissues of the sows. Preferential routing of folates to mammary glands has been suggested previously in women and was accentuated in cases of folic acid deficiency (Metz et al., 1968; EK, 1983). Serum folates increased markedly in both treatments by the end of lactation. This general trend probably is due to increased intake of dietary folate acid and to the decline in plasma volume observed later in lactation in sows (Anderson et al., 1970). As lactation progressed, the difference in serum folates between treatments became more accentuated. These results could be explained by restoration of reserves in treated sows, gradually leaving more folates in circulation.

Milk folates concentrations decreased from d 7 to d 21 of lactation. Despite this decrease, the quantitative transfer of folates to mammary glands probably is similar between the two stages of lactation because milk production increases from the 1st to the 3rd wk of lactation by about 30% (ARC, 1981; Noblet and Etienne, 1986), which corresponds to the difference in milk folates concentrations we observed between the two stages of lactation.

It is unlikely that the marked increase in milk folates following folic acid administration was sufficient to saturate the folate-binding proteins; the typical value for the binding capacity of sow's milk for folates is 80 ng folic acid/ml (Ford et al., 1975). Consequently, milk folates would represent only 10% and 15% of the total binding capacity of milk in control and treated sows, respectively. The effect of folic acid injections on milk folates probably is relevant in estimating physiological folate acid requirements of lactating sows. In other species, milk folates are insensible to folic acid supplementation except when the supply of folic acid is deficient (Metz et al., 1968; Tamura et al., 1980; Cooperman et al., 1982). If true for lactating sows, our results on milk folates would reflect a suboptimal folates status in these animals. Moreover, this change of folates status occurred despite the presence of 1.1 mg/kg of folic acid in the diet, a level almost twice the level (.6 mg/kg) suggested by NRC (1979).

Serum folates increased in piglets of dams receiving folic acid administration during lactation. The response to treatment was more immediate than that observed in sows and probably reflects what was observed in milk folates changes during lactation. In breast-fed infants, serum folates concentration is a reliable index of folates status and is directly proportional to the folates content of mother's milk (Tamura et al., 1980).

No effect of folic acid treatments of sows on piglet growth to 8 wk of age was observed. Similar observations on growth rate of suckling piglets were reported recently by Pharazyn and Ahern (1987), who added .45 mg/kg of folic acid to a basal diet containing .45 mg/kg. The authors concluded that a level of .45 mg/kg diet should be sufficient for lactating sows. On the basis of the mean daily intake of feed by the sows in the present experiment and the results obtained with gestating sows by Tremblay et al. (1986), our treated animals in this experiment received a supplement equivalent to about 2 mg of folic acid per kilogram of diet. Including what was already present in the basal diet, this would correspond to a total of about 3.1 mg/kg of folic acid. Dietary concentrations were lower than the 5 mg/kg diet used by Tremblay (1988) that reduced embryo mortality by 15% in gestating sows.

The absence of a treatment effect on growth rate of suckling piglets may indicate that folic acid is not required for growth at a level higher than that of control treatment. This assumption conflicts with the concept of a suboptimal physiological folates status suggested by effects of supplementation of milk folates concentrations. However, these results, taken together also suggest that the level of supplementation did not sufficiently increase the saturation level of folates in milk to induce a response on growth rate of piglets. Such assumption also might apply to the results reported by Pharazin and Ahern (1987). Further studies in progress in our laboratory should elucidate the practical significance of folates status measurements to evaluate folic acid requirements of sows and gilts.

In conclusion, the administration of folic acid during lactation increased serum and milk folates of sows as well as serum folates of piglets; however, injections had no effect on growth rate of piglets from birth to 8 wk of age. More studies are needed to clarify the metabolic and practical significance of these changes in folates status in establishing the folic acid requirements of lactating sows.
Literature Cited


