Summary

Two-hundred nine sows were used in a $2 \times 2$ split-plot unbalanced design to measure the effect of folic acid against control, and flushing against a normal level of feeding, between weaning and mating on the following variables: serum folates at weaning and at 60 d of gestation, blood hemoglobin (Hb) and hematocrit (Ht) after 15 wk of gestation and reproductive performance at farrowing. Folic acid was administered im according to a schedule that maintained serum folates at approximately the same level between weaning and 60 d of gestation. The treatments had no effect on Hb or Ht after 15 wk of gestation. Average live litter size was 12.0 piglets/litter for sows receiving the folic acid and flushing treatments as compared with 10.5 for sows without any treatment; the main effect of folic acid was significant ($P<.04$). Intralitter variation in birth weight and total litter weight tended to be increased by folic acid administration. Results showed that the administration of folic acid during gestation could appreciably improve the reproductive performance of sows.

(Key Words: Swine, Folic Acid, Flushing, Prolificacy, Hemoglobin, Hematocrit.)

Introduction

In swine production, litter size is one of the most important economical variables to consider. Litter size at birth is controlled by ovulation rate, fertilization rate and losses during gestation (Vangen, 1981; Flint et al., 1982). Ovulation rate, as measured by the count of corpora lutea, could reach 15 to 20 eggs under normal management conditions (Pond and Houpt, 1978; Vangen, 1981). The number of eggs ovulated has been increased by genetic selection (Vangen, 1981) and by direct hormonal stimulation (Hunter, 1980; Weibel and Day, 1982). Sudden changes in feeding level (flushing) before estrus, have also been considered as efficient means of increasing ovulation rate and litter size, at least in gilts (den Hartog and van Kempen, 1980). In sows, however, the efficacy of this practice on ovulation rate (Hardy and Lodge, 1969; Brooks and Cole, 1971; ARC, 1981) and litter size at farrowing (Brooks and Cole, 1971) has not been clearly demonstrated.

Prenatal mortality usually ranges between 20 and 45% after normal pregnancies (Hunter, 1980; Flint et al., 1982). Mortality occurs mostly during the first 25 d of gestation (Pond and Houpt, 1978). This could be explained by many complex physiological events occurring during this period: recognition of pregnancy, formation of blastocysts, attachment of conceptuses and secretion of several peculiar endometrial proteins (Flint et al., 1982; Bazer and First, 1983). Embryonic mortality occurring during these events is believed to be the most important factor controlling litter size variation (Paterson et al., 1980; Vangen, 1981). The influence of nutrition on embryonic mortality has not been thoroughly studied, but Hartog and van Kempen (1980), in reviewing this subject, concluded that a high level of feeding during the rearing and mating periods and during early gestation could increase embryonic mortality. When particular feed ingredients were studied, it was observed...
that alfalfa meal tended to increase the survival rate of embryos (O'Bannon et al., 1966) as well as litter size at birth (Pollman et al., 1980).

Matte et al. (1982) recently observed a drastic decrease in serum folates between weaning and mating, and during pregnancy in multiparous sows. This drop in serum folates could be prevented by im injections of folic acid (Matte et al., 1984). They concluded that the decrease in serum folates was suggestive of a temporary folic acid deficiency that, at this particular time of the reproductive cycle, may be associated in part with embryonic mortality. It was hypothesized that maintaining a high level of serum folates from weaning to mid-gestation would result in larger litter size at farrowing, through a decrease in embryonic mortality. If this maintenance of high levels of serum folates occurs with stimulation of ovulation, the effect on prolificacy of sows should be more marked. To verify this hypothesis, a flushing treatment was applied in an attempt to increase ovulation rate and a predetermined schedule of folic acid injections was followed to maintain serum folate at approximately the same level between weaning and 60 d of gestation. These treatments were investigated in relation to their possible effects on the reproductive performance of sows at farrowing.

Materials and Methods

Treatments. A lot of 240 sows was selected at random from four sections of a commercial unit and assigned to four treatments according to a 2 x 2 split-plot design. In the main plot, a comparison was made between two levels of feeding, a flushing level and a normal level from weaning to mating. During that period, in each section, sows were group-fed in a mating pen. In this pen, the folic acid treatment (im injections vs a control without injection) was administered randomly to animals. After breeding, the sows retained were transferred to a gestation pen with other pen-mates not necessarily involved in the experiment.

During the flushing treatment, the sows were fed ad libitum from the day after weaning through the first day of behavioral estrus. Sows assigned to the normal level of feeding were restricted throughout the weaning and mating period to about 2 kg/d of a diet previously described (Matte et al., 1984). Thereafter, all animals were restricted to approximately 2 kg/d of the same diet. The calculated folic acid content of the diet was about .6 mg/kg, a level corresponding to National Research Council suggested requirements (NRC, 1979).

The folic acid treatment consisted of 10 im injections of 15 mg folic acid\(^4\). One injection of folic acid was administered at each of the following time periods: day of weaning, first day of behavioral estrus, weekly during the first 4 wk of gestation and every fortnight for the following 8 wk. This injection schedule was chosen on the basis of previous research (Matte et al., 1984) and was expected to be adequate to maintain serum folates at approximately the same level between weaning and 60 d of gestation. The sows assigned to the control treatment received no folic acid in addition to what was present in the diet.

Mating Schedule. All sows were mated twice, once on the first day of behavioral estrus (d 0) and once on the following day (d 1). When this mating schedule could not be met, sows were discarded from the experiment. Furthermore, the period between weaning and mating was restricted to 7 d in order to avoid too large a decrease in serum folates following folic acid injection at weaning. This was based on previous observations with regard to changes in serum folates immediately following weaning (Matte et al., 1984). Accordingly, sows showing behavioral estrus after d 7 post-weaning were also discarded.

Animals. The animals used were all Yorkshire x Landrace sows of second parity or more. They were housed in a commercial unit and kept on slatted flooring throughout their reproductive cycle. They were transferred to farrowing cages about 1 wk before parturition. Of the 240 sows originally selected, 209 farrowed and remained on the trial, resulting in an unbalanced distribution among groups. Discarded sows were not out of a particular treatment. Pregnancy was verified on all sows by ultrasonic examination, at approximately 30 and 45 d of gestation.

At parturition, the total number of piglets born and alive was registered. Individual birth weight of live piglets was measured and used

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\(^4\)Folvite\textsuperscript{®} Parenteral Solution No. 4154, Lederle, Cyanamid Canada Inc., Montréal, Québec.
to calculate intralitter coefficient of variation in birth weight (SD within litter/mean birth weight of litter) and total litter weight.

Blood Sampling and Hematological Measurements. Blood samples were taken from each sow at weaning, before initiation of the treatments, and at 60 d of gestation, for serum folate determinations. The procedure used for blood collection, serum storage and folate analysis has been previously described (Matte et al., 1984). An additional blood sample was taken from all sows at 15 wk of gestation for hematocrit (Ht) and hemoglobin (Hb) measurements. Vacuum tubes containing .07 ml of a 15% EDTA (K$_3$) solution and .014 mg potassium sorbate were used. Hematocrit was measured in duplicate on fresh blood. Blood samples were then transferred into polypropylene tubes and stored at -20 C. Hemoglobin was determined in duplicate on thawed blood according to the method of Drabkin (Manet, 1968).

Statistical Analysis. Data were analyzed as a completely random split-plot design using the General Linear Model procedure of Statistical Analysis System (Freund and Littell, 1981). The main plot error term, sections within flushing, was used to test the flushing effect, but the degrees of freedom for this error term were judged insufficient to comment on this effect. The residual error term was used to test the folic acid effect and the flushing × folic acid interaction.

Results and Discussion

Serum Folates. The levels of serum folates observed in this experiment (figure 1) were similar to those previously reported (Matte et al., 1984). Mean serum folates at weaning were near 90 ng/ml and uniform (P>.05) among groups (figure 1). At 60 d of gestation, serum folates had dropped by a factor of over 50% in sows without folic acid injection, but had remained at weaning levels in sows receiving the folic acid treatments. The folic acid effect at that time was highly significant (P<.0001). The adopted injection schedule of folic acid was therefore adequate to maintain weaning serum folates levels for at least 60 d of gestation.

Hematological Measurements. Blood Ht and Hb after 15 wk of gestation were similar in all treatments (table 1). Therefore, the injection of folic acid to maintain the weaning levels of serum folates during the first 60 d of gestation, had no effect on these variables. It is recognized that in humans, the requirements for folates is increased by pregnancy (Hillman, 1980) and a prolonged folate deficiency may be conducive to megaloblastic anemia (Chanarin and Perry, 1977). It would appear then that in the present study, the low serum folate level observed at 60 d of gestation in groups without folic acid treatment were not indicative of a severe folic acid deficiency or of a deficiency status sufficient to affect the blood Ht and Hb levels.

Reproductive Performances. The folic acid treatments (table 1) significantly increased the number of piglets born (P<.03) and the number of piglets born alive (P<.04). The folic acid effect, however, was less marked on live litter weight (P<.12) than on litter size. The intralitter variation in birth weight tended (P<.09) to be increased by folic acid treatments. In flushed sows injected with folic acid, the number of piglets born alive was increased by 1.5 piglets/litter as compared with unflushed sows receiving no folic acid. Although folic acid increased the number of piglets born and born alive in both main treatments, flushing and normal level of feeding, such an effect was more pronounced when folic acid was administered to flushed sows. In fact, the number of piglets born alive was increased by 1.1/litter when folic acid was administered to flushed sows, as compared with .2 when folic acid was ad-
ministered to unflushed animals. It is apparent, therefore, that the folic acid treatment was much more efficient when a flushing treatment was applied concomitantly.

In groups without folic acid injections, flushing appeared to be inefficient in increasing litter size at farrowing. This would be consistent with previous reports dealing with sows kept under normal management conditions (Brooks and Cole, 1971, 1972). In gilts, as compared with older sows, flushing is often considered as an efficient means to stimulate ovulation rate, but the effect on embryonic mortality remains controversial. den Hartog and van Kempen (1980) suggested that embryonic mortality would not be affected by flushing; an increase in ovulation rate would therefore lead to an increase in litter size at farrowing. However, Bazer et al. (1968) reported a higher embryonic mortality in flushed gilts. They concluded that embryos from flushed gilts exhibited viability equal to those produced by unflushed animals. Flushing may reduce the uterine ability to support an increased number of embryos, so that litter size at birth would remain unchanged in both flushed and unflushed gilts. This last hypothesis would explain the apparent failure of flushing to increase litter size at farrowing when compared with the normal level of feeding used in the present study. It would also explain the marked effect of folic acid on litter size in the flushed animals; flushing could have increased ovulation rate, but litter size was increased only when proper uterine conditions prevented an increase in embryonic mortality. These conditions would be associated with an adequate supply of folic acid at a crucial moment during gestation. The fact that folic acid was inefficient when administered to sows on a normal level of feeding, could similarly be explained by this hypothesis. Under the conditions of normal ovulation rate, folic acid supplementation would have no effect on embryonic mortality and consequently on litter size at birth. Further research is needed to confirm these explanations.

Embryonic mortality would appear to be the most important factor controlling variation in litter size (Paterson et al., 1980; Vangen, 1981). In pigs, placentation is epitheliocorial and attachment of the trophoblast to the endometrium is superficial. Embryos are entirely dependent upon secretions of specific endometrial products such as uteroferrin (Bazer and First, 1983) and other constituents of histotroph (Flint et al., 1982) provided by surface and glandular epithelium of the uterus during early stages of gestation (Amoroso, 1956). Embryonic mortality or viability would depend on the competition among embryos for these uterine secretions (Ulberg and Rampacek, 1974). The increased secretory functions of the uterus (Basha et al., 1979) associated with the differentiation of placental structures, during the first one-half of gestation (Bazer and First, 1983), would suggest the possibility of increased requirements of metabolites essential for the synthesis of new tissues. During early

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### Table 1. Hematological Measurements and Reproductive Performance of the Sows

<table>
<thead>
<tr>
<th>Level of Feeding</th>
<th>Folic acid Treatment</th>
<th>Previous Farrowings, No.</th>
<th>Ht, %</th>
<th>Hb, g/dl</th>
<th>Piglets Born, No.</th>
<th>Piglets Born Alive, No.</th>
<th>Live Litter Wt, kg</th>
<th>Intralitter Variation, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>50</td>
<td>4.8</td>
<td>35.2</td>
<td>12.4</td>
<td>11.2</td>
<td>10.5</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>55</td>
<td>4.9</td>
<td>35.1</td>
<td>12.4</td>
<td>11.4</td>
<td>10.7</td>
<td>14.6</td>
</tr>
<tr>
<td>Flushing</td>
<td>Control</td>
<td>53</td>
<td>5.0</td>
<td>34.6</td>
<td>12.3</td>
<td>11.5</td>
<td>10.9</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>51</td>
<td>4.6</td>
<td>35.0</td>
<td>12.5</td>
<td>12.8</td>
<td>12.0</td>
<td>15.6</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td></td>
<td>58.5</td>
<td>8.6</td>
<td>9.5</td>
<td>21.7</td>
<td>21.1</td>
<td>19.6</td>
</tr>
</tbody>
</table>

Probabilities

| Main Effect Folic acid | .63 | .81 | .65 | .03 | .04 | .12 | .09 |
| Interaction Folic acid X flushing | .60 | .54 | .76 | .12 | .15 | .80 | .51 |

*Standard deviation within a litter/mean litter birth weight.*
gestation, the developing embryos go through rapid and intensive metabolism. The protein content of embryos, between d 6 and 9, for example, increases by a factor of 100 (Grammer and Wright, 1979). It has also been reported that the intracellular concentration of RNA of embryos was highly correlated with survival during the first 12 d of gestation (Martinenko, 1977). It would therefore appear logical to assume that the metabolism of these tissues going through a high rate of cell turnover would be dependent on an adequate supply of folic acid, a nutrient essential for DNA and RNA synthesis (Valencia, 1974; Hillman, 1980). The role of folic acid in preventing embryonic mortality could be explained in this manner.

Otel et al. (1972) reported that injection of folicysteine at mating and 9 d postmating increases litter size from 8.5 to 10.0 piglets/litter. The lack of details reported makes a valuable comparison between their results and ours impossible.

The im injections of folic acid tended (P<.12) to increase total live litter weight at birth. This would indicate that a greater quantity of pig tissues was produced due to the folic acid treatment. Pond and Houpt (1978) suggested that litter weight would be dependent on the growth pattern of the placenta, the transfer of nutrients across it and the intrauterine environment. The metabolic function of folic acid mentioned above could explain these phenomena.

The variability in birth weight of piglets within litter (table 1) tended to be greater (P<.08) when folic acid was administered. Fahmy and Bernard (1971) reported that survival rate at weaning may be influenced by intralitter variation in birth weight. English and Smith (1975) reported that mortality rate was 13.7 and 20.1% in groups where the calculated intralitter variation was 112 and 198 g, respectively. In the present research, intralitter variation in birth weight was 199 and 184 g for the folic acid treated and control groups, respectively. It is not known whether a difference of 15 g would be sufficient to influence survival rate at weaning. It was impossible to study this aspect under the conditions of the present experiment.

The results of this research indicate that folic acid plays an important role in controlling litter size at birth. More research is needed to determine the requirements for this nutrient under different management conditions, and to simplify means to maintain the weaning serum folate levels for at least 60 d of gestation.

Literature Cited


J. Reprod. Fertil. 19: 555.