

# Effect of Gestational Folic Acid Supplementation of Sows on Offspring Muscle Development and Postnatal Growth Response

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### Summary and Implications

Pairs of littermate, primiparous sows were penned individually and fed daily 1.9 kg of a low folic acid (FA) (.28 mg/kg) basal diet supplemented with 0 or 8 mg of FA from mating through parturition. All sows were fed the basal diet for 112 days prior to breeding to minimize the sows initial body folic acid stores. FA supplementation in sows during gestation resulted in elevated concentrations of serum FA. However, FA supplementation did not affect litter birth weight, litter muscle, fat, or bone weights, or litter DNA and protein content of three individual muscles at birth. FA supplementation of the sow also did not affect the offspring's body weight gain, feed intake, and gain:feed ratio from body weights of 13 to 107 kg. Based on these data, a dietary folic acid regimen of .3 ppm (.53 mg/day) during pregnancy supports normal muscle growth in pigs pre- and postnatally.

### Introduction

Economically important goals in swine production include the production of viable offspring that grow muscle tissue rapidly and produce highly muscled carcasses. Numerous environmental factors, including the dam's nutritional status, influence whether the offspring will survive and what proportion of an offspring's genetic capacity for growth will be expressed both pre- and postnatally.

Folic acid (FA) has a fundamental role in de novo synthesis of DNA. Therefore, the amount of FA available to the sow at critical times during pregnancy can determine the amount of hyperplastic growth (DNA accretion) of fetal and placental tissues as well as proliferation of immunoglobulin-producing cells. It has been demonstrated that serum FA levels decline during pregnancy in sows to minimal levels during mid-pregnancy (1). It also has been shown that the rate of FA catabolism increases during pregnancy in the rat (2). These findings would support the hypothesis that the FA requirement is increased during pregnancy, presumably to support the rapid rate of DNA synthesis in both fetal and maternal tissues. In a previous report, the sow's FA regimen during gestation was shown to influence the immune responsiveness of the offspring (3).

The objective of this experiment was to determine the influence of dietary FA supplementation for sows during

pregnancy on the muscle development and subsequent performance of the offspring.

### Materials and Methods

Nineteen pairs of littermate primiparous sows from a single genetic strain (moderate lean growth) and source of origin were used for this experiment. In an effort to ensure uniform and minimal FA stores, all sows were put on a low FA regimen for 112 days prior to breeding. During this period, sows were fed 1.9 kg/day of a low FA basal diet (.28 mg of FA/kg diet) (Table 1). The basal diet consisted of a corn/casein mixture supplemented with minerals and vitamins (except FA) at levels that exceeded the gravid sow's estimated nutrient needs (3).

**Table 1. Dietary feeding regimens.**

Stage	Diet	Analyzed FA <sup>a</sup> , mg/kg	Feed offered
Prebreeding	Basal	.28	1.9 kg/d
Pregnancy	Basal (-FA)	.28	1.9 kg/d
	Basal (+FA)	4.4	1.9 kg/d
Lactation	Corn/SBM	1.2	Ad lib
Nursery	Corn/SBM/Whey	2.4	Ad lib
Grower	Corn/SBM	2.1	Ad lib

<sup>a</sup>FA analyzed via Quantaphase II B<sub>12</sub>/folate radioassay; Bio-Rad.

After 98 days on the basal diet, sows within each littermate pair were synchronized by feeding 14 mg of Regumate per day per sow for 14 days and then artificially inseminated with semen from the same boar (high lean growth genotype). Thus, genetic variation in offspring of littermate sows was minimized.

On the day following insemination, sows within each littermate group were randomly allocated to treatment groups (0 or 8 mg supplemented FA per day) and feeding of experimental diets (Table 1) was initiated.

At 5 ± 2 days post-breeding, ovulation rate was determined via laparotomies and visually counting the number of corpus lutea present on each ovary. During the gestation period, sows were individually penned in 2 × 7 ft-stalls on a slotted floor. Sows consumed water ad libitum. Sow serum FA concentrations were determined weekly in 15 representative sows during the 112 depletion period (with the exception of depletion d 105), and weekly in all sows from d 28 to 105 of pregnancy.

On approximately day 109 post-breeding, sows were moved to farrowing rooms. At parturition, sows were allowed to consume a fortified, corn-soybean meal diet (1.2

mg of FA/kg diet) ad libitum from parturition through weaning (Table 1). Each pig farrowed was caught prior to nursing the dam, weighed, and numbered via ear notches. Litters were standardized to  $10 \pm 2$  pigs in an attempt to minimize variability in milk demand among litters.

At birth, two piglets from each litter were sacrificed via jugular injection followed by intracardial injection of sodium pentobarbital immediately post-weighing. These pigs were exsanguinated and frozen for later dissection. All subsequent pigs born were allowed to nurse immediately after weighing.

At  $11 \pm 2$  days of age, two pigs per litter, previously chosen based on the proximity of their birth weight to the mean birth weight of the litter, were treated with ivermectin (Ivomec; Merck-AG Vet, Rahway, NJ), weaned, and transported to nursery rooms isolated from the original sow complex. Each pig was individually penned on a slotted floor in .48 × 1.22-m pens and allowed to consume feed and water ad libitum. Pigs were fed a commercial milk based diet (Soweena® Litter Bites #1; Merrick's, Middleton, WI) for the initial 7 days and then a fortified corn-soybean meal-whey diet through d  $60 \pm 3$  post weaning (Table 1). The corn-soybean meal-whey diet was formulated to meet or exceed the NRC (1998) estimated nutrient requirement for a pig fed from 5 to 20 kg of body weight.

At d  $60 \pm 3$  post-weaning all pigs were moved to an isolated growth facility and individually penned on slotted floors in 2 ft × 7 ft pens. Pigs were fed a fortified corn-soybean meal diet formulated to meet or exceeded all NRC (1988) requirements for pigs 20 to 110 kg body weight (NRC, 1988; Table 1). Body weights and feed consumption were determined weekly during each stage of growth.

#### *Assessment of pre- and postnatal muscle development*

From the whole bodies (with blood removed) of pigs sacrificed at birth, three muscles (longissimus dorsi, rectus femoris, and semimembraneous muscles) were physically isolated, weighed, and analyzed for DNA and protein content. The remainder of the body was physically dissected into tissue components of muscle, fat, bone, skin, viscera, head, feet, and tail and each component was weighed. The dissected muscle was sampled and then all tissue components were combined, ground in liquid nitrogen, and analyzed for DNA and protein content. Composition of the total litter at birth was estimated by multiplying the mean concentration of each component present in the two pigs per litter sacrificed at birth by the litter weight.

When the two pigs per litter reached a body weight of  $110 \pm 5$  kg, they were transported 3 km from the growth facility to the Iowa State University Meat Laboratory. Pigs were then weighed, electrically stunned, and killed via exsanguination. After scalding and removal of the head, the thymus, spleen, heart-lungs, liver, kidneys, gastrointestinal tract (with digesta), reproductive tract, and jowl trim were removed and weighed. Carcasses were then split in halve, reweighed, and chilled for 20 to 24 hours at  $-2^{\circ}$  C.

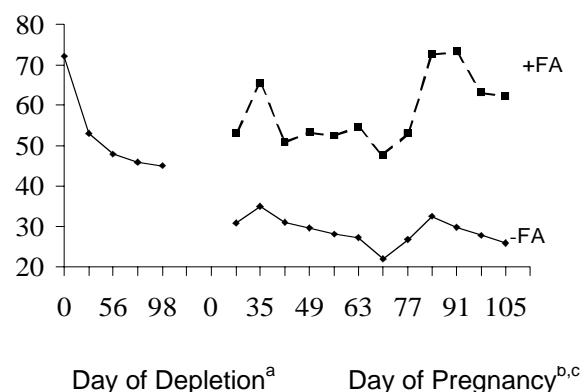
After chilling, cold carcass weight; longissimus muscle area; backfat at the 10th rib midline; backfat thickness at the first rib, last rib, and last lumbar vertebrae; and carcass length were taken on each halve of the carcass. Three muscles (longissimus dorsi, rectus femoris, and semimembraneous) were then isolated from the right side of the carcass, weighed, and frozen for subsequent analysis of DNA and protein content.

Data were analyzed by analysis of variance techniques with general linear model (GLM) procedure of SAS (1996). The sow litter was considered the experimental unit. Pig body weight at weaning was used as a covariate in the analysis of body weight gain, feed intake, and gain:feed data. Pig body weight at slaughter was used as a covariate in the analyses of carcass traits, organ weights, and muscle characteristics of pigs killed at 110 kg of body weight. Least square means are reported.

### **Results and Discussion**

Feeding the basal, low FA diet during the 112-day prebreeding depletion period resulted in a progressive reduction ( $P < .05$ ) in the sow serum FA concentration (Figure 1). Based on these data, the goal of minimizing body FA prior to the initiation of the experiment was achieved. Minimal sow serum FA concentrations were detected at day 70 of pregnancy in both the basal and supplemented groups (21.5 and 47.5 ng/ml, respectively). After this decline, serum levels increased through day 84 or 91, and then again declined preparturition. The initial decline in sow serum FA from day 28 to 70 of pregnancy corresponded to the period of hyperplastic muscle fiber growth in the fetus, whereas the latter decrease corresponds to the period of initial colostrum synthesis. Dietary supplementation of 8 mg/sow/d during pregnancy increased ( $P < .05$ ) serum FA concentration in sows independent of stage of pregnancy (Figure 1). Low gestational serum FA levels have been associated with low birth weight, increased incidence of fetal growth retardation, and increased preterm delivery in humans.

## Sow Serum Folic Acid Concentrations, ng/ml



<sup>a</sup>Number of observations=15.

<sup>b</sup>Number of observations=39.

<sup>c</sup>Effect of FA supplementation,  $P < .05$ .

**Figure 1. Serum folic acid concentration in sows fed the basal diet prior to pregnancy and the basal diet supplemented with 0 (-FA) or 8 (+FA) mg of FA per day during pregnancy.**

The number of pigs born (8.10 vs 8.15) and litter birth weight (10.49 vs 11.52 kg) were not altered by gestational FA supplementation. It is possible that although the sow's serum FA levels differed significantly, the small litter size observed in all sows did not place a great enough demand on the sow for DNA precursor material to result in a difference in either litter size or birth weight.

Gestational FA supplementation did not influence the total weight of muscle, fat, or bone at birth (Tables 2). Gestational FA supplementation resulted ( $P < .10$ ) in greater whole-body DNA and protein content of the litter at birth (Table 3), however, gestational FA supplementation did not alter total litter weight or DNA content of the longissimus dorsi, semimembraneous, or rectus femoris muscles (Table 3). These particular muscles were chosen due to their temporal diversity in fetal development (semimembraneous and rectus femoris muscles developing during mid-gestation, longissimus muscle during mid-to-late gestation). The fact that gestational FA supplementation did not affect development of any of these muscle would imply that at no point during gestation was FA the limiting factor in development of these fetal muscles.

**Table 2. Total neonatal litter tissue weights at birth (presuckle).<sup>ab</sup>**

Criteria	Sow FA Suppl., mg/kg	
	0	8
Number of litters	17	19
Total litter weight, kg	9.92	11.85
Carcass tissue weights		
Muscle, kg	2.87	3.47
Fat, kg	0.05	0.06
Bone, kg	1.31	1.52
Skin, kg	0.87	1.03
Visceral organs, <sup>c</sup> kg	1.40	1.83
Head, kg	1.80	2.17
Feet and tail, kg	1.09	1.28

<sup>a</sup>Litter tissue weights were calculated by multiplying the concentration of each tissue in the individual pigs sacrificed by the litter birth weight.

<sup>b</sup>Values reported are least square means.

<sup>c</sup>Difference due to FA supplementation,  $P < .05$ .

Gestational FA supplementation also did not alter individual muscle weight or DNA and protein content of offspring at 107 kg body weight (Table 4), nor did it affect carcass traits or organ weights of offspring slaughtered at 107 kg body weight (Table 5). These results were not unexpected because, as previously discussed, gestational FA supplementation did not affect tissue distribution at birth.

Gestational FA supplementation did not affect pig weight at weaning ( $3.16 \pm 0.57$  kg), nor did it affect pig daily body weight gain, feed intake, or feed:gain ratio from day 0 to  $56 \pm 3$  postweaning (Table 6) or from day  $56 \pm 3$  post-weaning to slaughter ( $106.6 \pm 2.5$  kg body weight). Daily muscle gain from birth to slaughter also was not affected by gestational FA supplementation (Table 6). Based on these data, the dietary FA regimen of .3 ppm (.53 mg/day) during pregnancy is sufficient to support the offspring's subsequent growth. In contrast, higher dietary FA intakes by sows during pregnancy have shown to allow the offspring to elicit greater immune responses.

**Table 3. Total neonatal litter muscle DNA and protein content.<sup>ab</sup>**

Item	Criteria	Sow FA Suppl., mg/kg	
		0	8
Whole body	DNA, g <sup>c</sup>	30.45	37.85
	Protein, kg <sup>c</sup>	1.04	1.28
Total Muscle	DNA, g	10.54	12.44
Rectus femoris	Wt, g	29.65	30.39
	DNA, mg	115.17	109.83
Semi-membraneous	Wt, g	40.36	47.89
	DNA, mg	144.49	172.08
Longissimus dorsi	Wt, g	99.20	120.94
	DNA, mg	361.21	420.41
	Protein, g	10.15	12.53

<sup>a</sup>Litter muscle DNA and protein were calculated by multiplying the concentration of each muscle component in the individual pigs sacrificed by the litter birth weight.

<sup>b</sup>Values reported are least square means.

<sup>c</sup>Differences due to gestational FA supplementation P<.10.

**Table 4. Individual muscle characteristics of offspring at 107 kg body weight.<sup>a</sup>**

Muscle	Sow FA Suppl., mg/kg	
	0	8
Number of litters	18	17
Longissimus dorsi		
Weight, kg	2.39	2.28
DNA concentration, mg/g	1.29	1.30
DNA content, g	3.05	2.95
Semimembraneous		
Weight, kg	0.74	0.79
DNA, mg/g	1.52	1.51
DNA content, g	1.11	1.20
Rectus femoris		
Weight, kg	0.40	0.40
DNA, mg/g	1.40	1.39
DNA content, g	0.55	0.55

<sup>a</sup>Least square means reported. Data adjusted for pig body weight.

**Table 5. Carcass traits and organ weights of offspring at 107 kg body weight.<sup>a</sup>**

Tissue	Sow FA Suppl., mg/kg	
	0	8
Number of litters	18	17
Pig weight, kg	107.02	106.95
Hot carcass weight, kg	80.74	80.34
Hot carcass yield, %	75.56	75.16
Average midline backfat, cm <sup>a</sup>	2.92	3.04
Tenth rib backfat, cm	2.64	2.79
Loin eye area at 10 <sup>th</sup> rib, sq in	5.68	5.59
Carcass length, cm	80.16	79.29
Carcass percent muscle, %	56.91	56.25
Organ weights, kg		
Heart and lungs	1.55	1.55
Liver	1.50	1.56
Leaf fat	1.44	1.56
Kidneys	0.32	0.33
Thymus	0.11	0.10
Spleen	0.15	0.14
Gastrointestinal tract	8.12	8.38
Head	5.91	6.00

<sup>a</sup>Least square means are reported. Prekill pig weight was used as a covariate in data analysis.

<sup>b</sup>Average of backfat measurements at the first rib, last rib, and last lumbar vertebrae.

**Table 6. Growth and efficiency of feed utilization of offspring post-weaning.<sup>a</sup>**

Criteria	Day post Weaning	Sow FA Suppl., mg/kg	
		0	8
No. of litters	0	19	19
	56	19	19
	Slaughter	18	17
Pig weight, kg	0	3.05	3.33
	56	28.5	30.0
	Slaughter	106.3	107.0
Weight gain, kg/day	0 to 56	0.47	0.49
	56 to slaughter	0.84	0.88
Feed intake, kg/day	0 to 56	0.77	0.81
	56-slaughter	2.59	2.71
Gain:feed ratio	0 to 56	0.61	0.61
	56-slaughter	0.33	0.32
Muscle gain, kg/day <sup>b</sup>	Birth-slaughter	0.28	0.29

<sup>a</sup>Least square means reported. Pig body weight at weaning (day 0) was used as a covariate.

<sup>b</sup>Pig body weight at birth was used as a covariate.

### References

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