INFLUENCE OF L-CARNITINE ON LITTER CHARACTERISTICS FROM GILTS HARVESTED AT DAY 40, 55, AND 70 OF GESTATION[1]

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Summary

A total of 59 gilts were used to determine the effects of supplemental L-carnitine on reproductive performance. Experimental treatments were arranged in a 2 × 3 factorial with main effects of L-carnitine (0 or 50 ppm) and day of gestation (40, 55, or 70). All gilts received a constant feed allowance of 3.86 lb/day and a top-dress containing either 0 or 88 mg of L-carnitine, starting on the first day of breeding and continuing until the day of harvest. Total litter size, total litter weight, and crown-to-rump length of fetuses were not different (P>0.10) between treatments at any gestation length. By d 70 of gestation, average fetus weight was heavier (P = 0.06) for fetuses from gilts fed L-carnitine, compared with fetuses from gilts fed the control diet. In addition, at d 70, fetal insulin-like growth factor-II (IGF-II) concentrations were lower (P = 0.09) for fetuses from gilts fed L-carnitine than for fetuses from gilts fed the control diet. Feeding L-carnitine may have decreased fetal IGF-II, therefore increasing cell proliferation and delaying cell differentiation. These results show that providing supplemental L-carnitine to gestating gilts has beneficial effects on average fetal weight, possibly observed because of its ability to reduce fetal IGF-II concentrations.

(Key Words: Fetus, Gestation, Gilts, IGF-II, L-carnitine.)

Introduction

Research has shown that supplementing sow's diets with L-carnitine during gestation improves performance criteria. Providing supplemental L-carnitine in a sow's diet has been shown to increase pig and litter weights at birth, reduce the number of stillborn pigs, and increase litter size. Research reported in a separate article (page 12) showed that supplementing L-carnitine to gestating gilts numerically increased weight gain at d 70 of gestation, but no differences were observed for maternal IGF-I. No data currently exist for the effects of L-carnitine on fetal IGF-II values.

The addition of dietary L-carnitine to gestation diets has resulted in inconsistent data on first-parity reproductive performance. Therefore, our objective of this study was to deter-

[1]The authors thank Lonza, Inc., Allendale, New Jersey, for their financial support. Sincere appreciation is expressed to Cassie Benz, Leah Bond, Alexa Hayes, Drew Taylor, Meghan Tindle, and Amanda Wetzel for their assistance in data collection

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mine the effects of supplementing with L-carnitine through the developmental stages of gestation. We examined total litter weight, average fetal weight, total fetus number, fetus number in the left and right uterine horn, fetal crown-to-rump length, fetal IGF-II, and gilt corpora lutea on each ovary at three gestation points.

Procedures

Animals and Feeding Protocol. All animal procedures used in this study were reviewed and approved by the Kansas State University Animal Care and Use Committee. Fifty-nine terminal gilts (PIC, Franklin, KY; L327 × 1050; BW = 303.6 lb) were artificially inseminated (PIC; MQ 280) 12, 24, and 36 h after the onset of the second observed estrus. Gilts were housed in individual gestation crates (7 ft × 22 in) and allowed *ad libitum* access to water in an environmentally controlled gestation barn at the Kansas State University Swine Teaching and Research Center. Gilts were randomly allotted to one of two dietary treatments and one of three harvesting dates (d 40, 55, or 70 of gestation) on the basis of weight at breeding. All gilts were fed a corn-soybean meal-based gestation diet (Table 1) once daily (3.86 lb/d), and received a 50-g top-dress containing either 0 (control, n = 30) or 88 mg (equivalent to approximately 50 ppm on an as-fed basis) of L-carnitine (Carniking 10 (10% of L-carnitine), n = 29; Lonza Group, Inc., Allendale, NJ) from d 1 to d 39, 54, or 69 of gestation.

Harvesting Protocol and Collection of Samples. Gilts were harvested at d 40, 55, or 70 of gestation. Fifteen hours before harvest, gilts were transported from the Kansas State University Swine Teaching and Research Center to the Kansas State University Meat Laboratory. Gilts were allowed *ad libitum* access to water, and collections were performed 24 h after the last feeding. Gilts were harvested by electrical stunning, followed by exsanguination. A mid-lateral incision was made to gain access to the abdominal cavity, and the uterus was removed. Once the uterus was removed, the number of fetuses was determined on both sides, and fetuses were immediately removed under aseptic conditions. The fetuses were transported to the Kansas State University Growth Laboratory for additional processing. The number of corpus lutea on each ovary was determined.

Table 1. Diet Composition Fed During Gestation[a]

Item	Gestation Diet
Ingredient, %	
Corn	81.22
Soybean meal (46.5% CP)	14.55
Monocalcium P (21% P)	2.03
Limestone	1.05
Salt	0.50
Vitamin premix	0.25
Trace mineral premix	0.15
Sow add pack	0.25
	100.00
Calculated analysis	
Lysine, %	0.65
ME, kcal/lb	1,483
Protein, %	13.7
Ca, %	0.85
P, %	0.75
Available P, %	0.48

[a]Gestation feeding of 3.86 lb/d, with or without a top-dress providing 88 mg/d (equivalent to approximately 50 ppm on an as-fed basis) added L-carnitine.

Fetal Blood Collection, Weights, and Lengths. Individual fetus weight and crown-

to-rump length were determined. Total litter weight was calculated as the sum of the individual fetus weights per litter. Fetal blood was collected from the heart of each fetus, and pooled with the other fetuses in the litter for determination of fetal IGF-II.

Statistical Analysis. Fetal weights, lengths, and circulating IGF-II, and gilt corpora lutea, were analyzed as a 2 × 3 factorial design with the MIXED procedure of SAS. Fixed effects included treatment, day of harvest, and their interaction. Kenward-Roger adjustment was used for the degrees of freedom. Proc Mixed of SAS was used to determine the slopes of average fetus weight vs. fetus number per litter at d 40, 55, and 70 of gestation. Fixed effects included treatment × day of harvest, and treatment × day of harvest × fetus number. Estimate statements were used to determine slope differences of average fetus weight vs. number of fetuses per litter. Regression was used to determine if the slopes were different from zero. The fixed effect included number of fetuses, separated by treatment and day of harvest. The Fisher's Exact method was used to determine p-values of a chi-square statistic between differences in the number of litters having detectable IGF-II for control gilts and those fed L-carnitine. The significance was declared at $P<0.05$ unless otherwise noted.

Results and Discussion

Total litter size and total litter weight were not different ($P>0.05$) at d 40, 55, or 70 of gestation for the gilts fed L-carnitine and those fed the control diet. In addition, no differences ($P>0.05$) were observed between treatments for number of fetuses in the right uterine or left uterine horn and fetal crown-to-rump length at the three gestation lengths. No differences ($P>0.05$) were observed in total, right, or left corpus lutea at d 40, 55, and 70 gestation for the gilts fed L-carnitine and those fed the control diet. The number of corpora lutea located on the left ovary decreased ($P = 0.07$) as gestation length increased. As gestation length increased, total litter weight, average fetal weight, and fetal crown-to-rump length increased ($P<0.05$), but total number of fetuses and number of fetuses in the right and left uterine horns decreased ($P<0.05$). At d 70 of gestation, fetuses from the gilts fed L-carnitine tended to be heavier ($P = 0.06$) than fetuses from the control gilts (Table 2; 236.6 g vs. 217.7 g, respectively). Fetal IGF-II concentrations tended to be lower ($P = 0.09$) at d 70 for the fetuses from the gilts fed L-carnitine than for the fetuses from the gilts fed the control diet (17.9 ng/mL vs. 22.9 ng/mL). In addition, fetal IGF-II was undetectable for ten of the twenty L-carnitine litters analyzed, and only one control litter had undetectable IGF-II (data not shown; $P = 0.0033$).

Average fetal weight was positively correlated ($R^2 = 0.68$) with number of fetuses for the control pigs at d 40 of gestation, whereas L-carnitine litters showed a weak positive correlation. Therefore, at d 40 of gestation, there was a strong correlation that the more fetuses per litter in the control sows, the heavier the average fetus was for those litters. At d 55 of gestation, there was no correlation (L-carnitine, $R^2 = 0.0001$; control $R^2 = 0.01$) between average fetus weight and number of fetuses per litter for the two treatments. At d 70 of gestation, negative correlations were observed (Figure 1). As number of fetuses per litter increased, the average fetus weight decreased. On d 70, correlations among average fetus weight and number of fetuses per litter were weak (L-carnitine, $R^2 = 0.17$; control $R^2 = 0.08$). Through gestation, correlations went from positive to negative. The correlations were different ($P = 0.06$) between d 40 and d 70 of gestation for the L-carnitine litters. The slopes of the litters from the gilts fed the control diet followed the same trend, but differences were not significant. In addition, the slopes at d 40, 55, and 70 were not different

between the two treatments (P>0.05; Figure 1A, B, and C).

In the current study, pigs from gilts fed supplemental L-carnitine had heavier average fetal weights at d 70 of gestation than the pigs from gilts fed the control diet, but no changes were observed in maternal IGF-I (see separate report, page 12). The role of maternal IGF-I in fetal muscle growth has been unclear. Unequivocal findings on the effects of maternal IGF-I from L-carnitine supplementation have shown that L-carnitine has increased and decreased maternal IGF-I concentrations. Researchers have found that supplemental L-carnitine increased maternal IGF-I and litter weights, concluding that maternal IGF-I played a role in increased fetal muscle proliferation. In contrast, other researchers have found no increase in circulating IGF-I in sows fed L-carnitine and having heavier litters. This suggests that the heavier litter weights from sows fed L-carnitine were not due to maternal IGF-I. The results of the current study suggest that circulating IGF-I is similar for gilts fed supplemental L-carnitine and gilts fed diets without supplemental L-carnitine. Therefore, the role of maternal IGF-I on impacting fetal muscle growth is unclear.

In this study, we found half of the litters from gilts fed L-carnitine had no detectable fetal IGF-II, and fetal IGF-II concentrations were lower at d 70 of gestation from the gilts fed L-carnitine. In muscle cells, IGF-I promotes muscle proliferation; IGF-II can promote muscle differentiation. The average fetus weight from the gilts fed L-carnitine was 18.9 g heavier than the average fetus weight from the gilts fed the control diet at d 70 of gestation. Feeding L-carnitine may have increased fetal IGF-I and decreased fetal IGF-II. Therefore, feeding L-carnitine may have increased cell proliferation, producing heavier fetuses, and caused a delay in muscle cell differentiation late in gestation.

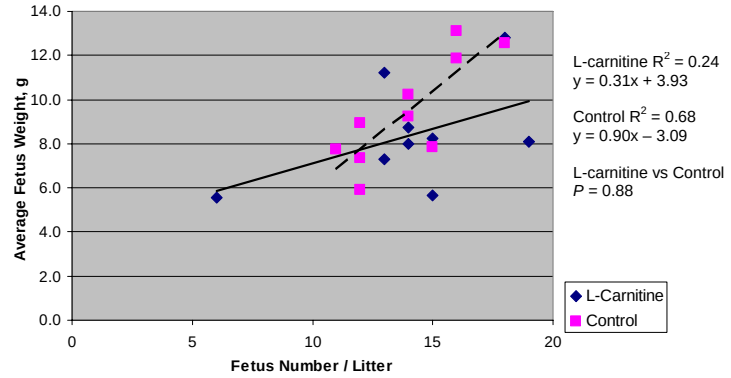
Previous research has reported that increased uterine crowding in prolific species in early gestation has negative consequences on muscle-fiber development, specifically secondary muscle-fiber development. At d 40 of gestation, we observed positive correlations for number of fetuses per litter vs. average fetus weight. Slopes were different for the L-carnitine litters at d 40 and 70 (P = 0.06), which suggests a biological change between d 40 and 70 of gestation. This may have contributed to the observed increase in fetal weight in the fetuses at d 70 from the gilts receiving supplemental L-carnitine. Fetal IGF-II concentrations were lower for the L-carnitine fetuses at d 55 and 70 of gestation. L-carnitine may be reducing fetal IGF-II concentrations and inhibiting uterine crowding early in gestation, therefore having beneficial consequences for muscle-fiber development.

Providing supplemental L-carnitine to gestating gilts increased gilt BW and average fetus weight at d 70 of gestation, and may have reduced uterine crowding at d 40 of gestation. L-carnitine may be inhibiting differentiation and positively regulating proliferation of myogenic cells in the fetus through gestation, therefore increasing fetal weight. Additional knowledge about developmental regulation of the growth-factor system in cultured myogenic cells from gilts fed L-carnitine will aid in our understanding of increased weight observed in fetuses from gilts fed L-carnitine. Therefore, our future research will focus on growth-factor expression from cultured porcine embryonic myoblasts at d 40, 55, and 70 of gestation. Results from the rest of this study will be published in a future report.

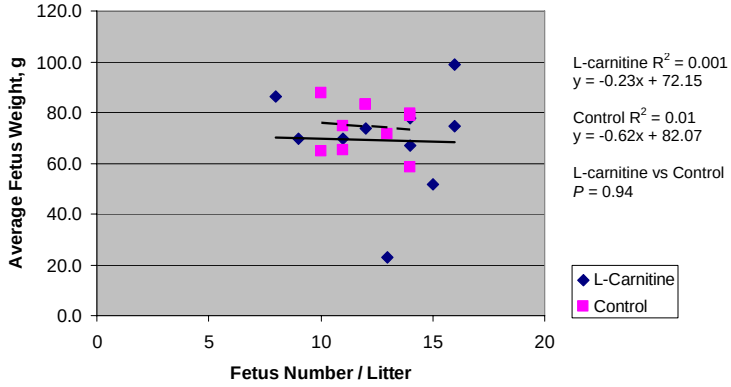
Table 2. Effects of L-carnitine on Litter and Ovary Characteristics

							Probability, $P <$					
	Control			L-carnitine			Control vs. L-carnitine			Trt SE		
Item	d 40	d 55	d 70	d 40	d 55	d 70	d 40	d 55	d 70	d 40	d 55	d 70
No. of Gilts	10	10	10	9	10	10						
Litter												
Total weight, g	136.7	901.0	2484.5	122.4	883.9	2657.1	0.94	0.93	0.35	188.20	183.18	183.18
Average fetal weight, g	9.5	74.6	217.7	8.4	69.2	236.6	0.92	0.59	0.06	10.30	9.95	9.95
Total fetus no.	14.0	12.1	11.5	14.1	12.8	11.4	0.93	0.56	0.93	1.23	1.20	1.20
Fetus no. right horn	7.4	5.7	5.8	6.9	6.1	6.2	0.51	0.60	0.60	0.77	0.75	0.75
Fetus no. left horn	6.6	6.3	5.7	7.1	6.7	5.1	0.49	0.63	0.45	0.76	0.74	0.74
Crown to rump, cm	5.3	12.0	16.8	5.2	11.6	16.8	0.81	0.32	0.91	0.33	0.32	0.32
IGF-II,	*	16.3	22.9	*	14.5	17.6	*	0.62	0.09	*	3.54	3.04
Corpora lutea												
Total	17.2	16.9	15.7	18.1	17.1	16.4	0.50	0.84	0.61	1.30	1.26	1.26
Right ovary	9.4	9.5	9.6	9.4	8.7	9.5	0.99	0.53	0.93	1.30	1.26	1.26
Left ovary	7.8	7.6	6.1	8.8	8.4	6.9	0.41	0.49	0.49	1.18	1.15	1.15

	Overall		
Litter	Trt	Day	Trt × Day
Total weight, g	0.66	<0.001	0.71
Average fetal weight, g	0.48	<0.001	0.19
Total fetus no.	0.74	0.01	0.89
Fetus no. right horn	0.83	0.05	0.63
Fetus no. left horn	0.80	0.02	0.54
Crown to rump, cm	0.44	<0.0001	0.80
IGF-II	0.14	0.05	0.45
Corpora lutea			
Total	0.42	0.20	0.94
Right ovary	0.68	0.88	0.90
Left ovary	0.20	0.07	0.99



A



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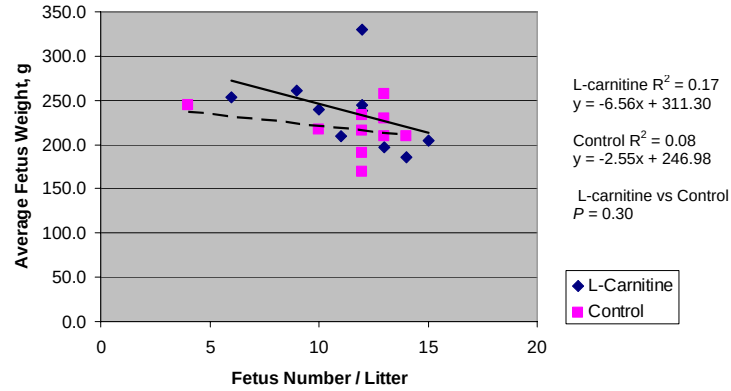

C

Figure 1. Relationship Between the Number of Fetuses per Gilt and Average Fetus Weight at Day 40 (A), 55 (B), and 70 (C) Gestation.