Growth characteristics, blood metabolites, and insulin-like growth factor system components in maternal tissues of gilts fed L-carnitine through day seventy of gestation[1,2]

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ABSTRACT: A total of 59 gilts (BW = 137.7 kg) from 3 breeding groups were used to assess the effects of feeding L-carnitine during gestation on gilt growth characteristics, blood metabolites, and uterine and chorioallantoic expression of IGF axis components at d 40, 55, and 70 of gestation. Experimental treatments were arranged in a 2×3 factorial, with main effects of added L-carnitine (0 or 50 ppm) and day after initial breeding (d 40, 55, or 70 of gestation). All gilts received a constant feed allowance of 1.75 kg/d and a top-dress containing 0 or 50 ppm of L-carnitine beginning on the first day of breeding through the assigned day of gestation. No dietary treatment differences were observed for gilt BW, backfat, or estimated protein or fat mass at any day of gestation. No differences were observed in circulating total and free carnitine at breeding, but concentrations increased ($P < 0.01$) as day of gestation increased for gilts fed diets containing L-carnitine compared with those fed the control diet. Maternal IGF-I concentration decreased ($P < 0.01$) from d 0 to 70 for all gilts, with no differences between treatments. Insulin-like growth factor binding protein-3 mRNA ($P = 0.05$) and IGFBP-5 mRNA ($P = 0.01$) increased in the endometrium of gilts supplemented with L-carnitine. These data demonstrate that L-carnitine supplementation and day of gestation alter the expression of the IGF axis by changing the expression of IGFBP at the fetal-maternal interface in swine. These changes in the IGF axis at the fetal maternal interface may aid in determining the reasons for the effects of L-carnitine on reproductive traits.

Key words: gestation, gilt, insulin-like growth factor, insulin-like growth factor binding proteins, L-carnitine, messenger RNA

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INTRODUCTION

Previous research has shown the addition of L-carnitine, a vitamin-like water-soluble quaternary amine, to maternal gestation diets may increase BW gain (Musser et al., 1999; Ramanau et al., 2002), last-rib backfat (Musser et al., 1999), and plasma IGF-II (Doberenz et al., 2006) of gestating sows. In addition, supplementing L-carnitine to gestating sows has shown increased total number of pigs born and born alive (Ramanau et al., 2004; Birkenfeld et al., 2005), decreased number of stillborn pigs (Musser et al., 1999; Doberenz et al., 2006), and increased average pig (Eder et al., 2001; Ramanau et al., 2002, 2004) and litter weights (Ramanau et al., 2002, 2004; Doberenz et al., 2006) at birth. However, research has not shown an equivalent response of maternal IGF-I due to L-carnitine supplementation (Musser et al., 1999; Waylan et al., 2005).

These changes in sow growth and reproductive performance might be the result of an influence on the IGF system. However, the role of L-carnitine in altering IGF and IGFBP at the fetal-maternal interface has not been

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thoroughly investigated. In 2005, Waylan et al. conducted a study using fourth parity sows to investigate the effects of L-carnitine on fetal growth and the IGF system in pigs at d 55 of gestation. Waylan et al. (2005) found no changes in IGF axis components in maternal uterine chorioallantoic tissues. Conversely, Waylan et al. did find differences in IGF-II mRNA and myogenin mRNA abundance in porcine embryonic myoblasts in sows fed L-carnitine compared with the controls.

Therefore, the objective of this study was to evaluate the effects of supplementing L-carnitine through developmental stages up to d 70 of gestation in gilts. In gestation, gilts and sows are fed for combinations of body growth, body maintenance, and fetal growth; gilts are growing at a greater rate compared with sows. Components of the IGF axis were evaluated at d 40, 55, and 70 of gestation in the myometrium, endometrium, and chorioallantois of gilts.

MATERIALS AND METHODS

Animals

All animal procedures used in this study were reviewed and approved by the Kansas State University Animal Care and Use Committee. Fifty-nine gilts (PIC, Franklin, KY; L327 × 1050; BW 137.7 kg; 190 d of age) received AI (PIC; MQ 280) 12, 24, and 36 h after the onset of their second observed estrus. Day one of gestation was considered 12 h after the first insemination. Gilts were housed in individual crates (1.83 × 0.55 m) in an environmentally controlled gestation barn at the Kansas State University Swine Teaching and Research Center from AI until d 39.5, 54.5, or 69.5 of gestation. Gilts were allowed ad libitum access to water and were randomly allotted to 1 of 2 dietary treatments and 1 of 3 slaughter dates based on BW at breeding.

All gilts were fed a corn-soybean meal, gestation diet (Table 1) once daily (1.75 kg/d; as-fed basis) and received a 50-g, ground corncob, top-dress containing 0 mg (control, n = 30) or 88 mg of L-carnitine (50 ppm L-carnitine, n = 29; Carniking 10, 10% L-carnitine, Lonza Group Inc., Allendale, NJ) from d 1 to d 39, 54, or 69 of gestation. The gestation diet was formulated based on NRC (1998) models to be slightly above the requirements for maintenance and fetal growth. Fetal and uterine gain throughout pregnancy was predicted to be 25 kg, with maternal gain predicted at an additional 13.6 kg (Aherne and Kirkwood, 1985; Williams et al., 1985). At this feeding level, gilts should have maintained backfat levels throughout gestation while increasing BW. The total gestation energy requirement was determined by summing the daily energy requirement, energy for maternal gain, plus energy for products of fetal and uterine gain.

Backfat was measured at the P2 position (last rib) on both sides of the backbone using a Lean-Meter (Renco Corporation, Minneapolis, MN), and BW were determined at breeding, and on d 39, 54, and 69 of gestation.

Table 1. Diet composition fed during gestation (as-fed basis)[1]

Item	
Ingredient, %	
Corn	81.22
Soybean meal, 46.5% CP	14.55
Monocalcium phosphate, 21% P	2.03
Limestone	1.05
Salt	0.50
Vitamin and trace mineral premix[2]	0.65
	100.00
Calculated analysis	
Lysine, %	0.65
ME, Mcal/kg	3.27
CP, %	13.7
Ca, %	0.85
P, %	0.75
Available P, %	0.48

[1]Gestational feeding levels of 1.75 kg/d, with a 50-g ground corncob top-dress containing 0 mg (control, n = 30) or 88 mg of L-carnitine (50 ppm L-carnitine, n = 29; Carniking 10, 10% L-carnitine, Lonza Group Inc., Allendale, NJ) from d 1 to d 39, 54, or 69 gestation. Predicted L-carnitine content of the basal diet was 43.8 ppm (Jacobs, 2002).

[2]Supplied per kilogram of diet: 11,025 IU of vitamin A; 1,654 IU of vitamin D_3; 55.1 mg of niacin; 44.1 IU of vitamin E; 33.1 mg of pantothenic acid; 9.9 mg of riboflavin; 4.4 mg of vitamin K (menadione); 0.04 mg of vitamin B_{12}; 551.3 mg of choline; 15.2 mg of pyridoxine; 1.65 mg of folic acid; 0.22 mg of biotin; 165 mg of Zn (oxide); 165 mg of Fe (sulfate); 39.7 mg of Mn (oxide); 16.5 mg of Cu (sulfate); 0.30 mg of I (as Ca iodate); and 0.30 mg of Se (as Na selenite).

Protein and fat mass were estimated using the prediction equations of Dourmad et al. (1998): protein mass = 2.28 + [0.178(BW, kg)] – [0.333(backfat, mm)], and fat mass = –26.40 + [0.221(BW, kg)] + [1.331(backfat, mm)].

Blood Collection

At 0, 39, 54 and 69 d of gestation, blood was collected by venipuncture for determination of circulating IGF-I and free and total carnitine. Blood samples were collected in heparinized and nontreated tubes and placed on ice until centrifuged (2,500 × *g* for 20 min at 4°C) or refrigerated (4°C) for 24 h before centrifugation, respectively. Plasma or sera were then obtained and frozen (–20°C) until analysis. The concentrations of free and total plasma carnitine (Parvin and Pande, 1977) and serum IGF-I (Active IGF-I with Extraction, DSL-5600; Diagnostics Systems Laboratory Inc., Webster, TX) were determined. For IGF-I, the assay sensitivity was 6.0 ng/mL, and the intraassay CV was 6.2% and the interassay CV was 9.0%.

Slaughter Protocol and Collection of Samples

Slaughter was on d 40, 55, or 70 of gestation. Fifteen hours before slaughter, the gilts were transported from the Kansas State University Swine Research and Teaching Center to the Kansas State University Meat Laboratory, where sample collections were performed

24 h after the last feeding. Gilts were allowed ad libitum access to water until slaughter. Gilts were killed by electrical stunning followed by exsanguination. A midlateral incision was then made to gain access to the abdominal cavity. The ovarian pedicles and the uterus at the level of the cranial cervix were cut, and the uterus was removed. Uterus (myometrium and endometrium) and chorioallantoic samples were excised at the level of the middle conceptus from each uterine horn. The tissues samples were immediately frozen in liquid nitrogen and stored at –80°C.

Sample Preparation and RNA Isolation

Total RNA was isolated from the endometrium and myometrium by using the RNeasy Mini Kit (Qiagen, Valencia, CA). The total RNA was isolated from the chorioallantoic samples using TRI Reagent (Sigma, St. Louis, MO). Samples isolated with TRI Reagent were treated with DNase to remove any contaminating genomic DNA using a commercially available kit (DNA-*free*, Ambion, Austin, TX). The concentration of RNA was determined by absorbance at 260 nm. Electrophoresis of total RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining to allow visualization of 28S and 18S ribosomal RNA (**rRNA**) was used to assess the integrity of RNA. One microgram of total RNA was then reverse-transcribed to produce the first-strand complementary DNA (**cDNA**) using TaqMan reverse transcription (Applied Biosystems, Foster City, CA) following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis.

Real-Time Quantitative PCR

Real-time quantitative PCR was used to measure the quantity of mRNA for IGF-I, IGF-II, IGFBP-3, IGFBP-5, and 18S rRNA in total RNA isolated from the myometrium, endometrium, and chorioallantois. Measurement of the relative quantity of cDNA was carried out using TaqMan Universal PCR Master Mix, 900 n*M* of the appropriate forward and reverse primers, 200 n*M* of the appropriate TaqMan detection probe, and 1 μL of the cDNA mixture. Sequences for primers and probes for IGF-I, IGF-II, IGFBP-3, and IGFBP-5 are presented in Table 2. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; GenBank Accession No. X03205). Assays were performed on an ABI Prism 7000 sequence detection system (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (50 cycles of 15 s at 95°C and 1 min at 60°C). Relative expression of mRNA for IGF-I, IGF-II, IGFBP-3, and IGFBP-5 were normalized to the 18S rRNA endogenous control and expressed as arbitrary units.

Statistical Analysis

All data are shown as means and SE. Statistical analyses for backfat, BW, and blood metabolites of gilts were performed with the MIXED procedure (SAS Inst. Inc., Cary, NC). Data were analyzed as repeated measures only for gilts slaughtered at d 70 of gestation (control, n = 10; L-carnitine n = 10). The model included treatment as the fixed effect and day of sampling as the repeated measure. Kenward-Roger adjustment was used for the degrees of freedom.

For all genes evaluated, mRNA concentrations were analyzed as a 2 × 3 factorial arrangement with the MIXED procedure of SAS. The significance was declared at $P < 0.05$ unless noted otherwise.

RESULTS

Gilt Growth Parameters

No differences between dietary treatments were observed for BW gain, backfat, estimated protein mass or estimated fat mass at any day of gestation. As day of gestation increased, these response criteria differed (Table 3; $P < 0.01$).

Plasma and Sera Analysis

No differences were observed in maternal plasma IGF-I collected at d 0, 40, 55, and 70 of gestation between the 2 treatments (Figure 1). Plasma IGF-I concentrations decreased ($P < 0.01$) as day of gestation increased from d 0 to 40 for L-carnitine and control-fed gilts.

As expected, no differences were observed in total and free (Table 3) carnitine between the gilts fed L-carnitine or the control diet at d 0 of gestation, but these increased ($P < 0.01$) on d 40, 55, and 70 of gestation for gilts fed additional L-carnitine. A treatment × day of gestation was detected ($P < 0.01$) for total and free carnitine.

No significant treatment × day of gestation interactions were noted for any genes of tissues evaluated. In the chorioallantois, IGF-I, IGF-II, IGFBP-3, and IGFBP-5 mRNA levels were not different between gilts fed diets with supplemental L-carnitine and the control diet, and no differences were observed in IGF-II, IGFBP-3, and IGFBP-5 mRNA expression as day of gestation increased (Table 4). In the endometrium, no differences were observed in IGF-I, IGF-II, IGFBP-3, and IGFBP-5 mRNA expression as day of gestation increased. However, IGFBP-3, and IGFBP-5 mRNA expression was greater in gilts fed diets with supplemental L-carnitine (IGFBP-3, $P = 0.05$; IGFBP-5, $P = 0.01$) compared with the control-fed gilts. In the myometrium, IGF-I, IGF-II, IGFBP-3, and IGFBP-5 mRNA expression was not different in gilts fed diets containing supplemental L-carnitine compared with the gilts fed the control diet. As day of gestation increased from d 40 to 55, there was a decrease in IGF-I mRNA ($P < 0.01$), IGFBP-3 mRNA ($P = 0.10$), and IGFBP-5 mRNA ($P < 0.01$) expression (Table 4).

Table 2. Primers and probes used for real-time quantitative PCR

Gene	GenBank accession number[1]		Sequence
IGF-I	M31175	Forward	TCTTCTACTTGGCCCTGTGCTT
		Reverse	GCCCCACAGAGGGTCTCA
		Probe	6FAM-CCTTCACCAGCTCTGCCACGGC-TAMRA
IGF-II	X56094	Forward	CCGGACAACTTCCCCAGATA
		Reverse	CGTTGGGCGGACTGCTT
		Probe	6FAM-CCCGTGGGCAAGTTCTTCCGC-TAMRA
IGFBP-3	AF085482	Forward	AGCACGGACACCCAGAACTT
		Reverse	CGGCAAGGCCCGTATTC
		Probe	6FAM-TCCTCTGAGTCCAAGCGCGAGA-TAMRA
IGFBP-5	U41340	Forward	GGCAGAGGCCGTGAAGAAG
		Reverse	CAGCTCCCCCCACGAACT
		Probe	6FAM-CCGCAGAAAGAAGCTGACCCAGTCC-TAMRA

[1]Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide&itool=toolbar (last accessed on 19 March 2007).

Table 3. Effects of L-carnitine on gilt growth characteristics and blood parameters[1] for gilts slaughtered on d 70 of gestation

Item	Control	L-Carnitine	*P*-value	SE
No. of gilts	10	10		
BW, kg				
D 0	136.4	135.5	0.86	5.08
D 40	150.5	154.2	0.47	5.08
D 55	158.5	162.4	0.44	5.08
D 70	166.2	170.3	0.43	5.08
Estimated protein mass,[2] kg				
D 0	23.0	22.8	0.82	0.91
D 40	25.3	25.8	0.66	0.91
D 55	26.7	27.3	0.50	0.91
D 70	28.2	29.0	0.40	0.91
Backfat, mm				
D 0	15.0	15.2	0.83	0.95
D 40	16.3	17.9	0.10	0.95
D 55	16.7	17.2	0.60	0.95
D 70	15.9	15.4	0.60	0.95
Estimated fat mass,[3] kg				
D 0	27.3	27.3	0.99	1.30
D 40	31.2	33.0	0.16	1.30
D 55	33.2	34.4	0.34	1.30
D 70	34.4	35.1	0.61	1.30
Total carnitine, μM				
D 0	8.3	8.6	0.77	1.15
D 40	10.1	13.6	<0.01	1.17
D 55	9.8	15.2	<0.01	1.15
D 70	10.9	18.3	<0.01	1.15
Free carnitine, μM				
D 0	7.0	7.1	0.86	0.96
D 40	8.4	11.5	<0.01	0.98
D 55	8.6	12.5	<0.01	0.96
D 70	9.6	15.4	<0.01	0.96
	Treatment	Day	Treatment × Day	
BW, kg	0.58	<0.01	0.11	
Estimated protein mass, kg	0.64	<0.01	0.20	
Backfat, mm	0.50	<0.01	0.31	
Estimated fat mass, kg	0.44	<0.01	0.10	
Total carnitine, μM	<0.01	<0.01	<0.01	
Free carnitine, μM	<0.01	<0.01	<0.01	

[1]D 0 to 70.
[2]Prediction equation from Dourmad et al. (1998): 2.28 + [0.178(BW, kg)] – [0.333(backfat, mm)].
[3]Prediction equation from Dourmad et al. (1998): –26.40 + [0.221(BW, kg)] + [1.331(backfat, mm)].

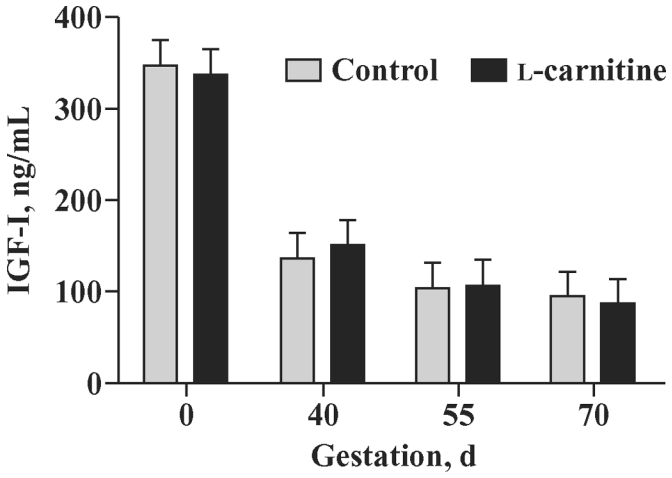

Figure 1. Plasma insulin-like growth factor-I concentrations of gilts fed diets unsupplemented (control) or supplemented with L-carnitine (50 mg/kg) at 3 d of gestation; day effect, $P < 0.01$.

DISCUSSION

Previous research has shown supplementing L-carnitine increases circulating carnitine concentrations in streptozotocin-induced diabetic rats (Heo et al., 2001), finishing pigs (Owen et al., 2001), and gestating sows at d 57 (Waylan et al., 2005), 60, and 90 of gestation (Musser et al., 1999). The results herein agree that supplementing L-carnitine to the gestating dam increases circulating free and total carnitine at greater concentrations at d 40, 55, and 70 of gestation compared with gilts not supplemented with L-carnitine. Increasing circulating L-carnitine concentrations via dietary supplementation has shown positive effects on carcass leanness (Owen et al., 2001), gilt growth (Musser et al., 1999; Ramanau et al., 2002), and reproductive performance (Musser et al., 1999; Eder et al., 2001; Ramanau et al., 2004).

In newborn pigs, Kempen and Odle (1995) found that L-carnitine increases fatty acid oxidation in hepatocytes and Owen et al. (2001) suggests L-carnitine reduces backfat in finishing pigs by the acceleration of the β-oxidation of fatty acids in the liver mitochondria and hepatocytes, in turn increasing the rate of acetyl CoA production. In this study, no differences were observed between treatments at d 40, 55, or 70 of gestation. In 1999, Musser et al. found an increase in backfat in sows fed supplemental L-carnitine at d 119 of gestation. Musser et al. (1999) suggest the increase in backfat observed in their study was due to the differences in partitioning of nutrients between finishing pigs and gestating sows and the ability of the sow to store more adipose tissue under conditions of supplemental L-carnitine.

In the current study, no differences were found in gilt BW at d 40, 55, or 70 of gestation, but significant increases in sow BW through gestation was observed by other researchers (Musser et al., 1999; Eder et al., 2001; Ramanau et al., 2002). Fetal and uterine gain throughout pregnancy was predicted to be 25 kg with

Table 4. Effects of L-carnitine on gilt chorioallantoic and uterine mRNA concentrations of IGF-system genes on d 40, 55, and 70 of gestation[1]

	Day of gestation									
	40		55		70					
	L-carnitine,[2] mg/kg							*P*-value		
Gene	0	50	0	50	0	50	SE	Treatment	Day[3]	Treatment × Day
Chorioallantois										
IGF-I	1.9	1.7	2.7	2.3	11.2	3.1	4.18 to 4.29	0.24	0.16	0.34
IGF-II	4,728	5,564	5,825	6,542	6,858	5,510	1,682 to 1,728	0.96	0.71	0.70
IGFBP-3	74.5	59.9	108.0	147.4	145.5	113.0	52.06 to 53.88	0.93	0.18	0.61
IGFBP-5	31.8	32.9	49.0	24.8	58.8	44.4	19.27 to 20.34	0.27	0.36	0.66
Endometrium										
IGF-I	3.3	5.43	4.10	4.34	2.61	3.77	1.39 to 1.44	0.16	0.44	0.66
IGF-II	490	1,776	11,050	1,237	655	740	713 to 752	0.22	0.61	0.44
IGFBP-3	358.2	722.4	540.5	742.8	592.2	1,042.2	290.05 to 305.78	0.05	0.41	0.84
IGFBP-5	188.1	208.5	126.8	228.8	134.2	246.0	49.51 to 51.19	0.01	0.85	0.38
Myometrium										
IGF-I	80.5	75.3	40.8	36.9	33.6	57.3	14.23 to 14.62	0.56	<0.01	0.28
IGF-II	591	527	263	266	1002	221	501 to 515	0.34	0.58	0.48
IGFBP-3	15.6	19.3	10.3	11.2	13.4	12.1	4.32 to 4.55	0.66	0.10	0.72
IGFBP-5	87.7	100.6	37.5	39.4	34.2	44.5	13.38 to 14.17	0.30	<0.01	0.84

[1]Values are least squares means and SED, and data are presented as arbitrary units expressed in millions. Uterus and chorioallantoic samples were excised at the level of the middle conceptus from each uterine horn.
[2]Gestation feeding levels of 1.75 kg/d, with a top dress providing 0 or 50 mg/kg added L-carnitine.
[3]Day = day of gestation.

maternal gain predicted at an additional 13.6 kg (Aherne and Kirkwood, 1985; Williams et al., 1985). At this feeding level, gilts should have maintained backfat levels throughout gestation while increasing BW. The total gestation energy requirement was determined by summing the daily energy requirement, energy for maternal gain, plus energy for products of fetal and uterine gain. From d 0 to 55 of gestation backfat increased, and from d 55 to 70 of gestation backfat decreased in gilts fed both dietary treatments. This suggests the gilts in this study were in an anabolic state up to d 55 of gestation and at their requirement from d 55 to 70. When gilts are fed at a maintenance level, they will gain protein mass and lose fat mass (and backfat). The change from an anabolic state to maintenance state is commonly observed when gilts are fed a constant feed allowance during gestation. Because gilts were gaining BW during gestation, the maintenance requirement was increasing with less energy being available for maternal BW and backfat gain.

Insulin-like growth factor-I concentrations did not differ in gilts fed supplemental L-carnitine compared with the gilts fed the control diet. This is in agreement with Waylan et al. (2005) who observed no change in circulating IGF-I levels in sows with or without supplemental L-carnitine. However, Musser et al. (1999) observed an increase in maternal circulating IGF-I at d 60 and 90 of gestation in sows fed supplemental L-carnitine compared with the sows fed no supplemental L-carnitine. The observed increase in maternal circulating IGF-I observed by Musser et al. (1999) may be due to the sows being above their energy requirement (Thissen et al., 1994). Doberenz et al. (2006) also found increased maternal circulating levels of IGF-I and IGF-II in gilts and sows fed supplemental L-carnitine at d 80 and 95 of gestation. The gilts and sows in the study of Doberenz et al. (2006) were allowed ad libitum feed consumption, whereas gilts in this study were limit fed.

The placental and uterine environment play an important role in optimal fetal development (Anthony et al., 1995; Fant et al., 1996), fetal BW and survival (Knight et al., 1977), and normal reproductive function. It has been shown physiological changes in this environment during pregnancy are coordinated by changes in expression of insulin-like growth factors and binding proteins (Correia da Silva et al., 1999). In this study, growth factor expression evaluated in the myometrium and chorioallantois were not affected by maternal supplementation of L-carnitine, but IGFBP-3 and IGFBP-5 gene expression increased in the endometrium in gilts fed diets with supplemental L-carnitine. No changes were observed for endometrium IGF-I and IGF-II gene expression.

Previous studies have reported the importance of the IGF system in uterine and fetal development. In vitro research has shown that IGF-I stimulates DNA synthesis in uterine tissues of rats (Murphy and Ghahary, 1990) and pigs (Simmen et al., 1990), and the expression of specific receptors for IGF-I indicate a role for IGF-I in implantation (Simmen et al., 1995), and uterine (Corps et al., 1990; Ohleth and Bagnell, 1995) and conceptus (Simmen et al., 1995) development. Insulin-like growth factor-I also increases nutrient supply to the porcine conceptus (Persson et al., 1997). Previous research showed no changes in IGF-I gene expression in the porcine uterus collected from sows fed diets with supplemental L-carnitine at midgestation (Waylan et al., 2005). In the current study, we excised these 2 uterine tissue layers and found differences in gene expression in the endometrium. Waylan et al. (2005) did not find any differences in uterine gene expression but also did not separate these 2 tissues, which may explain the lack of a similar response. We cannot draw specific conclusions on our contrasting results because data are nonexistent on IGF system components in the pig myometrium. In addition, it is difficult to determine whether the IGF axis is involved in the myometrium across species because some of the IGF components are not found at detectable levels in some species (Han et al., 1999).

A number of research studies have concluded that IGFBP inhibit the biological actions of IGF (Knauer and Smith, 1980). In addition to these biological effects, researchers have also suggested IGF independent effects observed from changes in IGFBP (Jones and Clemmons, 1995). Specifically, researchers have shown human IGFBP-1 enhances the action of IGF-I in the endometrium (Elgin et al., 1987). Although IGFBP-1 and IGFBP-2 are major carriers of the growth factors (Ohleth and Bagnell, 1995), some researchers have focused on IGFBP-3 expression in fetal development because of its importance in porcine reproductive tissues (Samaras et al., 1992). We did not elucidate gene expression of IGFBP-1, IGFBP-2, IGFBP-4, or IGFBP-6 in the endometrium, only IGFBP-3 and IGFBP-5.

In this study, we observed an increase in IGFBP-3 and IGFBP-5 mRNA expression in the endometrium of gilts fed diets with supplemental L-carnitine. Maternal hormonal changes have been shown to increase levels of IGFBP-3 secretions in humans during pregnancy (Guidice et al., 1991). In follicular and amniotic fluid, IGFBP-3 has been shown to have potentiative and inhibitory effects on IGF-I and IGF-II concentrations (Rajaram et al., 1997). Other researchers have found IGF-I stabilizes IGFBP-3 in myometrial cells, therefore initiating a mitogenic response (Huynh, 2000) in this cell type. Little is known about IGFBP-5 expression in reproductive tissues in the pig (Nicholson et al., 1999). Insulin-like growth factor-II is coexpressed with IGFBP-5 during vascularization of the placenta (Han et al., 1999), and increases in IGF-I have been shown to stimulate the expression of IGFBP-5 mRNA and protein expression in ovarian cultured porcine granulosa cells (Grimes et al., 1994; Leighton et al., 1994).

The uterus provides the conceptus with a nourishing environment in gestation through changes in hormones and other biological molecules, such as IGF and IGFBP. In this study, we observed increases in IGFBP-3 and

IGFBP-5 mRNA expression in the endometrium of gilts fed diets with supplemental L-carnitine. The exact mechanism by which L-carnitine affects these 2 binding proteins in uterine development is not completely clear. One can speculate these binding proteins exert IGF-dependent and -independent effects on the uterine environment, enhancing endometrium growth similar to IGF-I, but further research is needed in this area before conclusions can be drawn. The increase in IGFBP-3 and IGFBP-5 mRNA expression we observed in the endometrium from gilts fed L-carnitine suggests that L-carnitine altered the IGF axis at the fetal maternal interface.

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