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Dietary L-Arginine Supplementation Enhances the Reproductive Performance of Gilts[1]

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Abstract

Arginine is a common substrate for the synthesis of nitric oxide and polyamines that are crucial for placental angiogenesis and growth in mammals. This study was conducted to test the hypothesis that dietary L-arginine supplementation may improve reproductive performance of pregnant gilts. Fifty-two pregnant gilts with body weight (BW) of 166.3 ± 1.8 kg were housed individually in gestation crates. At d 30 of gestation, gilts were assigned randomly to corn-soybean-based diets supplemented with 1.0% L-arginine-HCl or 1.7% L-alanine (isonitrogenous control). Both diets contained 13.0 MJ metabolizable energy/kg and 12.2% crude protein and were fed to gilts at 1 kg twice daily during gestation. Backfat thickness and BW were measured and blood samples were obtained on 30, 70, 90, and 110 d of gestation. At d 110 of gestation, gilts were transferred to individual farrowing crates. The numbers of total piglets born and born alive, as well as birth weights of piglets, were recorded immediately after farrowing. Throughout the gestation, BW or backfat thickness of gilts did not differ between treatment groups. Plasma urea concentrations were lower in arginine-supplemented than in control gilts at d 90 ($P < 0.010$) and d 110 ($P < 0.001$) of gestation. Compared with the control group, arginine supplementation increased the number of pigs born alive by 22% (11.40 vs. 9.37, $P = 0.032$) and live litter birth weight of piglets by 24% (16.38 vs. 13.19 kg, $P = 0.016$). This exciting finding provides the first evidence for a marked increase of live-born piglets by 2 per litter through nutritional intervention in gilts. J. Nutr. 137: 652–656, 2007.

Introduction

Intrauterine growth retardation is a significant problem in both animal and human nutrition (1). Particularly, embryonic loss and fetal deaths during gestation account for 30 to 50% of the total number of fertilized ova (2,3), thereby limiting the number of piglets born at farrowing. Modern sows have higher fetal growth rates than those in the past breeds due to intensive genetic selection (4,5). However, higher fetal growth rates may require an increased provision of nutrients for supporting the metabolic needs of both the sow and her fetuses (6). Moreover, uterine and umbilical blood flow per fetus decrease as litter size increases (7,8), which can lead to an unequal delivery of substrates among fetuses. Further, larger fetal litter sizes may limit uterine capacity, which can decrease fetal growth, increase fetal death, and reduce litter size at birth (9–12).

Arginine plays multiple roles in animal metabolism by serving as a substrate for protein synthesis, an intermediate in the hepatic urea cycle, and a precursor for the synthesis of various important metabolic molecules, including nitric oxide (NO)[6] and polyamines (13,14). Several studies have demonstrated a crucial role for NO in enhancing blood flow during ovine pregnancy (15–18), therefore increasing the delivery of essential nutrients from maternal to fetal blood. Polyamines are synthesized from ornithine via the arginase pathway and are important determinants of embryogenesis and placental growth (19–21). Available evidence shows that both polyamines and NO play key roles in angiogenesis, which is a critical event during placental growth and fetal development (22). Remarkably, we recently discovered an unusual abundance of arginine (4–6 mmol/L) in porcine allantoic fluid during early gestation when placental growth is most rapid (23,24). Therefore, we hypothesized that increasing L-arginine provision may enhance the reproductive performance of pigs. This hypothesis was tested using dietary supplementation with L-arginine-HCl to gilts between 30 and 114 d of gestation.

Materials and Methods

Animals and diets. This study was approved by the Texas Tech University Animal Care and Use Committee. A total of 52 gilts (Camborough 22, Pig Improvement) with initial body weight (BW) of 166.3 ± 1.8 kg and backfat (BF) of 13.3 ± 0.2 mm were used in this study. They were housed individually in gestation crates (2.12 × 0.61 m).

All gilts were checked for estrus once daily in the morning and inseminated twice with unfrozen semen via artificial insemination during

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[6] Abbreviations used: BF, backfat thickness; BW, body weight; NO, nitric oxide.

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estrus (18–24 h apart). At d 30 of gestation, gilts were assigned randomly to 1 of the 2 treatment groups representing supplementation with 1% L-arginine-HCl or 1.7% L-alanine (isonitrogenous control) to a corn- and soybean meal-based diet (**Table 1**). The supplemental level of 1% L-arginine-HCl was chosen because it was shown in our preliminary study to increase plasma concentration of arginine by 70% at 2 h after feeding. L-Arginine-HCl or L-alanine (Ajinomoto) was added to the basal diet at the expense of molasses cane. Alanine was chosen for the isonitrogenous control diet, because it is neither toxic nor a substrate for arginine synthesis but is extensively catabolized by pigs (25,26). There were 28 and 24 gilts in the control and arginine-supplemented groups, respectively. The number of gilts differed between the 2 treatment groups, because some gilts initially allotted to the study were nonpregnant. All gilts received 2 kg diet (on an as-fed basis) daily as 2 equal-sized meals (0700 and 1800) during the entire gestation period. Both diets provided 13.0 MJ metabolizable energy/kg and 12.2% crude protein (on an as-fed basis). Pregnant gilts had free access to drinking water and ate all the feed offered throughout the experiment.

BW and BF thickness were measured on d 30, 70, 90, and 110 of gestation. BF thickness was measured by ultrasound (Keiki LS-1000, Tokimec) at the P2 position (left side of the 10th rib and 6 cm away from the spine). Blood samples were collected at 2 h after feeding via jugular venepuncture into heparinized tubes (Becton-Dickinson Vacutainer Systems) on d 30, 70, 90, and 110 of gestation. Samples were centrifuged at 2000 × *g*; 15 min at 4°C. Plasma was transferred to 1.5 microcentrifuge tubes (National Scientific) and stored at −20°C until analysis. At d 110 of gestation, all pregnant gilts were transferred to individual farrowing crates (1.5 × 2.2 m). We counted the total number of piglets and recorded their BW at birth. The piglets were further classified as born alive or dead. The piglets born dead referred only to stillborns, which were recorded within 12 h after parturition as any piglets found dead during or shortly after farrowing. These stillborns were mostly fully-formed pigs, which had no characteristics of mummified fetuses, such as dark color, bloated stomach, sunken eyes, loose skin, or bad odor. The piglets found dead under or near the mammary gland were assumed to have died by crushing and were not included as stillborns. An attempt to thoroughly inspect all placentae was made; however, the placentae from 4 gilts fell through the slatted floors and could not be recovered. Among 48 gilts examined, only 3 mummified fetuses were observed. Thus, the number of mummified fetuses (early gestation deaths) was negligible and the number of piglets born dead represented almost entirely fetal deaths during late gestation.

TABLE 1 Composition of gestation diets (on an as-fed basis)[1]

Item	Gestation diets, %	
	Control	Arginine
Corn grain	71.20	71.20
Soybean meal	10.50	10.50
Alfalfa meal	5.00	5.00
Molasses cane	4.30	5.00
Potassium chloride	0.75	0.75
Salt	0.35	0.35
Vitamin-mineral premix[2]	3.00	3.00
Oil, vegetable	0.50	0.50
Dicalcium phosphate	2.20	2.20
Limestone	0.50	0.50
L-Arginine HCl	—	1.00
L-Alanine	1.70	—
Calculated analysis		
Dry matter, %	89.3	
Metabolizable energy, *MJ/kg*	13.0	
Crude protein, %	12.2	
Calcium, %	0.94	
Available phosphorus, %	0.47	
Total phosphorus, %	0.69	

[1] The analyzed contents (% of diet; on an as-fed basis) of amino acids in the basal diet were: Ala, 0.78; Asp+Asn, 1.34; Arg, 0.70; Cys, 0.23; Glu+Gln, 2.29; Gly, 0.55; His, 0.33; Ile, 0.51; Leu, 1.17; Lys, 0.58; Met, 0.18; Phe, 0.62; Pro, 1.03; Ser, 0.50; Thr, 0.49; Trp, 0.13; Tyr, 0.45; and Val, 0.65.
[2] Provided the following per kg of the complete diet: manganese, 46.7 mg; iron, 75 mg; zinc, 103.8 mg; copper, 9.5 mg; iodide 0.72 mg; selenium, 0.23 mg; retinyl acetate, 2600 μg; cholecalciferol, 20.6 μg; D-α-tocopherol, 41.5 mg; menadione sodium bisulfate, 2.7 mg; vitamin B-12, 54.9 μg; riboflavin, 13.7 mg; niacin, 54.9 mg; and choline, 1650 mg.

Chemical analyses. Plasma samples were assayed for urea concentrations using a colorimetric method involving reaction with phenol and hypochlorite, as previously described (27). Plasma concentrations of amino acids were analyzed by HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde, as described by Wu et al. (27). Amino acid standards and other chemicals were obtained from Sigma Chemical.

Statistical analysis. Data were analyzed using MIXED procedures of SAS (SAS Institute) following a randomized complete block design. Gilt was considered as the experimental unit. Data for the number of piglets born dead were analyzed using the Friedman test of SAS. Separation of means was performed using the PDIFF option of SAS. $P < 0.05$ was considered significant. Values in the text are means ± pooled SEM.

Results

Gestation performance. Initial BW or BF thickness of gilts did not differ between the arginine-supplemented and control groups. Similarly, BW and BF did not differ between arginine-supplemented and control gilts throughout the experimental period. The BW of gilts were 166.3 ± 1.8, 183.4 ± 2.3, 193.6 ± 2.9, and 204.6 ± 4.25 kg at d 30, 70, 90, and 110 of gestation, respectively. The BF thickness of gilts was 13.3 ± 0.2, 13.8 ± 0.4, 14.0 ± 0.4, and 15.6 ± 0.3 mm at d 30, 70, 90, and 110 of gestation, respectively. Gestation length (114 ± 0.3 d, $n = 52$) did not differ between control and arginine-supplemented gilts.

Gilts farrowed during June (25%), July (29%), August (21%), and September (25%) in 2004. The proportion of farrowing that occurred in each of these months did not differ between control and arginine-supplemented gilts. The total number of piglets born did not differ between the 2 groups of gilts (**Table 2**). However, the total number of piglets born alive was 22% higher ($P = 0.032$) for arginine-supplemented gilts compared with gilts fed the control diet (Table 2). The average birth weights of all piglets born or of piglets born alive did not

TABLE 2 Reproductive performance of gilts fed diets supplemented with or without 1% L-arginine HCl[1]

Parameters of reproductive performance	Treatment		SEM
	Control	Arginine	
Total piglets born per litter, *n*	11.27	11.94	0.96
Total piglets born alive per litter, *n*	9.37	11.40*	0.56
Birth weight of all piglets born, *kg*	1.39	1.43	0.04
Birth weight of all piglets born alive, *kg*	1.41	1.46	0.04
Litter birth weight of all piglets born, *kg*	15.54	16.85	1.31
Litter birth weight of all piglets born alive, *kg*	13.19	16.38*	0.74
Piglets born dead per litter, *n*	1.86	0.66*	0.147
Birth weight variation of all piglets born,[2] *kg*	0.293	0.257	0.021
Birth weight variation of all piglets born alive,[3] *kg*	0.240	0.253	0.017

[1] Values are means with pooled SEM, $n = 52$. *Different from the control group, $P < 0.05$.
[2] Variation in birth weights of piglets based on the total number of piglets born.
[3] Variation in birth weights of piglets based on the total number of piglets born alive.

differ between the 2 treatment groups. Total litter birth weights, based on the total number of piglets born per litter, did not differ between control and arginine-supplemented gilts; however, total live litter birth weights, based on the total number of piglets born alive per litter, were 24% higher ($P = 0.003$) for the arginine-supplemented gilts compared with the control group (Table 2). The number of piglets born dead was 65% lower ($P = 0.037$) for the arginine-supplemented gilts compared with the control group. Variations in birth weights of piglets did not differ between the 2 groups of gilts, whether based on the total number of piglets born or total number of piglets born alive.

Plasma urea concentrations. Concentrations of urea in plasma did not differ between the control and arginine-supplemented groups at d 30 or d 70 of gestation (**Table 3**). However, concentrations of urea in plasma were 14% and 21% lower in arginine-supplemented gilts than in the control group of gilts at d 90 ($P < 0.010$) and d 110 ($P < 0.001$) of gestation, respectively (Table 3).

Plasma concentrations of amino acids. Plasma concentrations of all amino acids were not different for the 2 treatment groups of gilts at the start of the study (d 30 of gestation; **Table 4**). However, at d 70 of gestation, plasma concentrations of proline, ornithine, and arginine were 31% ($P < 0.001$), 44% ($P < 0.001$), and 79% ($P < 0.001$) higher, respectively, in the arginine-supplemented gilts compared with the control group. Plasma concentrations of glutamine were 13% ($P = 0.019$) lower in arginine-supplemented gilts compared with the control group at d 70 of gestation. Similar results were obtained at d 90 of gestation. At d 110 of gestation, concentrations of proline, ornithine, and arginine in the plasma of arginine-supplemented gilts increased by 29% ($P = 0.001$), 52% ($P = 0.002$), and 77% ($P < 0.001$), respectively, compared with gilts fed the control diet (Table 4). Notably, at d 110 of gestation, concentrations of glutamine in the plasma of arginine-supplemented gilts were 26% lower ($P = 0.028$) in comparison with the control group. At d 70 and d 110 of gestation, concentrations of alanine in the plasma of the control (alanine-supplemented) gilts were 91 and 35% higher ($P < 0.05$), respectively, than in arginine-supplemented gilts. At all selected days of gestation, plasma concentrations of other amino acids were not different between the 2 treatment groups of gilts (Table 4).

Discussion

Embryonic and fetal losses due to unfavorable intrauterine conditions during gestation represent a major obstacle in maximizing the reproductive efficiency of breeding animals (1,28). As a major factor in influencing the intrauterine environment, maternal nutrition plays an important role in regulating fetal growth, development, and survival (22). Thus, providing the pregnant dam with proper nutrition, including adequate amounts of amino acids, is vital for the growing fetus (4,29,30). Arginine is not only required for protein synthesis and ammonia detoxification but is also a precursor of many metabolically important molecules, including proline, ornithine, polyamines, and NO (13,14). Additionally, arginine is the most abundant nitrogen carrier in fetal pigs and is 1 of the most abundant amino acids deposited in fetal tissues (31) and in allantoic fluid of the porcine conceptus during early gestation (23), reflecting its importance in the survival, growth, and development of fetal pigs. Furthermore, amino acid malnutrition in gestating sows results in lower concentrations of arginine in the placenta and fetal plasma (24), as well as reduced activities of placental NO synthase and ornithine decarboxylase (32). The NO synthase is responsible for the synthesis of NO (the endothelium-derived relaxing factor) from L-arginine, whereas ornithine decarboxylase catalyzes the first and rate-controlling step in the synthesis of polyamines (33). Impaired placental synthesis of both NO and polyamines is considered a major factor contributing to intrauterine growth retardation (1,22). However, it was unknown whether arginine supplementation could improve gestation performance in gilts. An availability of data to address this question will provide the necessary foundation for guiding future studies to define the molecular and cellular mechanisms whereby arginine supplementation enhances placental development as well as fetal survival and growth.

The results from this study demonstrate for the first time, to our knowledge, that arginine supplementation to gestation diets for pregnant gilts improved pregnancy outcome by increasing the total number of live-born piglets and total live litter birth weight without any reduction in the average birth weight of piglets. Plasma concentrations of arginine and its metabolites (ornithine and proline) were also increased in arginine-supplemented gilts between d 70 and d 110 of gestation, which coincides with the period of rapid fetal growth (4). Previous reports have shown that uterine capacity starts to become limiting for embryonic survival at as early as 30 d of gestation, thereby affecting fetal growth (3,34) and leading to losses of viable fetuses. Although the majority of the conceptus loss occurs during the periimplanation period, there is evidence that significant losses also occur during later gestation (35). This is in agreement with the finding of this study that almost all piglets born dead were fully formed, suggesting that these fetal deaths occurred during late gestation. Dietary arginine supplementation reduced the number of stillborn piglets, probably due to an improved uterine environment for fetal growth and development (1). Importantly, no differences were noted in plasma concentrations of lysine and histidine between the control and arginine-supplemented gilts at d 70 to 110 of gestation (Table 4), indicating a lack of an imbalance among basic amino acids. Although plasma concentrations of alanine were increased in the isonitrogenous control group of gilts due to the addition of alanine to the basal diet, their reproductive performance was similar to that of nonsupplemented pregnant gilts at the Texas Tech Swine Research Farm (6), indicating that alanine had no adverse effect on the performance or metabolism of pregnant gilts. Notably, the gilts in this study farrowed during the 2004 summer (June to September) with high ambient temperatures (35–40°C) in Lubbock, Texas. The relatively high number of stillborns that occurred in the control group was typical of gilts that farrowed in the summer months on the Texas Tech Swine Research Farm. For example, our records indicate that, in recent years (2004–2006),

TABLE 3 Plasma concentrations of urea in gilts fed diets supplemented with or without 1% L-arginine HCl[1]

	Treatment		
Gestation	Control	Arginine	SEM
d	*mmol/L*		
Initial[2]	1.70	1.70	0.07
70	2.21	2.06	0.10
90	2.81	2.43*	0.07
110	3.17	2.52**	0.06

[1] Values are means with pooled SEM. **$P < 0.001$, *$P < 0.05$: different from the control group.
[2] Initial values at 30 d of gestation.

TABLE 4 Concentrations of amino acids in the plasma of pregnant gilts fed diets supplemented with or without L-arginine HCl[1]

	Gestation, *d*								
	Initial[2]			70			110		
Amino acid	Control	Arginine	SEM	Control	Arginine	SEM	Control	Arginine	SEM
	μmol/L								
Proline	282	287	5.6	278	365**	14.0	289	372*	14.7
Cysteine	279	293	8.5	276	288	7.6	275	287	8.2
Aspartate	17	16	1.3	17	18	1.0	31	32	1.7
Glutamate	157	138	12.9	101	102	8.4	143	131	10.1
Asparagine	82	65	7.1	54	51	4.2	64	56	3.7
Serine	158	161	12.1	133	118	5.4	168	167	4.0
Glutamine	304	308	17.3	320	278*	13.4	391	291*	23.6
Histidine	86	84	2.5	81	74	2.5	88	85	1.9
Glycine	581	530	20.5	817	705	39.2	622	556	25.0
Threonine	138	151	10.3	130	120	9.2	122	112	4.6
Citrulline	80	77	3.4	72	71	3.7	72	80	4.6
Arginine	202	203	8.6	194	349**	23.7	193	341**	21.1
β-Alanine	28	27	2.0	19	19	0.9	24	23	0.9
Taurine	75	72	7.1	63	56	3.8	63	64	4.2
Alanine	416	408	14.5	743	388*	62.5	701	518*	42.8
Tyrosine	113	112	5.9	86	87	4.6	93	94	3.8
Tryptophan	61	54	4.0	60	54	3.3	54	51	2.4
Methionine	54	48	3.0	43	38	2.2	42	42	2.1
Valine	225	212	10.2	212	203	13.3	177	176	7.4
Phenylalanine	97	92	5.0	71	76	4.7	76	79	2.3
Isoleucine	126	121	7.5	93	105	8.2	97	97	4.8
Leucine	218	202	9.9	210	208	10.8	166	161	7.5
Ornithine	117	104	6.4	105	152**	7.6	95	145*	9.1
Lysine	184	151	14.7	148	163	14.9	123	138	8.4

[1] Values are means with pooled SEM, $n = 52$. $^{**}P < 0.001$, $^{*}P < 0.05$: different from the control group.
[2] Initial day for arginine supplementation (d 30 of gestation). Blood samples were obtained from gilts before the addition of alanine or arginine to the basal diet.

the numbers of total piglets born, total piglets born alive, and total piglets born dead per litter were 11.30 ± 0.28 (ranging from 7 to 17 heads), 9.80 ± 0.37 (ranging from 6 to 16 heads), and 1.58 ± 0.29 (ranging from 0 to 8 heads), respectively ($n = 270$ gilts). Thus, in this study, heat stress may explain the relatively high mean rate of stillbirths (1.86 per litter) in the control group of gilts. Importantly, dietary supplementation with arginine to gilts between d 30 and 114 of gestation enhances the viability of fetal pigs even under this adverse environmental condition.

It is currently unknown how dietary supplementation with L-arginine increased the number of piglets born alive or live litter birth weight. The arginine treatment may enhance placental angiogenesis and growth during early- to mid-gestation, thereby promoting an optimal intrauterine environment throughout pregnancy (22). Additionally, previous studies showed that uterine uptake of arginine may not be sufficient to meet fetal growth requirements during late gestation in pigs (31). In pregnant gilts, as reported previously for pregnant sheep (36), an increase in plasma concentrations of arginine likely resulted in enhancement of its placental transport from mother to fetus. This would provide adequate amounts of arginine from the pregnant gilt to her fetuses, thereby supporting their optimal metabolism and growth during the period of most rapid fetal growth. Indeed, results of a recent study showed that arginine infusion into ewes during late gestation increased protein accretion in fetal lambs (37).

Arginase activity is virtually absent from the porcine placenta (38). Thus, the conversion of arginine into ornithine and proline via the arginase pathway in other maternal tissues and the subsequent catabolism of proline to yield ornithine in the porcine placenta play an important role in placental synthesis of polyamines (1). In support of this notion, plasma concentrations of both ornithine and proline increased in arginine-supplemented gilts (Table 4). In addition, proline and hydroxyproline (major components of collagen) are the only amino acids that exhibit a progressive increase in fetal pigs between d 40 and 114 of gestation (31). The high concentrations of proline in plasma are closely associated with maximal placental growth at d 60 to 70 of gestation (38). Also, elevated levels of plasma proline were observed in arginine-supplemented gilts at d 70 and 110 of gestation (Table 4), which coincide with the progressive increase in proline concentrations in porcine allantoic fluid between d 60 and 110 of gestation (38). This increase in plasma proline concentration further gives credence to the beneficial effect of arginine supplementation, because the uterine uptake of both proline and hydroxyproline only marginally meets fetal pig growth requirements during late gestation (31). Therefore, it is possible that dietary supplementation with arginine increased the provision of ornithine and proline for the synthesis of NO, polyamines, and collagen in the placenta and fetus, as reported for adult rats (13,39). The outcome would be to enhance placental angiogenesis and growth (including vascular growth), utero-placental blood flow, the transfer of nutrients from mother to fetus, and, therefore, fetal survival, growth, and development (1,22,40).

Finally, the finding that concentrations of glutamine and urea were reduced in the plasma of arginine-supplemented gilts

(Tables 3 and 4) deserves comments. Arginine supplementation may reduce whole-body amino acid degradation and, thus, urea production as well as the endogenous synthesis of glutamine from branched-chain amino acids and ammonia. Our finding that live litter size and live litter birth weights increased in arginine-supplemented gilts compared with control gilts despite their same feed intake (Table 2) suggests an improvement in the efficiency of utilization of dietary amino acids for fetal growth. Similarly, dietary supplementation with arginine reduced urea concentrations in the plasma of milk-fed piglets and increased the efficiency of utilization of dietary protein for neonatal growth (25).

In conclusion, dietary supplementation with L-arginine markedly enhanced the reproductive performance of gilts by increasing fetal survival and live litter birth weight. This novel and important finding has important practical implications for enhancing fetal survival and growth in pigs and other mammalian species. Further, our results provide a necessary foundation for future studies to define the mechanisms responsible for the beneficial effect of arginine in improving pregnancy outcome in gilts.

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