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ORIGINAL ARTICLE

Dietary L-arginine supplementation enhances placental growth and reproductive performance in sows

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Abstract Suboptimal embryonic/fetal survival and growth remains a significant problem in mammals. Using a swine model, we tested the hypothesis that dietary L-arginine supplementation during gestation may improve pregnancy outcomes through enhancing placental growth and modulating hormonal secretions. Gestating pigs (Yorkshire × Landrace, $n = 108$) were assigned randomly into two groups based on parity and body weight, representing dietary supplementation with 1.0% L-arginine–HCl or 1.7% L-alanine (isonitrogenous control) between days 22 and 114 of gestation. Blood samples were obtained from the ear vein on days 22, 40, 70 and 90 of gestation. On days 40, 70 and 90 of gestation, concentrations of estradiol in plasma were higher ($P < 0.05$) in arginine-supplemented than in control sows. Moreover, arginine supplementation increased ($P < 0.05$) the concentrations of arginine, proline and ornithine in plasma, but concentrations of urea or progesterone in plasma did not differ between the two groups of sows. Compared with the control, arginine supplementation increased ($P < 0.05$) the total number of piglets by 1.31 per litter, the number of live-born piglets by 1.10 per litter, the litter birth weight for all piglets by 1.36 kg, and the litter birth weight for live-born piglets by 1.70 kg. Furthermore, arginine supplementation enhanced ($P < 0.05$) placental weight by 16.2%. The weaning-to-estrus interval of sows was not affected by arginine supplementation during gestation. These results indicate that dietary arginine supplementation beneficially enhances placental growth and the reproductive performance of sows.

Keywords L-Arginine · Sow · Placenta · Estradiol · Reproductive performance · Piglet

Abbreviations

BF	Backfat
IUGR	Intrauterine growth restriction
NO	Nitric oxide
NOS	Nitric oxide synthase
ODC	Ornithine decarboxylase

Introduction

Suboptimal embryonic/fetal survival and growth remains a significant problem in mammals (Bazer et al. 2010). Because of ethical concerns over human studies, animal models are widely used to improve pregnancy outcomes. Work with domestic animals also has important implications for enhancing livestock production worldwide (Kim et al. 2007; Wu et al. 2010). A notable example is the successful application of L-arginine to reduce embryonic and fetal mortality in first-parity pigs (gilts) (Mateo et al. 2007). At present, it is unknown whether arginine also exerts benefits on primiparous and multiparous sows.

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Arginine, which is an unusually abundant amino acid in the conceptus, (Wu 2009) is utilized by multiple pathways, including the synthesis of protein, nitric oxide (NO), polyamines, and creatine (Blachier et al. 2011; Wu and Morris 1998; Wu et al. 2007, 2009). Several studies have demonstrated that polyamines and NO are essential to placental growth and angiogenesis, therefore increasing uterine and placental–fetal blood flow (Gardner et al. 2001; McCrabb and Harding 1996; Wu et al. 2006). These physiological processes are critical for fetal growth and development (Wu et al. 2004, 2008). Furthermore, arginine administration during gestation could result in changes in hormonal secretions, which may affect fetal and maternal metabolism (Chew et al. 1984; Kensinger et al. 1986). Thus, arginine-free diet results in fetal resorption, intrauterine growth retardation (IUGR), and fetal death, while dietary arginine supplementation increases the number of live-born litter in rats and gilts (Greenberg et al. 1997; Mateo et al. 2007; Zeng et al. 2008). The mechanisms responsible for the effect of arginine on pregnancy remain largely elusive.

We hypothesized that dietary L-arginine supplementation during gestation may improve pregnancy outcomes through enhancing placental growth and modulating hormonal secretions. The present study was conducted with both primiparous and multiparous sows to test this hypothesis.

Materials and methods

Animals and diets

This study was approved by Guangdong Academy of Agricultural Science. A total of 108 Yorkshire × Landrace mixed-parity sows ($n = 66$ primiparous; $n = 42$ multiparous, parities 2–3) were used in this experiment on a commercial 1,000-sows unit with a farrow-to-wean operation in Guangdong Wen's Foodstuffs Group CO. Ltd. (Guangdong, China). The experiment began in June 2009 and ended in November 2009. The sows were housed individually in gestation crates (2.12 × 0.61 m) in a double curtain sided barn. All sows were checked for estrus daily in the morning and artificially inseminated three times with diluted semen from Pietrain × Duroc boars during estrus (10–12 h apart). On the day of breeding, sows were assigned randomly to one of the two groups based on parity and body weight. At days 18–21 post breeding, sows were checked for pregnancy by B-mode ultrasound scanner (ULTRASOUND WED-2000, Beijing, China). Beginning at day 22 of gestation when implantation of the embryo in the uterus has been completed, pregnant sows were fed daily a commercial diet (Table 1) supplemented with 1% L-arginine–HCl or 1.7% L-alanine (isonitrogenous control). There were 52 sows ($n = 31$ primiparous; $n = 21$ multiparous, parities 2–3) in the arginine-supplemented group and 56 sows ($n = 35$ primiparous; $n = 21$ multiparous, parities 2–3) in the control. The number of sows differed between the two treatment groups, because some sows initially assigned to the study were nonpregnant at day 22 post breeding. The supplemental level of 1% L-arginine–HCl and L-alanine as isonitrogenous control was used, as described by Mateo et al. (2007) and Kim and Wu (2004). L-Arginine–HCl and L-alanine met the AJI92 standard and were produced in Ningbo Zhenhai Haide Biochem CO. Ltd. (Ningbo, China).

All sows received an increasing amount of diet during the entire gestation period. On days 22–90 of gestation, sows were restricted to 2 kg diet (on an as-fed basis; Table 1) daily, and after day 90 of gestation, sows were fed

Table 1 Composition and nutrient levels of the basal diet (as-fed basis)

Items	Content (%)
Ingredients	
Corn grain	67.20
Soybean meal	13.00
Wheat bran	11.00
Soybean oil	3.50
Salt	0.30
Premix[a]	2.00
Limestone	1.00
Dicalcium phosphate	1.85
Choline chloride (50%)	0.15
Nutrient levels	
Dry matter[b] (%)	89.60
CP[b] (%)	13.24
Metabolizable energy[c] (Mcal/kg)	3.11
Arg[b] (%)	0.88
Glu + Gln[b] (%)	2.58
Pro[b] (%)	1.00
Lys[b] (%)	0.65
Met + Cys[b] (%)	0.52
Val[b] (%)	0.68
Thr[b] (%)	0.54
Ca[c] (%)	0.95
Available P[c] (%)	0.45
Total P[c] (%)	0.68

[a] Provided per kg of diet: 10,500 IU vitamin A, 1,600 IU vitamin D_3, 45 IU vitamin E, 2.5 mg vitamin K_3, 0.75 mg vitamin B_1, 6.5 mg vitamin B_2, 2.4 mg vitamin B_6, 0.05 mg vitamin B_{12}, 50 mg niacin, 30 mg calcium pantothenate, 2.5 mg folic acid, 0.2 mg biotic, 40 mg manganese, 85 mg iron, 75 mg zinc, 1.5 mg copper, 0.09 mg iodine, and 0.03 mg selenium

[b] Analyzed values

[c] Calculated values

3 kg of the diet daily. Primiparous and multiparous sows were fed the same amounts of diet to ensure equal intake of L-arginine daily. On day 110 of gestation, sows were moved to individual farrowing crates (1.5 × 2.2 m). Pregnant sows were provided free access to drinking water and consumed all the feed offered throughout the experiment.

Backfat (BF) thickness of sows was measured on days 22 and 110 of gestation. BF thickness for the left and right sides was measured at P2 position (6 cm from the mid line at the head of the last rib) with an ultrasonic device (Agroscan A16, France), and the average was calculated. On days 22, 40, 70 and 90 of gestation, blood samples were collected, at 2 h after feeding on the morning, from the ear vein into heparinized tubes. Samples were centrifuged at 3,000×*g* for 15 min at 4°C, and plasma was stored at −20°C for assays of biochemical parameters (Hou et al. 2010; Kong et al. 2009). After farrowing, the numbers of piglets born, born alive, stillborn and mummified were recorded. Individual body weights of piglets were obtained at parturition. After all the placentae were expelled, their weights were recorded.

Chemical analyses

Samples of the basal diet were analyzed for the contents of dry matter and crude protein according to AOAC (1996) methods. After acid hydrolysis (Li et al. 2011) amino acids in the diet were analyzed by amino acid analyzer (L-8900, HITACHI, Japan), as described by Ma et al. (2010) and Yin et al. (2009).

Porcine progesterone and estradiol ELISA kits (Uscnlife science & technology CO. Ltd., Wuhan, China) were used to determine plasma progesterone and estradiol concentrations. The sensitivity of progesterone ELISA assays was 0.4 ng/ml with the intra- and inter-assay coefficient of variation being 6.8 and 8.0%, respectively. Concentrations of progesterone in the range 1.6–100.0 ng/ml could be measured. The sensitivity of estradiol ELISA assays was 3.9 pg/ml with the intra- and inter-assay coefficient of variation being 7.4 and 8.9%, respectively. Concentrations of estradiol in the range 15.6–1,000.0 pg/ml could be measured. All samples were measured in duplicates and the mean values were used for statistical analysis.

Plasma urea concentration was measured by an automated chemistry analyzer (Spotchem EZ SP-4430, Arkray, Japan), using multi-type reagents (Arkray Inc., Japan). Amino acids in plasma were analyzed by amino acid analyzer (L-8900, HITACHI, Japan), as described by Ma et al. (2010) and Yin et al. (2009, 2010).

Statistical analysis

All statistical analyses were performed using the SAS software package (version 9.0, SAS Institute Inc., Cary, NC, USA). Each sow was used as the experimental unit for analysis. There were no interactions between parity and arginine treatment using the GLM procedure; therefore, data were analyzed by the student's *t* test, (Wei et al. 2011) except for the number of stillborn and mummified per litter, to determine the effect of arginine supplementation on reproductive parameters in sows. The data on stillborn and mummified were analyzed by the Mann–Whitney's *U* test because these data were not normally distributed. $P < 0.05$ was taken to indicate statistical significance. Values are expressed as means with pooled SEM.

Results

Reproductive performance

Changes in BF thickness between days 22 and 110 of gestation (1.16 ± 0.30 mm) did not differ ($P > 0.05$) between the arginine-supplemented and control groups. Gestation length (114 ± 0.34 days) did not differ ($P > 0.05$) between arginine-supplemented and control sows.

Sow performance, as affected by diets, is shown in Table 2. The total numbers of piglets born and of piglets born alive were higher ($P < 0.05$) for arginine-supplemented primiparous and multiparous sows when compared with the control group by 1.31 and 1.10 per litter, respectively. The number of stillborn or mummified piglets born did not differ ($P > 0.05$) between the two groups. The total litter birth weight and total weight of pigs born alive per litter were greater ($P < 0.05$) in primiparous and multiparous sows fed the L-arginine-supplemented diet than in the control (17.79 vs. 16.43 kg; and 17.52 vs. 15.82 kg, respectively). The average individual weight of pigs born alive within litters was not different ($P > 0.05$) between the treatment groups. Notably, the total litter placental weight for pigs born alive was greater ($P < 0.05$) in primiparous and multiparous sows fed the L-arginine-supplemented diet, compared with the control sows (3.53 vs. 3.04 kg). The average placental weight of piglets born alive within litters was not affected ($P > 0.05$) by the arginine treatment. Meanwhile, birth weight variation of all piglets born alive did not differ ($P > 0.05$) between arginine-supplemented and control sows.

The percentages of sows returning to estrus in arginine-supplemented and control-group sows were 98.6 and 97.2%, respectively. The weaning-to-estrus intervals (4.87 ± 0.44 days) did not differ ($P > 0.05$) between arginine-supplemented and control sows.

Biochemical parameters in plasma

Concentrations of plasma urea or progesterone did not differ ($P > 0.05$) between arginine-supplemented and

Table 2 Effects of dietary L-arginine supplementation on the reproductive performance of sows

Items	Treatment		Pooled SEM
	Control	Arginine	
Number of piglets per litter (*n*)			
Total born	12.46	13.77*	0.35
Born alive	11.25	12.35*	0.51
Stillborn and mummified	1.21	1.42	0.36
Birth weights (kg)			
All piglets born per litter	16.43	17.79*	0.68
Piglets born alive per litter	15.82	17.52*	0.72
Average for piglets born alive	1.41	1.45	0.06
Placental weight for all live-born piglets (kg)	3.04	3.53*	0.17
Placental weight per live-born piglet (kg)	0.240	0.259	0.011
Birth weight variation of all piglets born alive[a] (kg)	0.229	0.239	0.014

Data are means with pooled SEM, $n = 108$

* $P < 0.05$ versus the control group

[a] Variation in birth weights of piglets based on the total number of piglets born alive

Table 3 Effect of dietary L-arginine supplementation on concentrations of biochemical parameters in the plasma of sows on days 40, 70 and 90 of pregnancy

Items	Treatment		Pooled SEM
	Control	Arginine	
Urea (mmol/L)			
Initial (day 22)	2.69	2.69	0.20
Day 40	3.69	3.36	0.37
Day 70	3.39	3.14	0.26
Day 90	3.60	3.59	0.33
Progesterone (ng/ml)			
Initial (day 22)	13.2	13.8	2.2
Day 40	14.7	13.7	3.2
Day 70	15.0	13.8	2.7
Day 90	12.3	13.6	2.0
Estradiol (pg/ml)			
Initial (day 22)	56.9	54.3	16.1
Day 40	55.7	140.6*	36.4
Day 70	124.5	320.9*	83.0
Day 90	220.3	483.5*	100.3

Data are means with pooled SEM, $n = 108$

* $P < 0.05$ versus the control group

control-group sows throughout the experimental period (Table 3). However, concentrations of estradiol in plasma were higher ($P < 0.05$) in arginine-supplemented sows than in the control group at days 40, 70 and 90 of gestation (Table 3).

Concentrations of amino acids in plasma are presented in Table 4. Compared with the control group, dietary arginine supplementation increased ($P < 0.05$) the concentrations of arginine, proline and ornithine in the plasma of sows at days 40, 70 and 90 of gestation. At all the selected time points of gestation, dietary supplementation with 1% L-arginine–HCl had no effect ($P > 0.05$) on the concentration of plasma lysine in sows.

Discussion

Approximately 5–10% of infants suffer from IUGR worldwide, but currently there is no treatment for this disorder (Wu et al. 2008). Likewise, the rate of mortality for multiple pregnancies remains high in human medicine (McKnight et al. 2011). Additionally, embryonic loss and fetal deaths during gestation claim 15–50% of the total number of fertilized ova (Geisert and Schmitt 2002; Holden and Ensminger 2006) and IUGR is a significant problem in pigs (Mateo et al. 2007; Perry 1954; Wu et al. 2004). These problems are more severe in modern sows which have higher fetal growth rates and litter size than those in the past breeds due to intensive genetic selection (De Boo et al. 2005; Reynolds et al. 2006; Wu et al. 2006). Recently, there is increasing interest in nutritional interventions to reduce embryonic loss and ameliorate IUGR in sows and other mammals (Prunier and Quesnel 2000; Whittemore 1996; Wu et al. 2010). For example, supplementing L-arginine to the maternal diet enhanced the reproductive performance of mice with porcine circovirus type-2 infection (Ren et al. 2011). Dietary supplementation of certain nutrients could enhance litter size and fetal growth in swine, such as chromium, L-carnitine, omega-fatty acid, lysine, and L-arginine (Mateo et al. 2007, 2009; Real et al. 2008; Farmer and Petit 2009; Yang et al. 2009). Among these studies, strategic arginine supplementation to improve pregnancy outcomes has been recognized as a major advance in nutrition research (Bérard and Bee 2010; Li et al. 2010; Mateo et al. 2007; Wu et al. 2010).

Wu and coworkers discovered that arginine was particularly abundant in porcine allantoic fluids (4–6 mmol/l) at day 40 of gestation (Wu et al. 1996, 2010). Also, concentrations of the arginine-family of amino acids (including ornithine and glutamine) in porcine allantoic fluids increased 20–40 times between days 30 and 40 of gestation (Wu et al. 1996, 1998a, b, 2007). Additionally, previous work has shown that dietary supplementation with arginine increases fetal survival and live-born litter weight in pregnant gilts (Mateo et al. 2007), prevents fetal growth retardation and enhances the litter weight of live-born rats (Vosatka et al. 1998; Zeng et al. 2008). All of these studies

Table 4 Effect of dietary L-arginine supplementation on concentrations of free amino acids in the plasma of sows on days 40, 70 and 90 of pregnancy

Items	Gestation (days)								
	40		Pooled SEM	70		Pooled SEM	90		Pooled SEM
	Control	Arginine		Control	Arginine		Control	Arginine	
Tau	0.052	0.056	0.005	0.065	0.066	0.007	0.082	0.094	0.008
Thr	0.191	0.167	0.014	0.179	0.172	0.013	0.210	0.194	0.011
Ser	0.154	0.149	0.011	0.167	0.166	0.015	0.200	0.193	0.013
Gln	0.494	0.486	0.045	0.618	0.589	0.038	0.571	0.621	0.044
Gly	0.362	0.360	0.011	0.382	0.390	0.009	0.421	0.418	0.010
Ala	0.755	0.683	0.056	0.858	0.827	0.071	1.020	0.992	0.099
Val	0.375	0.369	0.029	0.355	0.346	0.016	0.448	0.393	0.034
Met	0.066	0.065	0.011	0.072	0.080	0.016	0.070	0.069	0.004
Ile	0.102	0.118	0.013	0.108	0.112	0.006	0.132	0.122	0.010
Leu	0.278	0.282	0.022	0.274	0.283	0.012	0.309	0.310	0.021
Tyr	0.108	0.102	0.007	0.101	0.111	0.009	0.129	0.124	0.007
Phe	0.074	0.072	0.006	0.077	0.079	0.005	0.073	0.083	0.006
Orn	0.115	0.169**	0.012	0.105	0.168**	0.005	0.117	0.213**	0.011
Lys	0.259	0.247	0.018	0.267	0.281	0.022	0.298	0.254	0.029
Arg	0.186	0.348**	0.019	0.177	0.405**	0.020	0.200	0.401**	0.026
Pro	0.206	0.272*	0.020	0.213	0.289*	0.016	0.232	0.331*	0.019

Data (mmol/L) are means with pooled SEM, $n = 18$

** $P < 0.01$, * $P < 0.05$ versus the control group

indicate that arginine plays an important role in embryonic and placental development in pregnancy (Xiao and Li 2005; Wu et al. 2007). Such effects of arginine were confirmed for the first time in the present study involving primiparous and multiparous sows, as indicated by increases in the total number of piglets born, the number of piglets born alive, litter birth weights of all piglets born, litter birth weights of piglets born alive, and placental weight (Table 2). Of particular note, the current experiment was carried out under practical conditions on a commercial farm, and the basal diet used was previously considered to provide adequate arginine for maximal reproductive performance of sows. Thus, the favorable pregnancy outcomes further underscore the significance of dietary arginine supplementation to enhance the litter size of mammals (Mateo et al. 2007; Zeng et al. 2008). Additionally, the findings will provide a much needed experimental database to revise the next version of nutrient requirements for gestating sows. Currently, results from independent studies in North America and the Europe have indicated that dietary arginine supplementation beginning after day 14 of gestation can enhance embryonic/fetal survival in gilts (Bérard and Bee 2010; Mateo et al. 2007; Wu et al. 2010). However, caution should be taken with regard to the optimal period for supplementing arginine to the gestating swine after breeding. This is epitomized by the recent report that dietary supplementation with 0.8% arginine between days 0 and 25 of gestation adversely affects the litter size of gilts (Li et al. 2010). Thus, arginine supplementation should not be conducted within the first 2 weeks of pregnancy in gilts or sows (Wu 2010).

Arginine is not only required for protein synthesis and ammonia detoxification, but is also metabolized to form glutamine, glutamate, proline, aspartate, asparagine, ornithine, and citrulline with enormous biological importance for placental development and fetal growth (Deutz 2008; Wu et al. 2007, 2008). L-Arginine also activates the mammalian target of rapamycin signaling to promote mRNA translation initiation in cells (Kim et al. 2011; Tan et al. 2010). Additionally, arginine is a physiological substrate for NO and polyamines syntheses via NOS and ODC, respectively (Blachier et al. 2011; Wu and Morris 1998). Polyamines and NO are essential to placental growth and angiogenesis, thereby regulating the placental-fetal blood flow and the transfer of nutrients, oxygen, ammonia, and metabolic waste between mother and fetus in pregnant mammals (Bird et al. 2003; Wu et al. 1998b, 2009). Importantly, the size and functional capacity of the placenta, and uteroplacental transfer of materials between mother and fetus, were the major factors to affect embryonic/fetal growth and development (Bazer et al. 1969; Gude et al. 2004; Wu et al. 2006, 2010). Previous reports

have shown that placental efficiency affects litter size and that placental size is highly correlated with fetal weight after day 60 of gestation in swine (Biensen et al. 1998; Wilson et al. 1999).

Another interesting and novel finding of the present work is that dietary arginine supplementation enhanced placental weight per sow (Table 2), indicating an increase in total placental growth. Notably, placental weight per fetus did not differ between control and arginine-supplemented sows despite an increase in the total number of fetal pigs (Table 2). This observation is significant because it indicates that both the placental mass per fetus and placental function should be considered in dissecting out the biochemical and physiological mechanisms responsible for the effects of arginine supplementation on pregnancy outcomes in mammals. Pigs exhibit severe naturally occurring IUGR because of the reduction of placental mass per fetus, resulting in natural uterine insufficiency (Père and Etienne 2000; Rumball et al. 2008; Wu et al. 2006). The arginine treatment affects expression of microRNAs that regulate angiogenesis in the vasculature (Liu et al. 2011) improves placental angiogenesis and development during gestation (Li et al. 2010), thus promoting optimal intrauterine conditions to minimize the losses of viable fetuses (Wu et al. 2004, 2007, 2009).

Available evidence shows that the average birth weight of piglets decreases and its variation increases with increasing litter size (Bérard et al. 2008; Quiniou et al. 2002). Thus, it is possible that the relative reduction in uterus mass per fetus, which results in reduced uterine blood flow per fetus, is the major factor limiting fetal survival and growth when litter size increases (Père and Etienne 2000). Clearly, results of our current study on the average birth weight of piglets born alive and the statistically non-significant variation of this parameter between control and arginine-supplemented sows further support a beneficial effect of dietary arginine supplementation on enhancing placental growth. Although the placental weight was measured in the present work, future research is warranted to determine placental blood flow as well as placental histopathology and immuno-histochemistry in sows.

In support of the above proposition, our results revealed that arginine supplementation altered the concentrations of arginine and related amino acids in the plasma of sows (Table 4). Specifically, the circulating levels of arginine and its metabolites (proline and ornithine) (Wu et al. 2011) were increased in arginine-supplemented sows during gestation, which is conducive to reduce blood ammonia (McKnight et al. 2010; Najarian and Harper 1956) and enhance protein synthesis in the fetus (Wu et al. 2009). This is consistent with the need for a large amount of the arginine-family of amino acids in the critical period of embryo/fetal development (Wu et al. 1996, 1998a, b, 2007). Furthermore, arginase activity is absent from the porcine placenta, (Wu et al. 2005) which make it impossible for the conversion of arginine into ornithine or polyamines within this organ (Wu et al. 2008). However, the higher concentration of ornithine and proline in plasma as a result of arginine catabolism in extraplacental tissues would make up for the lack of arginase in the porcine placenta and support a high rate of polyamine synthesis in the placenta (Wu et al. 2008).

Another novel and exciting finding from this work is that plasma concentration of estradiol was higher in arginine-supplemented sows compared with the control group, but there was no difference in plasma concentrations of progesterone between the two groups of sows during pregnancy (Table 3). Notably, both estrogen and progesterone are essential for the initiation and maintenance of pregnancy in mammals (Matsuura et al. 2004). While it is known that progesterone does not affect growth and development of either the embryo or the fetus within a wide range of concentrations in maternal plasma, elevated levels of estrogen are correlated with the increase in the rate of fetal-placental development, as well as fetal growth and litter size (Edgerton et al. 1971; Kensinger et al. 1986). Previous reports have shown that arginine stimulates the secretion of pancreatic hormones (insulin and glucagon), anterior pituitary hormones (growth hormone and prolactin), and placental lactogen in humans and animals (Alba-Roth et al. 1988; Chew et al. 1984; Davenport et al. 1995). These findings raise an important question regarding a role for arginine in regulating hormone secretions and whole-body metabolism in pregnant sows.

In conclusion, results of this study indicate that dietary supplementation with 1% L-arginine–HCl between days 22 and 114 of gestation enhances pregnancy outcomes in primiparous and multiparous sows under practical production conditions. The beneficial effects of arginine supplementation are associated with enhanced placental weight as well as elevated levels of estrogen and the arginine-family of amino acids in the maternal circulation. These findings support the notion that arginine plays a crucial role in nutrition and physiology of pregnant mammals and that the gestation diet must provide adequate arginine for optimal survival and growth of the embryo and fetus. Our results also have important implications for improving pregnancy outcomes in humans.

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