Revista Brasileira de Zootecnia
Brazilian Journal of Animal Science

ISSN 1806-9290
www.sbz.org.br

Full-length research article

R. Bras. Zootec., 47:e20170283, 2018
https://doi.org/10.1590/rbz4720170283

Non-ruminants

Arginine improves nutritional quality of sow milk and piglet performance

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ABSTRACT - The objective of this study was to evaluate the effect of L-arginine supplementation in lactation diets on the productive and reproductive performance of pluriparous sows and their litters. Seventy-six sows of the same genetic line were assigned to four treatments in a completely randomized design with 19 replicates. The experimental unit was the sow and its respective litter. Treatments comprised a control diet (no L-arginine supplementation) and other three diets obtained by top dressing the control diet with 0.5, 1.0, and 1.5% of L-arginine. L-arginine supplementation had no effect on any performance variables, body condition, milk production, or weaning-estrus interval. There was a quadratic effect on percentage of protein and fat in milk as well as on the daily production of these components. Protein and fat percentage declined during lactation. Adding L-arginine to the diet had a quadratic effect on piglet weight at 13 and 21 days, the optimal level of L-arginine supplementation being estimated as 0.64% and 0.71%, respectively. L-arginine supplementation had a quadratic effect on the weight gain of piglets during the first 13 days and on total period of lactation, the optimal level of L-arginine supplementation being estimated as 0.60% and 0.70%, respectively. Supplementing lactation diets with 0.70% of L-arginine, corresponding to 45 g day^{-1}, improves the weight gain of piglets by improving the nutritional quality of sow milk.

Key Words: amino acid, lactation, milk composition, reproduction, swine

Introduction

Genetic improvement has allowed a greater number of piglets to be born alive per litter; however, it has generated many negative aspects for lactating sows including body weight loss, increased weaning-estrus interval, and fewer piglets born in the subsequent farrowing (Boyd et al., 2000). Thus, it is necessary to determine the nutritional requirements of sows in lactation, as frequent changes in genetic lines result in increased productivity of sows (Paiva et al., 2006).

Various options have been tested to overcome the challenges mentioned above, such as adjustments of amino acid levels in the diet, which, in addition to being required for maintenance, milk production, and mammary gland growth, participate in important metabolic pathways. Among these so-called functional amino acids, arginine is particularly important because, besides being a precursor for protein synthesis, it is also a precursor for the synthesis of urea, citrulline, creatine, polyamines, ornithine, proline, agmatine, and nitric oxide (Wu et al., 2004). Nitric oxide contributes to the formation and branching of blood vessels (Matsunaga et al., 2002). Furthermore, arginine stimulates the secretion of prolactin and growth hormone and is required for mammary gland development (Zhu et al., 2017), which may affect the milk production capacity of the sow. Thus, the objective of this study was to evaluate the effect of L-arginine supplementation in lactation diets on the productive and reproductive performance of pluriparous sows and their litters.

Material and Methods

The local bioethics committee approved this study (case no. 43/13). The experiment was conducted with 76 lactating sows

Received: October 27, 2017
Accepted: January 3, 2018
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(parity 2 to 6) on a commercial pig farm located in Oliveira, Minas Gerais, Brazil (latitude: 20°50'50.7444"S, longitude: 44°48'51.7428"W, and 973 m above sea level). Sows were selected based on their reproductive history of 12-13 piglets/farrowing and insemination records using the same boars.

Sows were assigned to four treatments in a completely randomized design with 19 replicates. Treatments comprised a control diet (no L-arginine supplementation) and other three diets obtained by top dressing the control diet with 0.5, 1.0, and 1.5% of L-arginine with 98.5% purity. The sow and its litter represented the experimental unit. The criteria for assigning sows to treatments were based on sows with similar weights and parity order in each treatment. The lactation diet (Table 1) was the diet used on the farm but with L-arginine supplementation, and with 7 kg of feed being offered each day.

Samples of diet were analyzed for crude protein using the Kjeldahl method (AOAC, 2000) and for amino acids by high performance liquid chromatography (Table 2). Sows and piglets had free access to water, and piglets received no feed during the experimental period. Independently of treatments, all newborn piglets were assisted to ensure colostrum intake, and litter size was standardized to 12-13 piglets/sow by cross-fostering 48 h post farrowing.

Each farrowing crate had a creep area for piglets equipped with heat lamps. Sows and their piglets were weighed individually on the 2nd, 13th, and 21st day of lactation to calculate the weight loss of sows as well as the body weight and weight gain of the piglets. On the 2nd and 21st day of lactation, the backfat and loin depth of sows were measured by ultrasound (ALOKA SSD-500) using a linear transducer of 3.5 MHz (model UST 5011) at two positions (P1 and P2). The first measurement was performed at 6.5 cm from the dorsolumbar and 6.5 cm from the last rib in the caudal direction (P1); the second measurement was performed at 6.5 cm from the dorsolumbar and 6.5 cm from the last rib in the cranial direction (P2). A local trichotomy was performed to improve the visualization of the points.

On the 2nd, 13th, and 21st day of lactation, piglets were separated from sows for 1 h, after which 1 mL of oxytocin was injected into the ear vein of 13 sows per treatment with similar body condition. A total of 80 mL of milk per sow was collected by manual extraction from the first eight mammary glands, four from each side, then labeled and stored at −20°C for later analysis of fat and protein content.

Six replicates per treatment were used to evaluate the percentage of fat, using methodologies described in Instruction no. 68 of 12/12/2006, which provides physical and chemical analytical methods for the official control of milk and dairy products (Brasil, 2006). For crude protein analysis,

Table 1 - Ingredient composition of lactation diet

Ingredient	Amount (g kg^{-1} as feed)
Corn	550.60
Soybean meal	318.68
Soybean oil	42.46
Sugar	39.96
Dicalcium phosphate	15.48
Limestone	10.64
Salt	5.00
Sodium bicarbonate	3.00
Kaolin	3.00
Choline chloride 60%	0.70
L-lisyne 78.8	1.50
L-threonine 99	0.74
DL-methionine 99	0.79
Mineral premix[1]	1.00
Vitamin premix[2]	0.40
Citric acid	2.00
Nutritional supplement[3]	4.05

[1]The mineral premix provided the following quantities of minerals per kilogram of complete diet: 45 mg kg^{-1} copper; 275 mg kg^{-1} iron; 8.5 mg kg^{-1} phosphorus; 85 mg kg^{-1} fluorine; 1.75 mg kg^{-1} iodine; 125 mg kg^{-1} manganese; 0.75 mg kg^{-1} selenium; 4.9 mg kg^{-1} sodium; 275 mg kg^{-1} zinc; 0.5 mg kg^{-1} chrome; 100 mg kg^{-1} zinc bacitracin.
[2]The vitamin premix provided the following quantities of vitamins per kilogram of complete diet: 9000 IU kg^{-1} retinol; 1500 IU kg^{-1} cholecalciferol; 60 mg kg^{-1} dl-α-tocopherylacetate; 3 mg kg^{-1} vitamin K; 25 mg kg^{-1} vitamin B12; 40 mg kg^{-1} niacin; 20 mg kg^{-1} pantothenic acid; 2.6 mg kg^{-1} folic acid; 0.27 mg kg^{-1} biotin; 336 mg kg^{-1} choline; 4 mg kg^{-1} pyridoxine; 6 mg kg^{-1} riboflavin; 1.3 mg kg^{-1} thiamine.
[3]Nutritionnal supplement: inactivating mycotoxins and antioxidant.

Table 2 - Analyzed amino acids and nutritional composition of basal diet

Amino acid	Basal diet (g kg^{-1} as fed)
Lysine	10.79
Threonine	8.32
Methionine	2.68
Cystine	2.73
Methionine + cystine	5.41
Alanine	8.97
Arginine	11.13
Aspartic acid	17.28
Glutamic acid	30.70
Glycine	7.28
Histidine	6.29
Isoleucine	7.26
Leucine	16.05
Phenylalanine	9.23
Serine	8.76
Tyrosine	6.79
Valine	8.10
Tryptophan	1.89
Crude protein (g kg^{-1})	173.40
Dry matter (g kg^{-1})	896.50
Calculated value	
Metabolizable energy (kcal kg^{-1})	3445.90
Sodium	2.16
Calcium	8.81
Available phosphorus	3.90
Crude fiber	26.30

seven replicates per treatment were evaluated; the N in milk was analyzed using the Kjeldahl method (AOAC, 2000).

The milk yield of sows was estimated using the equation:

$$\text{Milk yield (g day}^{-1}) = \frac{(0.718 \times \text{piglet daily weight (g)} - 4.9) \times \text{number of piglets}}{0.19}$$

(Noblet and Etianne, 1989). Based on daily milk yield and the concentration of protein and fat in the milk, daily fat and protein production were estimated.

After weaning, all sows received the same management for detection of return to estrus, which consisted of daily use of boar contact at 9.00 h. The day after weaning, on which sows were inseminated, was recorded to evaluate the weaning-estrus interval (WEI). Farrowing room temperature and relative humidity during the lactation period were recorded every 10 min by a data logger (model HT-500) placed in the middle of the room at half the height of the body of sows.

The analysis of variance was performed according to the statistical model below:

$$Yij = \mu + Gi + \varepsilon ij,$$

in which Yij = observation of the effect of L-arginine level i and at replication j, μ = overall mean, Gi = effect of L-arginine inclusion levels, and εij = random error associated with each observation.

Data were analyzed using SAS (Statistical Analysis System, version 9.0) and subjected to the Shapiro-Wilk test ($\alpha = 0.05$) to ensure normality. Data with normal distribution were subjected to regression analysis to determine the optimum level of L-arginine supplementation. The Tukey test ($\alpha = 0.05$) was performed to compare the mean days of lactation. Data that were not normally distributed were normalized, when possible, using the PROC RANK procedure of SAS, and non-normalized data were compared using the Kruskal-Wallis test ($\alpha = 0.05$).

Results

L-arginine supplementation during lactation did not affect (P>0.05) average daily feed intake (ADFI), body condition, or the weaning-estrus interval of sows (Table 3). There was no change (P>0.05) in the WEI, which was an average of 4.08 days.

L-arginine supplementation during lactation did not affect milk yield (P>0.05) (Table 4), which is directly related to ADFI and body condition. Moreover, treatments had a quadratic effect (P<0.05) on the percentage of protein and fat in milk, estimated using the following equations, respectively:

$$\text{Milk protein (g kg}^{-1}) = 56.20 + 5.76x - 4.21x^2 \ (R^2 = 0.95)$$

$$\text{Milk fat (g kg}^{-1}) = 69.90 + 6.49x - 5.30x^2 \ (R^2 = 0.98)$$

Table 3 - Performance and body condition of sows fed diets with L-arginine supplementation

Variable	L-arginine supplementation (%)				P-value	SEM
	0.0	0.5	1.0	1.5		
Daily feed intake (g)	6,415	6,398	6,409	6,597	0.714	434.36
Sow weight (kg)						
2nd day	254.89	254.79	255.21	255.10	0.999	18.13
13th day	249.32	247.42	249.74	254.47	0.647	17.41
21st day	241.10	238.32	238.18	240.95	0.929	17.68
Sow weight loss (kg)						
2 to 13 days	5.58	7.37	5.47	6.30	0.079	8.43
2 to 21 days	7.26	10.51	8.24	6.48	0.553	8.22
Sow weight loss (g kg^{-1})						
2 to 13 days	21.1	28.5	20.7	17.0	0.072	3.29
2 to 21 days	30.4	47.8	36.7	29.2	0.513	3.67
Loin depth at 2nd day (cm)						
P1	4.95	5.07	4.94	5.09	0.931	0.86
P2	4.59	4.67	4.74	4.61	0.925	0.70
Loin depth at 21st day (cm)						
P1	4.52	4.58	4.31	4.54	0.593	0.65
P2	4.40	4.47	4.36	4.34	0.905	0.59
Backfat depth at 2nd day (cm)						
P1	1.28	1.28	1.42	1.39	0.449	0.30
P2	1.20	1.18	1.27	1.29	0.741	0.27
Backfat depth at 21st day (cm)						
P1	1.34	1.34	1.39	1.39	0.969	0.27
P2	1.24	1.23	1.27	1.38	0.760	0.35
Weaning estrus interval (days)	3.94	4.22	4.18	4.00	0.913	1.01

SEM - standard error of the mean.

The treatments also had a quadratic effect (P<0.05) on daily production of protein and fat in the milk, estimated using the following equations respectively:

$$\text{Production of milk protein (g kg}^{-1}) = 0.54 + 0.16x - 0.12x^2 \ (R^2 = 0.94)$$

$$\text{Production of milk fat (g kg}^{-1}) = 0.78 + 0.17x - 0.12x^2 \ (R^2 = 0.97)$$

The highest levels of concentration and production were obtained at 0.68, 0.61, 0.66, and 0.66% L-arginine supplementation, respectively.

There was an effect (P<0.05) of days of lactation on the percentage of protein and fat in milk (Table 4). The percentage of milk protein declined from the 2nd to the 13th day of lactation (by 22.92%), and from this to the 21st day of lactation (by 4.92%) (P<0.05) (Table 4); the same pattern was observed for percentage of milk fat (P<0.05), with reductions of 12.16 and 3.98%, respectively (Table 4).

L-arginine did not affect (P>0.05) the number of piglets at 21 days of age (Table 5). However, adding L-arginine to the diets had a quadratic effect (P<0.05) on piglet weight at day 13 of lactation, as estimated by the equation:

$$\text{Weight of piglets at 13 days (g)} = 3988.53 + 490.89x - 385.40x^2 \ (R^2 = 0.78)$$

and on piglet weight at day 21 of lactation (P<0.05), as estimated by the equation:

$$\text{Weight of piglets at 21 days (g)} = 6287.09 + 917.31x - 642.20x^2 \ (R^2 = 0.82),$$

with weights at 13 and 21 days being highest for 0.64 and 0.71% L-arginine, respectively. Similarly, L-arginine influenced (P<0.05) piglet weight gain during the first 13 days of lactation in a quadratic way, as estimated by the equation:

$$\text{Weight gain at 13 days (g day}^{-1}) = 222.32 + 34.47x - 28.64x^2 \ (R^2 = 0.85)$$

Table 4 - Sow milk yield and nutritional composition according to lactation period and L-arginine supplementation in the diet

Variable	L-arginine supplementation (%)				Average	SEM	P-value[1]		
	0.0	0.5	1.0	1.5			L	Q	C
Milk yield (kg day^{-1})	11.14	11.65	11.55	11.16		1.51	0.975	0.231	0.844
Milk protein concentration (g kg^{-1})									
2nd day	68.7	73.0	65.4	67.8	68.5A	10.4	0.339	0.296	0.299
13th day	51.9	53.2	57.6	48.9	52.8B	5.7	0.446	0.151	0.073
21st day	48.7	52.1	51.4	48.5	50.2C	6.2	0.850	0.912	0.775
Average[2]	56.4	59.4	58.1	55.0	56.8	5.4	0.728	0.035	0.636
Daily protein production (kg day^{-1})[3]	0.64	0.67	0.67	0.62	0.65	0.06	0.206	0.002	0.396
Milk fat concentration (g kg^{-1})									
2nd day	76.3	79.1	79.6	74.1	77.3A	4.3	0.565	0.889	0.559
13th day	68.1	69.6	68.0	65.8	67.9B	3.1	0.421	0.501	0.641
21st day	64.9	67.5	64.9	63.6	65.2C	2.7	0.064	0.092	0.150
Average[4]	69.8	72.1	70.8	67.8	70.1	6.2	0.036	0.001	0.587
Daily fat production (kg day^{-1})[5]	0.78	0.84	0.82	0.76	0.80	0.07	0.469	0.022	0.693

SEM - standard error of the mean.
Different letters in the column differ by Tukey test (P<0.05).
[1] L, Q, and C = linear, quadratic, and cubic effects, respectively, concerning the inclusion of L-arginine in the diet.
[2] $\hat{Y} = 56.20 + 5.76x - 4.21x^2$ ($R^2 = 0.95$).
[3] $\hat{Y} = 0.54 + 0.16x - 0.12x^2$ ($R^2 = 0.94$).
[4] $\hat{Y} = 69.90 + 6.49x - 5.30x^2$ ($R^2 = 0.97$).
[5] $\hat{Y} = 0.78 + 0.17x - 0.12x^2$ ($R^2 = 0.97$).

Table 5 - Live weight and average daily gain of piglets from lactating sows fed diet with L-arginine supplementation

Variable	L-arginine supplementation (%)				SEM	P-value[1]		
	0.0	0.5	1.0	1.5				
	n = 16	n = 15	n = 16	n = 14		L	Q	C
Piglets/sow at 2nd day	13.12	12.87	12.94	13.14	0.88	0.944	0.318	0.850
Piglets/sow at 21st day	12.75	12.53	12.81	12.71	1.13	0.900	0.846	0.509
Weight at 2nd day (g)	1540	1600	1560	1560	170	0.846	0.430	0.435
Weight at 13th day (g)[2]	3988	4137	4094	3858	382	0.367	0.048	0.246
Weight at 21st day (g)[3]	6287	6585	6562	6218	522	0.791	0.016	0.248
ADG from 2-13 days (g)[4]	222.3	232.4	228.2	209.6	27.3	0.205	0.039	0.306
ADG from 2-21 days (g)[5]	249.7	263.4	262.0	245.6	24.8	0.714	0.017	0.326

SEM - standard error of the mean.
[1] L, Q, and C = linear, quadratic, and cubic effects, respectively, concerning the inclusion of L-arginine in the diet.
[2] $\hat{Y} = 3988.53 + 490.89x - 385.40x^2$ ($R^2 = 0.78$).
[3] $\hat{Y} = 6287.09 + 917.31x - 642.20x^2$ ($R^2 = 0.82$).
[4] $\hat{Y} = 222.32 + 34.47x - 28.64x^2$ ($R^2 = 0.85$).
[5] $\hat{Y} = 249.69 + 42.40x - 30.09x^2$ ($R^2 = 0.86$).

and over the total period ($P<0.05$), as estimated by the equation:

$$\text{Weight gain at 21 days (g day}^{-1}) = 249.69 + 42.40x - 30.09x^2 \ (R^2 = 0.86)$$

(Table 5), with weight gains being highest for 0.60 and 0.70% L-arginine, respectively.

Discussion

The temperature at which the sows were housed was close to that suggested by Nääs (2000), ranging from 12 to 25°C; the relative humidity was consistent with Veit and Troutt (1982), ranging from 55 to 75% (Figure 1). Thus, it can be inferred that animals were in their thermal comfort zone for most of the time.

The ADFI of sows was 6,455 g day^{-1}. As the daily supply of feed was fixed at 7 kg day^{-1}, it can be inferred that sows were able to reach their physiological limit of intake. According to Mello (1993), an imbalance of amino acids in the diet reduces voluntary feed intake; however, sows are unaffected if the digestible arginine:lysine ratio is less than 3:1 (Wu et al., 2013). In this study, the maximum ratio between these two amino acids was 2.89, which could explain why there was no effect of L-arginine on ADFI. The ADFI of lactating sows can be affected by diet flavor, parity, body weight, litter size, and environment (Domiciano et al., 2008; Mosnier et al., 2010; Wang et al., 2014). In this study, these factors were kept unchanged among treatments.

The weight of sows was not affected by L-arginine. According to Schenkel et al. (2010), sows lose weight during lactation, and the magnitude of this loss is important for their future reproductive performance. It has been assumed that if weight loss is less or around 13%, it does not affect the future reproductive performance of sows (Hoving et al., 2011).

In this study, sows in all treatments lost less than 5.0% of their weight at weaning, indicating minor body mobilization during lactation. This can be explained by the fact that sows were not subjected to dietary restrictions during the study, given that feed leftovers were observed in all treatments. Another important factor is the nutritional quality of the diet, especially in relation to lysine, which is the major limiting amino acid for lactating sows, affecting both milk yield and the extent of body mobilization (Nunes et al., 2006). In this study, the average digestible lysine intake was 61.3 g day^{-1}, meeting the requirements for this amino acid according to Rostagno and Gomes (2011).

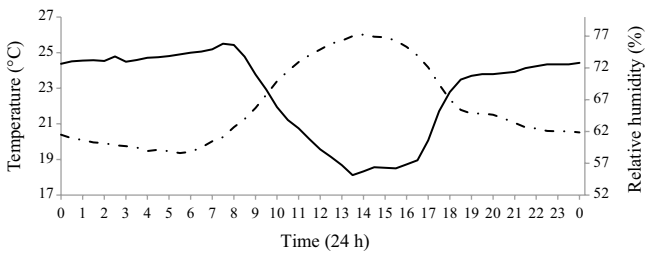

Figure 1 - Average temperature (°C, dashed line) and average air relative humidity (%, continued line) during the trial period.

Weaning to estrus interval was consistent with the results observed by Mateo et al. (2008), who used 1% L-arginine, and with those obtained by Schenkel et al. (2010), who found that 90% of the sows returned to estrus between three and five days after weaning. Based on reports by Schenkel et al. (2010), sows may lose up to 10% of their body weight during lactation without affecting subsequent reproductive performance, and thus the observed WEI was considered normal and was not affected by the weight loss of the sows (average loss of 3.60%).

Milk production is directly related to ADFI and body condition. L-arginine supplementation during lactation did not affect these variables; however, it did affect milk nutritional quality (protein and fat). Milk nutrients are derived from two sources: feed and body tissues (fat and muscle) mobilized during lactation (Lima et al., 2011). As neither ADFI nor the mobilization of tissues, as indicated by backfat thickness and loin depth, were affected by supplementation with L-arginine, it is possible that the observed improvement in milk nutritional quality was due to the metabolic roles of arginine in the mammary glands and improvements in the use efficiency of dietary protein, as arginine level in the diets was the only factor that differed between treatments.

Mammary blood flow and angiogenesis are regulated by nitric oxide, which is a product of arginine metabolism (Lacasse and Prosser, 2003). In this sense, arginine supplementation in the diet of sows may improve blood flow and enhance nutrient flow to the mammary glands, contributing to milk protein synthesis due to increased nitric oxide synthesis in the endothelial cells of blood vessels (Wu and Meininger, 2002). Furthermore, milk production is highly correlated with growth and development of mammary glands (Kim et al., 2000). Zhu et al. (2017) found that 1% L-arginine increased the secretion of prolactin and growth hormone, both of which are necessary for mammary gland development. Moreover, Mateo et al. (2008) found that 1% L-arginine in the diet of gilts increased the flow of amino acids to the mammary glands through the action of arginine on anabolic hormones such as insulin, which was present at higher concentrations in the plasma of gilts fed diets supplemented with this amino acid, especially during the first week of lactation.

According to Trottier et al. (1997), 188.5 g of essential amino acids are absorbed by mammary glands every day, and only 49 g or 25% of these amino acids are retained in the mammary glands. These amino acids can be used for the synthesis of structural proteins and remodeling of mammary tissue cells, which may have influenced the increase in percentage milk protein.

Amino acids retained in the mammary glands can also serve as energy substrates. It is possible that arginine helped increase milk fat, since it has been proven that arginine increases the availability of amino acids to mammary glands. Zhu et al. (2017) supplemented the diet of gilts with 1% L-arginine and found an increase in the amount of fat in the milk; this result is similar to the results of the present trial. Another important factor is the ratio of amino acids in the feed (Trottier et al., 1997), the recommended ratio of digestible arginine:digestible lysine of 1.43:1 to ensure the maximum amino acid absorption by the mammary glands (Guan et al., 2004) was achieved in this study.

Furthermore, supplementation with 1% L-arginine stimulates insulin production, which participates in lipoprotein lipase activation (Mateo et al., 2008). The increased insulin activates lipoprotein lipase enzyme, which releases glycerol and fatty acids of triglycerides that may be absorbed into the peripheral tissues, especially muscle and fat for energy and storage or for milk fat synthesis.

The changes in milk composition on the days of evaluation were consistent with several other studies (Daza et al., 2004; Aguinaga et al., 2011; Wang et al., 2013) that observed a reduction in the nutritional quality of milk as lactation progressed.

The higher weight gain of piglets from sows fed L-arginine-supplemented diets in this study may be attributed to the improvement in the nutritional quality of milk (protein and fat), as piglets did not have access to another source of feed. Besides, milk production did not vary among treatments, and tissue mobilization among sows was similar.

According to Kim and Wu (2009), supplementing the diet of lactating gilts with 0.83% L-arginine increased piglet growth. This amount of L-arginine was a little less than the observed level in our study; considering that gilts have higher nutritional requirements, as they are still developing and in full reproductive activity, this suggests that a higher level of L-arginine would be needed to keep increasing the weight of piglets for gilts.

Conclusions

Supplementation of 0.70% L-arginine in the lactation diet, corresponding to the intake of 45 g day^{-1} of L-arginine, improves the weight gain of piglets during the total trial period, which may be explained by the improvement in nutritional quality of the milk of pluriparous sows.

Acknowledgments

The authors want to acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA/CNPq), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for the scholarship granted to the first author, and the Ajinomoto Animal Nutrition.

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