Relative bioavailability of L-lysine sulfate is equivalent to that of L-lysine HCl for nursery piglets¹

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ABSTRACT: Supplementary L-lysine sources include L-lysine HCl and L-lysine sulfate. L-Lysine sulfate contains at least 50% L-Lys and other components as residues from the fermentation process, other amino acids, and other organic and inorganic substances, being an alternative to L-Lys HCl. The aim of this study was to evaluate the relative bioavailability (RBV) of L-Lys sulfate in comparison with L-Lys HCl and its effects on performance, blood parameters, intestinal functionality, and the apparent total tract digestibility in nursery piglets. A total of 168 female piglets (DB90 × PIC337), weaned at 22 d (BW = 6.29 ± 0.41 kg), were distributed in seven dietary treatments and eight replicates, with three pigs per pen. The experimental period of 42 d was divided into two phases (phase 1, days 0–21; phase 2, days 21 to 42). The basal diet (CON) was lysine-deficient formulated to meet 73% of standardized ileal digestible Lys requirements. For the

other diets, the CON was supplemented with three levels (80%, 90%, and 100% of standardized ileal digestible Lys requirements) of L-Lys sulfate (70% L-Lys) or L-Lys HCl (79% L-Lys). There were no significant differences ($P > 0.05$) in the performance and concentrations of plasma urea and creatinine between the L-Lys sources. The RBV of L-Lys sulfate relative to L-Lys HCl was 106%, 119%, and 117% for effects on ADG, G:F, and plasma urea, respectively. Lys deficiency resulted in a greater ($P < 0.05$) incidence of diarrhea, while pigs supplemented with Lys sulfate or Lys HCl showed greater ($P < 0.05$) villus height in the jejunum when compared to those receiving the CON. Diets supplemented with L-Lys sulfate had greater ($P < 0.05$) apparent total tract digestibility of dry matter, gross energy, and crude protein. In conclusion, the RBV of L-Lys sulfate for effects on ADG, G:F, and plasma urea is equivalent to that of L-Lys HCl for nursery piglets.

Key words: diarrhea, intestinal morphometry, limiting amino acid, nutrition, piglet

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INTRODUCTION

The main objective of pork production is to provide lean tissue for human consumption. Lean tissue growth is the factor that most greatly influences the pigs' amino acid requirements, especially for Lys (NRC, 2012). Lys is the first limiting amino acid in diets for weaned piglets, and is essential for carcass muscle growth (Wu, 2013). Its adequate balance in the diet becomes

important to meet the amino-acid requirements in pigs' different stages of growth.

Supplementary Lys is used to supply probable deficits of this amino acid in diets (Baker et al., 1993; Eklunda et al., 2010), to reduce CP content of the diet (Hansen et al., 1993; Nyachoti et al., 2006), and reduce nitrogen (N) excretion (Jin et al., 1998; Shriver et al., 2003). In many cases, supplemental Lys increases feed efficiency and consequently is cost effective (Junghans et al., 2007; Zhao et al., 2014). Supplementary Lys sources include L-Lys HCl (78% L-Lys) and L-Lys sulfate (>50% L-Lys). L-Lys sulfate is obtained in a fermentation process similar to L-Lys HCl, but the processing forms differ mainly in the recovery and purification steps. At the end of processing, L-Lys sulfate contains at least 50% L-Lys in the base and some components such as other amino acids, residues from the fermentation process (dried microbial cells), macromolecules, and other organic and inorganic substances that could have some beneficial effect on the digestive and absorptive processes of piglets in the postweaning period and improve their performance (Whittemore and Moffat 1976; Smiricky-Tjardes et al., 2004; Liu et al., 2007).

For the best utilization and strategic use of these products, knowledge about their relative bioavailability (RBV) is necessary, especially when formulating diets that provide the greatest economic return. Bioavailability studies necessitate feeding graded levels of the nutrient in question and the linear response area is defined by a minimum of three levels of the independent variable (Baker, 1986). However, studies on RBV of Lys from L-Lys sulfate and L-Lys HCl in the postweaning phase are limited for nursery piglets. The aim of the present study was to evaluate the RBV of L-Lys sulfate in comparison with L-Lys HCl and its effects on performance, blood parameters, intestinal functionality, and the apparent total tract digestibility in nursery piglets.

MATERIALS AND METHODS

All experimental procedures for the present study were approved by the Ethics Committee on Animal use of the Federal University of Lavras under the Protocol 076-16.

The experiment was performed in the weaning facilities of the Experimental Center on pig farming (*Centro Experimental de Suínos*) at the Department of Animal Science of the Federal University of Lavras, in Lavras, MG, Brazil. A total of 168 female piglets from a commercial lineage of high genetic value (DB90 × PIC337) were weaned at 22 d of age (mean BW = 6.29 ± 0.41 kg) and placed on trial.

The pigs were housed in raised decks pens (120 cm × 114 cm) with fully plastic slatted floor. Each pen had an adjustable nipple drinker and a galvanized steel trough. The rooms underwent a cleaning and disinfection program prior to the arrival of pigs.

To characterize the environment, a thermo-hygrometer associated with a black globe was installed in each room. Data were recorded twice a day (0700; 1600 hours) for the subsequent wet-bulb globe temperature (WBGT). Temperature and ventilation of rooms were controlled using heaters and adjustment of windows. The average minimum and maximum temperatures in the first 21 d of the experiment were, respectively, 22.4 and 28.3 °C, while in the second period (21 to 42 d) were 21.7 and 25.3 °C. The calculated WBGT was 71.88 and 69.72 for the first and second phase of the experiment.

The experimental period of 42 d was divided into two phases (phase 1, days 0 to 21; phase 2, days 21 to 42). The experimental design was a randomized complete block, with seven dietary treatments and eight replicates per treatment, with three pigs per experimental pen. The basal diet (CON) was lysine-deficient formulated to meet 73% of standardized ileal digestible Lys requirements, but adequate in other AA and energy (NRC, 2012). For the other dietary treatments, the CON was supplemented with three levels (80%, 90%, and 100% of standardized ileal digestible Lys requirements) of L-Lys sulfate (70% L-Lys) or L-Lys HCl (79% L-Lys). For the initial phase, the CON diet was formulated containing 0.987% SID-Lys, the diets supplemented with either L-Lys sulfate or L-Lys HCl had 1.080%, 1.215%, and 1.350% SID-Lys, respectively, and replaced kaolin in the CON diet. For the second phase, the CON diet was formulated containing 0.90% SID-Lys, the diets supplemented with L-Lys sulfate or L-Lys HCl had 0.984%, 1.107%, and 1.230% SID-Lys, respectively. The L-Lys sulfate and L-Lys HCl were obtained from a commercial company and had 70% and 79% of L-Lys in the base (CJ do Brasil Ind. e Com. de Produtos Alimentícios Ltda. São Paulo, Brazil). Ingredients and nutritional composition of diets are presented in Tables 1 and 2. The analyzed free Lys content of the diets was used in all calculations. The pigs had *ad libitum* access to feed and water throughout the experimental period.

Pigs were weighed on the first day of the experiment, and at 7, 14, 21, 28, and 42 d. The same scale was used for all weighing. The provided diet and the leftovers were quantified daily in order to calculate ADG, ADFI, and G:F. At days 14, 28, and 42, blood samples from one pig per pen were collected for analysis of plasma urea and creatinine. Approximately

Table 1. Experimental diets of phase 1, days 0 to 21 (as-fed basis)

Ingredients, %	CON	L-Lys sulfate			L-Lys HCl		
		80%	90%	100%	80%	90%	100%
Corn, 7.88% CP	52.55	52.55	52.55	52.55	52.55	52.55	52.55
Micronized soy	11.66	11.66	11.66	11.66	11.66	11.66	11.66
Soybean meal	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Plasma, spray dried	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Whey dried, 70% lactose	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean oil	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral-vitamin premix ¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate, 18.5%	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Calcitic limestone	0.98	0.98	0.98	0.98	0.98	0.98	0.98
Common salt	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Zinc oxide, 72%	0.34	0.34	0.34	0.34	0.34	0.34	0.34
L-Lys HCl, 79%	0.00	0.00	0.00	0.00	0.12	0.29	0.46
L-Lys sulfate, 70%	0.00	0.13	0.33	0.52	0.00	0.00	0.00
L-Threonine	0.13	0.13	0.13	0.13	0.13	0.13	0.13
L-Tryptophan	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L-Valine	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-Methionine	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Kaolin	0.60	0.47	0.27	0.08	0.48	0.31	0.14
Calculated values ²							
ME, kcal/kg	3,400 (4,093)	3,400 (4,093)	3,400 (4,093)	3,400 (4,093)	3,400 (4,093)	3,400 (4,093)	3,400.00 (4,093)
CP, %	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)	19.19 (19.31)
SID ³ Lys, %	0.99 (1.15)	1.08 (1.24)	1.22 (1.38)	1.35 (1.51)	1.08 (1.24)	1.22 (1.38)	1.35 (1.51)
SID Met, %	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)	0.41 (0.43)
SID Met + Cys, %	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)	0.74 (0.86)
SID Thr, %	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)	0.79 (0.96)
SID Trp, %	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)	0.22 (0.24)
SID Arg, %	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)	1.12 (1.36)
SID Val, %	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)	0.86 (0.98)
SID Ile, %	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)	0.69 (0.87)
SID Leu, %	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)	1.49 (1.71)
SID His, %	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)	0.48 (0.58)
SID Phe, %	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)	0.80 (1.00)
Lactose, %	10.50	10.50	10.50	10.50	10.50	10.50	10.50
Total calcium, %	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)	0.80 (0.83)
Total phosphorus, %	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)	0.59 (0.60)
Available phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium, %	0.35	0.35	0.35	0.35	0.35	0.35	0.35

¹Levels per kg of diet, Mineral Premix: 0.08 g of iron, 0.04 g of manganese, 0.2 mg of cobalt, 0.11 g of zinc, 1.2 mg of iodine, and 0.35 mg of selenium. Vitamin premix: 15,000 IU of vitamin A, 3,125 IU of vitamin D3, 87.5 IU of vitamin E, 3.13 mg of vitamin K3, 2.75 mg of vitamin B1, 0.01 g of vitamin B2, 0.025 g of vitamin pantothenic acid, 3.75 mg of vitamin B6, 37.5 mg of vitamin B12, 0.038 g of nicotinic acid, 3.75 mg of folic acid, and 0.5 mg of biotin.

²Values in parentheses are analyzed = gross energy (kcal/kg); total AA contents (%); total calcium and phosphorus (%).

³SID = standardized ileal digestible.

8 mL blood was collected by jugular vein puncture in heparinized tubes. In the laboratory, plasma samples were then collected by centrifuging at $3,000 \times g$, 15 min, 4 °C, allocated into 1.5 mL microcentrifuge tubes. Plasma urea and creatinine analyses were performed through an automated analyzer (Labmax 240, Hirose Electric System Co., Ltd. 1-9-6, Ebisuminami, Nbf Ebisuminami Building, Gf. Shibuya-ku 150-0022, Japan), using the enzymatic and kinetic methods, respectively.

Daily, throughout the 42 d experiment, fecal consistency was visually examined at the same time, by the same person, and was scored on a scale of 1 to 5 (Halas et al. 2010). Diarrhoea was recorded when a piglet developed pasty or watery fecal consistency (scores 4 to 5). The incidence of diarrhea (%) was calculated as the sum of the total number of daily diarrheal piglet observations over the period divided by the number of piglet days in the period, and the quotient multiplied by 100. The

Table 2. Experimental diets for phase 2, days 21 to 42 (as-fed basis)

Ingredients, %	CON	L-Lys sulfate			L-Lys HCl		
		80%	90%	100%	80%	90%	100%
Corn, 7.88% CP	61.94	61.94	61.94	61.94	61.94	61.94	61.94
Micronized soy	6.79	6.79	6.79	6.79	6.79	6.79	6.79
Soybean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Mineral-vitamin premix ¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate, 18.5%	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Calcitic limestone	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Common salt	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Zinc oxide, 72%	0.26	0.26	0.26	0.26	0.26	0.26	0.26
L-Lys HCl, 79%	0.00	0.00	0.00	0.00	0.11	0.26	0.42
L-Lys sulfate, 70%	0.00	0.12	0.30	0.47	0.00	0.00	0.00
L-Threonine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Kaolin	0.70	0.58	0.40	0.23	0.59	0.44	0.28
Calculated values ²							
ME, kcal/kg	3350 (4047)	3350 (4047)	3350 (4047)	3350 (4047)	3350 (4047)	3350 (4047)	3350 (4047)
CP, %	19.00 (19.49)	19.00 (19.49)	19.00 (19.49)	19.00 (19.49)	19.00 (19.49)	19.00 (19.49)	19.00 (19.49)
SID ³ Lys, %	0.90 (0.97)	0.98 (1.05)	1.11 (1.18)	1.23 (3.30)	0.98 (1.05)	1.11 (1.18)	1.23 (1.30)
SID Met, %	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)	0.41 (0.39)
SID Met + Cys, %	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)	0.68 (0.76)
SID Thr, %	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)	0.73 (0.86)
SID Trp, %	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)	0.20 (0.25)
SID Arg, %	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)	1.21 (1.29)
SID Val, %	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)	0.81 (0.93)
SID Ile, %	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)	0.73 (0.87)
SID Leu, %	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)	1.52 (1.65)
SID His, %	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)	0.48 (0.54)
SID Phe, %	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)	0.86 (0.94)
Total calcium, %	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)	0.70 (0.67)
Total phosphorus, %	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)	0.56 (0.57)
Available phosphorus, %	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Sodium, %	0.28	0.28	0.28	0.28	0.28	0.28	0.28

¹Levels per kg of diet, mineral premix: 0.08 g of iron, 0.04 g of manganese, 0.2 mg of cobalt, 0.11 g of zinc, 1.2 mg of iodine, 0.35 mg of selenium. Vitamin premix: 15,000 IU of vitamin A, 3,125 IU of vitamin D3, 87.5 IU of vitamin E, 3.13 mg of vitamin K3, 2.75 mg of vitamin B1, 0.01 g of vitamin B2, 0.025 g of vitamin pantothenic acid, 3.75 mg of vitamin B6, 37.5 mg of vitamin B12, 0.038 g of nicotinic acid, 3.75 mg of folic acid, and 0.5 mg of biotin.

²Values in parentheses are analyzed = gross energy (kcal/kg); total AA contents (%); total calcium and phosphorus (%).

³ SID = standardized ileal digestible.

incidence of diarrhea was calculated in the first week and throughout the experimental period.

On day 42 of the experiment, one pig with BW closest to the pen average weight was chosen from each experimental pen and euthanized, totaling 56 pigs. The slaughter was performed through stunning by electronarcosis (>300 V, 1.25 A, for 6 s) followed by exsanguination. The slaughter was performed in a commercial slaughterhouse in the municipality of Lavras (Minas Gerais, Brazil) accompanied by a veterinarian. After evisceration, samples of about 3 cm long were collected from the jejunum (location relative to the of ileo-cecal junction) to perform histological analyses (villus height,

crypt depth, and villus:crypt ratio). After careful removal of the luminal content and washing with saline solution, the jejunum samples were fixed in 10% formaldehyde for 24 h and transferred to 70% alcohol solution until the slides were prepared. The histological analysis was performed in paraffin-embedded segments, sectioned at 4 µm and stained with hematoxylin and eosin stain, based on Luna (1968). The slides were photographed through the trinocular microscope (CX31, Olympus Optical do Brasil Ltda., São Paulo, SP, Brazil) and digital image capture camera (SC30, Olympus Optical do Brasil Ltda., São Paulo, SP, Brazil). The villus height and crypt depth were measured through the

AxionVision SE64 4.9.1 software, using 15 well-oriented villi and crypts per tissue. The villus:crypt ratio was calculated and all analyses were performed by a single person.

The apparent total tract digestibility (ATTD) of dry matter, crude protein, and gross energy of experimental diets were calculated according to the indirect evaluation method with the chromium oxide (Cr_2O_3) indicator. In the last week of the experiment, chromium oxide at 0.3% was added to the feed at the expense of corn. After the presence of the indicator was detected in the feces through the color change in all the pens, samples were collected once a day for three consecutive days in each pen (days 37, 38, and 39). The fecal samples were processed (homogenized, dried, and ground) and together with samples of experimental diets, were subjected to the analysis of dry matter, crude protein, gross energy, and chromium quantification. The following formula was used to calculate ATTD of CP, for example: $\text{ATTD} = 100 - [100 \times (\text{DC}/\text{CF} \times \text{PF}/\text{PD})]$, where ATTD is the apparent total tract digestibility; DC—% Cr_2O_3 in the diet; CF—% Cr_2O_3 in feces; PF—% crude protein in feces; and PD—% crude protein in the diet (Zhang and Adeola, 2017).

The diets and fecal samples were analyzed for dry matter and N content following the methods of AOAC (2000). Crude protein content was calculated by multiplying the N content by 6.25. Gross energy was determined using bomb calorimetry (IKA C5000). The total AA content of the diets was quantified by high-performance liquid chromatography, as outlined by Liu et al. (2007). Calcium and phosphorus in diets were determined according to the methods of AOAC (2000). The chromium was measured by atomic absorption spectrometry, using a spectrophotometer (Varian SpectraAA 100, Varian Australia Pty Ltd, Mulgrave, Victoria, Australia) according to the method reported by Williams et al. (1962).

Statistical analyses of the data were performed by ANOVA, using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Differences were considered significant if $P \leq 0.05$ and were described as tendencies if $0.05 < P \leq 0.10$. Orthogonal-polynomial contrasts were used to determine the linear and quadratic effects of L-Lys levels and sources on the studied variables. Data were fitted in the multivariate linear regression model with the following equation: $y = a + b_1x_1 + b_2x_2$, where y = performance criterion (ADG, G:F, plasma urea), a = common intercept, b_1 = slope of L-Lys HCl, x_1 = value for L-Lys HCl, b_2 = slope of L-Lys sulfate, and x_2 = value for L-Lysine sulfate. The RBV of L-Lys sulfate as

compared with L-Lys HCl was calculated for the 42-d experimental period and using the analyzed free Lys content of the diets. The RBV was calculated as the ratio of their linear slopes (i.e., $b_2/b_1 \times 100$) as described by Littell et al. (1997). The results were considered significant if $P < 0.05$. For the variable incidence of diarrhea, the influence of each treatment on the occurrence of diarrhea was analyzed by applying the generalized linear model in the GENMOD procedure of SAS.

RESULTS AND DISCUSSION

Maximum and minimum temperatures in the first 21 d of the experiment were, respectively, 22.4 and 28.3 °C, while in the second period (21 to 42 d) were 21.7 and 25.3 °C. The calculated WBGT was 71.9 and 69.7 for the first and second phase of the experiment. These environmental parameters indicate that the pigs were kept in a comfortable thermoneutral environment, so that the environment did not represent an influencing factor in the test results.

The animal performance results are presented in Table 3. In the first week, the pigs supplemented with L-Lys sulfate had higher ($P < 0.05$) feed intake than pigs supplemented with L-Lys HCl, with a tendency for greater daily weight gain. There were no significant differences in piglet performance when comparing supplementation of L-Lys sources and CON. When considering the two nursery periods (days 0 to 21 and days 21 to 42), pigs supplemented with L-Lys sulfate or L-Lys HCl had higher ($P < 0.05$) feed efficiency in relation to animals fed with the CON diet. Moreover, there were no significant differences in piglet performance between the tested L-Lys sources and levels.

In swine production, weaning is a stressful event in which pigs are subjected to nutritional changes and immunological and psychological challenges that lead to low feed intake, diarrhea, and decreased performance (Smith et al., 2010). In the present study, the consequences of these stressors were similar for all pigs. Pig performance was not significantly different among the treatments. However, supplementation of L-Lys sulfate produced a greater DFI, which is extremely important in this first week after weaning and could promote a positive residual effect on subsequent performance. For the two nursery periods, supplemented pigs had improved feed efficiency. This was expected as Lys is the first limiting amino acid for pigs, and deficiencies in the intake of this amino acid reduce pig performance.

Plasma urea concentrations were significantly lower ($P < 0.05$) for pigs supplemented with L-Lys

sulfate or L-Lys HCl in relation to those fed with the CON diet (Table 4). These differences were found in the three collection dates (14, 28 and 42 d). Creatinine levels were not influenced ($P > 0.05$) by Lys supplementation in the experimental diets. There were no significant effects of Lys source on plasma urea and creatinine concentrations.

Due to its close relationship with urinary nitrogen excretion, plasma urea can be used as an important metabolic indicator in swine (Zervas and Zijlstra, 2002). According to Cai et al. (1994), the

concentration of plasma urea is inversely correlated with the use of proteins, which in turn is influenced by the quality and quantity of the ingested protein. Thus, the lower the plasma urea concentrations, the greater the use of nitrogenous components of the diet. In the present study, pigs fed with CON had greater plasma urea concentrations. Lys is the first limiting amino acid, and deficiency in Lys would affect the amino acid balance and increase excretion of the remaining amino acids of the diet in the form of urea.

Table 3. Weight, daily weight gain (ADG), daily feed intake (ADFI), and feed efficiency (G:F) of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to CON¹

Item	CON ¹	L-Lys sulfate ²			L-Lys HCl ³			SEM	P-value ⁴		
		80%	90%	100%	80%	90%	100%		C ₁	C ₂	C ₃
Initial BW, kg	6.288	6.288	6.291	6.291	6.288	6.288	6.29	0.0024	0.42	0.78	0.45
Period, days 0 to 7											
BW, kg day 7	6.935	7.033	7.025	6.958	6.778	6.810	6.999	0.0968	0.53	0.52	0.08
ADG, kg	0.109	0.124	0.121	0.113	0.081	0.090	0.119	0.0160	0.58	0.52	0.09
ADFI, kg	0.175	0.194	0.210	0.164	0.146	0.146	0.186	0.0152	0.42	0.39	0.02
G:F, kg/kg	0.604	0.643	0.583	0.601	0.541	0.498	0.586	0.0528	0.94	0.32	0.14
Period, days 0 to 21											
BW, kg day 21	11.696	11.833	12.215	12.116	11.559	11.635	12.153	0.1102	0.14	0.72	0.1174
ADG, kg	0.258	0.264	0.280	0.279	0.253	0.255	0.278	0.0042	0.15	0.69	0.1409
ADFI, kg	0.428	0.431	0.443	0.418	0.409	0.411	0.423	0.0062	0.88	0.54	0.2808
G:F, kg/kg	0.603	0.615	0.640	0.670	0.615	0.620	0.650	0.0048	<0.01	0.04	0.1443
Period, days 0 to 42											
BW, kg day 42	21.233	21.485	22.085	22.715	21.125	21.611	22.729	0.4699	0.12	0.28	0.48
ADG, kg	0.365	0.371	0.385	0.401	0.362	0.374	0.401	0.0115	0.12	0.29	0.48
ADFI, kg	0.661	0.655	0.664	0.636	0.619	0.630	0.662	0.0205	0.70	0.33	0.40
G:F, kg/kg	0.553	0.566	0.581	0.630	0.586	0.593	0.606	0.0097	<0.01	<0.01	0.73

¹CON = basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

²Supplementation of L-Lys sulfate meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

³Supplementation of L-Lys HCl meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

⁴P-values of the orthogonal contrasts: C₁ = CON vs. L-Lys sulfate; C₂ = CON vs. L-Lys HCl; C₃ = L-Lys sulfate vs. L-Lys HCl.

Table 4. Plasma urea and creatinine, in milligram per deciliter, of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to a basal diet

Item	CON ¹	L-Lys sulfate ²			L-Lys HCl ³			SEM	P-value ⁴		
		80%	90%	100%	80%	90%	100%		C ₁	C ₂	C ₃
Urea, day 14	24.38	21.75	14.75	11.63	18.38	12.25	9.25	1.8967	<0.01	<0.01	0.08
Creatinine, day 14	0.94	1.01	0.94	1.02	1.01	0.98	0.89	0.0488	0.39	0.71	0.49
Urea, day 28	27.88	20.38	18.75	15.50	21.50	18.13	15.00	1.7674	<0.01	<0.01	0.99
Creatinine, day 28	0.95	0.99	0.93	0.96	0.89	0.93	0.94	0.0352	0.88	0.47	0.25
Urea, day 42	22.75	18.88	17.00	14.63	19.88	17.25	15.50	1.4409	<0.01	<0.01	0.55
Creatinine, day 42	1.03	1.04	1.10	1.14	1.15	1.11	1.10	0.0473	0.26	0.10	0.41

¹CON = basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

²Supplementation of L-Lys sulfate meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

³Supplementation of L-Lys HCl meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

⁴P-values of the orthogonal contrasts: C₁ = CON vs. L-Lys sulfate; C₂ = CON vs. L-Lys HCl; C₃ = L-Lys sulfate vs. L-Lys HCl.

Creatinine is a nonprotein nitrogen, produced from creatine metabolism in the muscle cells and excreted in urine (Deminice et al., 2009). Creatinine has been related to muscular mass (Deguchi 1997) and its levels associated with the quality of dietary protein (Awosanya et al., 1999). Moreover, increased in serum levels of creatinine was observed with dietary L-glutamine supplementation in weaned piglets (Xiao et al., 2012). The results of this study indicate that creatinine is not a suitable indicator of the amino acid utilization of the diet.

The RBV curves in response to supplementary levels of L-Lys sulfate and L-Lys HCl are shown in Figures 1, 2, and 3. The variables ADG, G:F, and plasma urea were fitted to the multivariate linear regression model described by Littell et al. (1997). Based on the ADG response, the RBV of Lys from L-Lys sulfate was 106% (76% to 136%) in relative to L-Lys HCl, with a coefficient of determination of 0.93 (Figure 1). Based on the G:F response to L-Lys supplementation level, RBV was estimated in 119% (92% to 146%) for L-Lys sulfate in relative

to L-Lys HCl, with a coefficient of determination equal to 0.96 (Figure 2). The RBV based on plasma urea concentrations was estimated in 117% (83% to 150%) for L-Lys sulfate in relation to L-Lys HCl, with a coefficient of determination equal to 0.96 (Figure 3). However, the values of RBV based on ADG, G:F, and plasma urea were not different ($P > 0.05$) from 100%, indicating that the RBV of L-Lys sulfate and L-Lys HCl are equivalents. That supporting the fact that they do not show differences when comparing their supplementation effects on pig performance and blood metabolites. These results agree with Smiricky-Tjardes et al. (2004), which evaluated the RBV of Lys from L-Lys sulfate (47.3% L-Lys) in relation to L-Lys HCl (78.5% L-Lys) in diets for piglets weighing 9.5 kg for 21 d. These authors found that RBV of Lys from L-Lys sulfate was not significantly different from RBV of Lys from L-Lys HCl based on the responses for ADG (99%) and G:F (97%).

In a similar study, Liu et al. (2007) evaluated the RBV of two supplementary sources of Lys in diets for pigs weighing from 10 to 20 kg, being L-Lys sulfate (50% L-Lys) and L-Lys HCl (78% L-Lys). In this research, the RBV of Lys from L-Lys sulfate in relation to L-Lys HCl was 101%, 105%, 104%, and 95% for the variables ADG, G:F, plasma urea nitrogen, and retained nitrogen, respectively. In a more recent study, RBV of Lys from L-Lys sulfate (54.5% L-Lys) in relation to L-Lys HCl (78% L-Lys) was estimated in 104% and 112% for the variables ADG and feed efficiency of growing pigs from 57 to 87 kg (Htoo et al., 2016). Together, these results corroborate with those of the present study, supporting the conclusion that there are no marked differences in RBV of Lys from L-Lys sulfate and L-Lys HCl when supplemented for growing pigs.

Figure 1. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on daily weight gain (ADG) of weaned pigs.

Figure 2. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on feed efficiency (G:F) of weaned pigs.

Figure 3. Bioavailability of L-Lys sulfate in relation to L-Lys HCl based on the plasma urea concentrations of weaned pigs.

Therefore, L-Lys sulfate can be used as a substitute for L-Lys HCl in the diet of growing pigs.

In previously cited articles, and in this study, L-Lys sulfate had different levels of Lys in the base product (47.3%, 50%, 54.5%, and 70%). Although the purity of the base product was variable, the RBV of the L-Lys sulfate relative to L-Lys HCl was not significantly different for these four trials. However, the relative contribution of nutrients from a given source may be related to purity level and therefore affect its bioavailability (Baker, 1986). Studies are needed to evaluate whether variation in the Lys percentage of L-Lys Sulfate may have some effect on RBV and how this would impact economic profitability by using a base produce with a higher or lower concentration of L-Lys.

The results for diarrhea incidence are shown in Figure 4. In the first week, there were differences in the diarrhea index among dietary treatments. Piglets fed with CON and those supplemented with L-Lys HCl at 80% had a greater ($P < 0.05$) rate of diarrhea in relation to pigs supplemented with L-Lys sulfate at 90% and 100% and with L-Lys HCl at 100%. For the total period, the pigs supplemented with L-Lys sulfate at 90% and 100% and with L-Lys HCl at 100% maintained a lower ($P < 0.05$) diarrhea rate.

There were significant treatment effects for villus height (Table 5). Pigs supplemented with L-Lys sulfate or L-Lys HCl had greater ($P < 0.05$) villus height in relation to those receiving the CON diet. The source and level of Lys did not affect villus height, crypt depth, and villus:crypt ratio in the pigs evaluated.

Lys deficiencies during the weaning phase could compromise the intestinal health of piglets, causing a greater incidence of diarrhea and damaged jejunal morphometry. These harmful events could be triggered by an immune system less prepared to combat pathogenic agents causing imbalances in the intestinal microbiota, since pigs subjected to Lys deficiency can have decreased immune responses (Li et al., 2007; Liao et al., 2015). This is likely due to impairment of the synthesis of proteins related to the animal inflammatory responses, including serum antibody, inflammatory cytokines, Tlr system, and ERK1/2 and NF- κ B signals (Han et al., 2018). It is also important to consider that the intestinal tissue has a high rate of cell turnover. Thus, for this process to occur efficiently, a constant supply of nutrients is necessary, including amino acids. Although Lys is more related to muscle protein synthesis, this amino acid is also metabolized by enterocytes, which may have direct or indirect effects on the dynamics of cell renewal and consequent state of intestinal villi. For

Figure 4. Incidence of diarrhea of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to a CON.

Table 5. Jejunal morphometry, in μ m, of piglets supplemented with L-Lys sulfate and L-Lys HCl in the weaning phase in relation to a basal diet

Item ¹	CON ²	L-Lys sulfate ³			L-Lys HCl ⁴			SEM	P-value ⁵		
		80%	90%	100%	80%	90%	100%		C ₁	C ₂	C ₃
A	344	439	389	419	412	418	419	22.9283	0.01	0.01	0.98
P	372	386	362	358	370	357	364	17.6582	0.87	0.69	0.74
R	0.99	1.14	1.13	1.25	1.18	1.25	1.21	0.0971	0.11	0.05	0.64

¹A = villus height; P = crypt depth; R = villus height/crypt depth ratio.

²CON = basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by NRC, 2012).

³Supplementation of L-Lys sulfate meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

⁴Supplementation of L-Lys HCl meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the NRC, 2012.

⁵P-values of the orthogonal contrasts: C₁ = CON vs. L-Lys sulfate; C₂ = CON vs. L-Lys HCl; C₃ = L-Lys sulfate vs. L-Lys HCl.

Table 6. Apparent total tract digestibility (ATTD) for dry matter (DM), gross energy (GE), and crude protein (CP) of diets supplemented with different levels and sources of L-lys for weaned pigs

Item	CON ¹	L-Lys sulfate ²			L-Lys HCl ³			SEM	P-value ⁴		
		80%	90%	100%	80%	90%	100%		C ₁	C ₂	C ₃
DM	76.12	78.27	79.29	79.81	75.31	78.87	76.28	0.8785	0.01	0.50	<0.01
GE	76.19	77.22	78.22	79.19	75.71	77.29	74.41	1.0246	0.09	0.75	0.01
CP	65.65	69.21	68.09	68.57	65.12	67.71	63.09	1.4677	0.09	0.84	0.01

¹CON = basal diet deficient in Lys (73% of standardized ileal digestible Lys requirements established by [NRC, 2012](#)).

²Supplementation of L-Lys sulfate meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the [NRC, 2012](#).

³Supplementation of L-Lys HCl meeting 80%, 90%, and 100% of standardized ileal digestible Lys requirements according to the [NRC, 2012](#).

⁴P-values of the orthogonal contrasts: C₁ = CON vs. L-Lys sulfate, C₂ = CON vs. L-Lys HCl, C₃ = L-Lys sulfate vs. L-Lys HCl.

instance, direct effects, being an important source of energy for the enterocyte ([Stoll et al., 1998](#)), or indirect, being a precursor for the amino acid synthesis of the glutamate family, main cellular fuels at the intestinal level.

[He et al. \(2013\)](#) found that dietary supplementation with Lys enhanced villus height and crypt depth in the jejunum and affected intestinal expression of cationic amino acid transporters, which could be related to the absorption of Lys and other basic amino acids. In a more recent study, Lys restriction (30% according to the [NRC 2012](#)) increased bacterial diversity ([Yin et al., 2017](#)). Thus, the greatest incidence of diarrhea presented in CON group, in the present study, might be associated with changes in the gut microbiome.

The ATTD of dry matter, gross energy and crude protein of diets supplemented with different levels and sources of L-Lys are presented in [Table 6](#). When comparing the L-Lys sources with the CON diet, the ATTD of dry matter was greater ($P < 0.05$) in diets supplemented with L-Lys sulfate, while the ATTD of the gross energy and crude protein ratio tended ($P = 0.09$) to be greater in diets supplemented with L-Lys sulfate. There were no significant differences when comparing treatments supplemented with L-Lys HCl and the CON diet. The diets supplemented with L-Lys sulfate had greater ($P < 0.05$) ATTD of dry matter, gross energy, and crude protein.

The fact that L-Lys sulfate favors ATTD could be related to the presence of other nutrients. During the production process of L-Lys sulfate, the fermented broth does not undergo the separation and purification processes, leading to a product containing other nutrients besides Lys, including mainly other essential amino acids ([Leuchtenberger et al., 2005](#)). There is also the presence of impurities from the fermentation process, such as dried microbial cells, macromolecules, and other organic and inorganic substances ([Kumon et al., 1991](#)). These additional components could influence the digestibility

of nutrients. However, the concentrations of these components must be known to explore their effects on the digestive processes and other functions, including pig growth performance. Furthermore, it is important to highlight the association of these results with the incidence of diarrhea and intestinal morphometry. The pigs fed L-Lys sulfate had decreased incidence of diarrhea and greater villus height during fasting, which may have contributed to better utilization of feed nutrients, reflecting higher nutrient digestibility coefficients.

The overall results of this experiment indicate that the RBV of L-Lys sulfate for effects on ADG, G:F, and plasma urea is equivalent to that of L-Lys HCl. Consequently, L-Lys sulfate can be used in substitution of L-Lys HCl as a supplementary source of Lys for nursery piglets. Lys deficiency may compromise the intestinal functionality of nursery piglets with a greater incidence of diarrhea and damaged jejunal morphometry.

LITERATURE CITED

- AOAC. 2000. Official methods of analysis. 17th ed. Washington, DC: Association of Official Analytical Chemists.
- Awosanya, B., J. K. Joseph, D. F. Apata, and M. A. Ayoola. 1999. Performance, blood chemistry and carcass quality attributes of rabbits fed raw and processed pueraria seed meal. *Trop. Anim. Sci. J.* 2:89–96. doi:10.4314/tjas.v2i2.49687
- Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. *J. Nutr.* 116:2339–2349. doi:10.1093/jn/116.12.2339
- Baker, D. H., J. D. Hahn, and T. K. Chung. 1993. Nutrition and growth: the concept and application of an ideal protein for swine growth. In: G. R. Hollis, editor, *Growth of the pig*. Wallingford, Oxon: CAB International. p. 133.
- Cai, Y., D. R. Zimmerman, and R. C. Ewan. 1994. Diurnal variation in concentrations of plasma urea nitrogen and amino acids in pigs given free access to feed or fed twice daily. *J. Nutr.* 124:1088–1093. doi:10.1093/jn/124.7.1088
- Deguchi, E. 1997. Relation between daily urinary creatinine and body weight in castrated male landrace pigs. *J. Vet. Med. Sci.* 59:477–478.
- Deminice, R., G. V. Portari, H. Vannucchi, and A. A. Jordao. 2009. Effects of creatine supplementation on homocysteine

- levels and lipid peroxidation in rats. *Br. J. Nutr.* 102:110–116. doi:10.1017/S0007114508162985
- Eklunda, M., R. Mosenthina, H. P. Piephob, and M. Rademacher. 2010. Estimates of dietary threshold levels for crude protein and amino acids to obtain plateau values of apparent ileal crude protein and amino acid digestibilities in newly weaned pigs. *Arch. Anim. Nutr.* 64:357–372. doi: 10.1080/1745039X.2010.492139
- Halas, D., C. F. Hansena, D. J. Hampsona, B. P. Mullanb, J. C. Kimb, R. H. Wilsoncd, and J. R. Pluske. 2010. Dietary supplementation with benzoic acid improves apparent ileal digestibility of total nitrogen and increases villous height and caecal microbial diversity in weaned pigs. *Anim. Feed. Sci. Technol.* 160:137–147. doi: 10.1016/j.anifeedsci.2010.07.001
- Han, H., J. Yin, B. Wang, X. Huang, J. Yao, J. Zheng, W. Fan, T. Li, and Y. Yin. 2018. Effects of dietary lysine restriction on inflammatory responses in piglets. *Sci. Rep.* 8:2451. doi:10.1038/s41598-018-20689-3
- Hansen, J. A., D. A. Knabe, and K. G. Burgoon. 1993. Amino acid supplementation of low-protein sorghum-soybean meal diets for 5- to 20-kilogram swine. *J. Anim. Sci.* 71:452–458.
- He, L. Q., H. S. Yang, Y. Q. Hou, T. J. Li, J. Fang, X. H. Zhou, Y. L. Yin, L. Wu, M. Nyachoti, G. Y. Wu. 2013. Effects of dietary L-lysine intake on the intestinal mucosa and expression of CAT genes in weaned piglets. *Amino Acids.* 45:383–391. doi:10.1007/s00726-013-1514-0
- Htoo, J. K., J. P. Oliveira, L. F. T. Albino, M. I. Hannas, N. A. A. Barbosa, and H. S. Rostagno. 2016. Bioavailability of L-lysine HCl and L-lysine sulfate as lysine sources for growing pigs. *J. Anim. Sci.* 94:253–256. doi.org/10.2527/jas.2015-9797
- Jin, C. F., I. H. Kim, K. Han, and S. H. Bae. 1998. Effects of supplemental synthetic amino acids to the low protein diets on the performance of growing pigs. *Asian-Aus. J. Anim. Sci.* 11:1–7. doi.org/10.5713/ajas.1998.1
- Junghans, P., M. Beyer, M. Derno, K. J. Petzke, U. Küchenmeister, U. Hennig, W. Jentsch, and M. Schwerin. 2007. Studies on persisting effects of soy-based compared with amino acid-supplemented casein-based diet on protein metabolism and oxidative stress in juvenile pigs. *Arch. Anim. Nutr.* 61:75–89. doi:10.1080/17450390601168424
- Kumon, S., and T. Kawakita. 1991. Amino acids. In: Goldberg I. and Williams R. *Biotechnology and food ingredients*. New York: Van Nostrand Reinhold. P. 125–152.
- Leuchtenberger, W., K. Huthmacher, and K. Drauz. 2005. Biotechnological production of amino acids and derivatives: current status and prospects. *Appl. Microbiol. Biotechnol.* 69:1–8. doi:10.1007/s00253-005-0155-y
- Li, P., Y. L. Yin, D. Li, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. *Br. J. Nutr.* 98:237–252. doi:10.1017/S000711450769936X
- Liao, S. F., T. Wang, and N. Regmi. 2015. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. *Springerplus* 4:147. doi:10.1186/s40064-015-0927-5
- Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. *J. Anim. Sci.* 75:2672–2683.
- Liu, M. S. Y., X. Qiao, J. M. Wangand, and X. S. Piao. 2007. Bioefficacy of lysine from L-lysine sulfate and L-lysine-HCl for 10 to 20 kg pigs. *Asian-Aust. J. Anim. Sci.* 20: 1580–1586. doi:10.5713/ajas.2007.1580
- Luna, L. G. 1968. *Manual of histologic staining methods of the armed forces institute of pathology*. 3rd ed. New York: McGraw-Hill Press. p. 249.
- NRC. 2012. *Nutrient requirements of swine*. 11th ed. Washington, DC: National Academic Press. p. 400.
- Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* 84:125–134. doi:10.2527/2006.841125x
- Shriver, J. A., S. D. Carter, A. L. Sutton, B. T. Richert, B. W. Senne, and L. A. Pettey. 2003. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. *J. Anim. Sci.* 81:492–502. doi:10.2527/2003.812492x
- Smiricky-Tjardes, M. R., I. Mavromichalis, D. M. Albin, J. E. Wubben, M. Rademacher, and V. M. Gabert. 2004. Bioefficacy of L-lysine sulfate compared with feed-grade L-lysine-HCl in young pigs. *J. Anim. Sci.* 82:2610–2614. doi:10.2527/2004.8292610x
- Smith, F., J. E. Clark, B. L. Overman, C. C. Tozel, J. H. Huang, J. E. Rivier, A. T. Blikslager, and A. J. Moeser. 2010. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298:G352–G363. doi:10.1152/ajpgi.00081.2009.
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr.* 126: 606–614. doi.org/10.1093/jn/128.3.606
- Whittemore, C. T., and I. W. Moffat. 1976. The digestibility of dried microbial cells grown on methanol in diets for growing pigs. *J. Agric. Sci.* 86: 407–410. doi:10.1017/S0021859600054885
- Williams, C., D. J. David, O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59: 381–385. doi:10.1017/S002185960001546X
- Wu, G. 2013. *Amino acids, biochemistry and nutrition*. New York: Taylor e Francis Group. p. 482.
- Xiao, Y. P., T. X. Wu, Q. H. Hong, J. M. Sun, A. G. Chen, C. M. Yang, and X. Y. Li. 2012. Response to weaning and dietary L-glutamine supplementation: metabolomic analysis in piglets by gas chromatography/mass spectrometry. *J. Zhejiang Univ. Sci. B* 13:567–578. doi:10.1631/jzus.B1100343
- Yin, J., H. Han, Y. Li, Z. Liu, Y. Zhao, R. Fang, X. Huang, J. Zheng, W. Ren, F. Wu, et al. 2017. Lysine restriction affects feed intake and amino acid metabolism via gut microbiome in piglets. *Cell. Physiol. Biochem.* 44:1749–1761. doi:10.1159/000485782
- Zervas, S., and R. T. Zijlstra. 2002. Effects of dietary protein and fermentable fiber on nitrogen excretion patterns and plasma urea in grower pigs. *J. Anim. Sci.* 80:3247–3256. doi.org/10.2527/2002.80123247x
- Zhang, F., and O. Adeola. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. *Anim. Nutr.* 3:344–352. doi:10.1016/j.aninu.2017.06.008
- Zhao, Y., A. C. Weaver, V. Fellner, R. L. Payne, and S. W. Kim. 2014. Amino acid fortified diets for weanling pigs replacing fish meal and whey protein concentrate: effects on growth, immune status, and gut health. *J. Anim. Sci. Biotechnol.* 5:57. doi:10.1186/2049-1891-5-57