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CROP SCIENCE

Effects of lysine levels on performance, blood parameters, and nutrient digestibility of Duroc barrows in the starter phase

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Abstract: The objective of this study was to evaluate the effects of different levels of digestible lysine, at fixed amino acid ratios, in performance, blood parameters and diet digestibility of barrows from 15 to 30 kg. Fifty barrows of the Duroc breed with an average initial body weight of 14.95±3.22 kg were assigned to five treatments in a completely randomized experimental design with five replicates and of two animals per experimental unit. Treatments consisted of crude protein and lysine levels, maintaining a fixed ratio with the other digestible amino acids (8.4, 9.4, 10.4, 11.4, and 12.4 g kg^{-1}lysine/kg of diet). The feed conversion decreased linearly according to lysine levels. The apparent digestibility coefficients of dry matter and crude protein increased linearly, and the same response was observed for serum total protein and urea. The results indicate that digestible lysine level for Duroc barrows in the starter phase is 12.4 g kg^{-1} of diet, as it improved feed conversion ratio and apparent nutrient digestibility.

Key words: Amino acid, ideal protein, nutrition, nutritional requirement.

INTRODUCTION

Swine of the Duroc breed stand out for their good production-performance indicators such as good feed conversion ability, elevated weight gain, and rapid growth (Kuhlers et al. 2003). Their production and carcass-related characteristics are similar or superior to those of white-line swine (Latorre et al. 2003). Duroc pigs have a higher of degree marbling in their meat and a higher growth rate than the Hampshire and Large White breeds (Lowe et al. 2011). Additionally, they may show higher carcass fat content when fed inadequate protein levels (Hamill et al. 2013).

The protein content of a diet depends on the supply of essential amino acids, given the concept of constant supply of free amino acids from the feed into the animal's metabolism. Free amino acids can be rapidly absorbed and are thus available sooner at the site where synthesis is taking place and with the other protein-derived amino acids. However, a transient deficiency of amino acids for the synthesis of enzyme proteins may be translated into decreased performance (Hanigan et al. 2018). Some research studies have shown that supplementation crystalline lysine to pigs in the starter phase provides improvements in their feed conversion, weight gain, and protein deposition in the carcass (Oliveira et al. 2006, Carvalho et al. 2010).

Therefore, the lysine requirement should be determined without excesses or deficiencies, since these changes in amino acid availability for the animal body will prompt alterations in

performance and carcass characteristics such as limitations in weight gain, development of lean muscle mass, and increases in fat deposition (Tous et al. 2014).

It is worth noting that the nutritional needs of animals can be influenced by factors like feed intake, nutrient concentration in the diet, genetic variations, sex, and environment (NRC 2012). The more aptitude a pig shows for lean meat deposition, the higher will be its digestible lysine requirements. Thus, these animals must be fed diets that meet their specific requirements.

One of the difficulties in comparing the nutritional requirements of distinct breeds is their distinguished voluntary feed intake ability. Pigs with high genetic potential for lean meat deposition have a lower feed intake than animals with low and medium deposition ability, which suggests that the amino acid concentration of diets for animals with high lean-meat deposition potential should be increased in order to meet the needs of this category (Nieto et al. 2002).Pigs of the Duroc breed reach the desired slaughter weight at the same age as pigs of the Landrace and Large White breeds (Tänavots et al. 2011), although their loin-eye area (LEA) is larger than that of Landrace animals and their *Longissimus thoracis* muscle is larger than that of crossbred pigs (Põldvere et al. 2015).

On these bases, the present study proposes to estimate the optimal digestible lysine level under the hypothesis that high levels of digestible lysine affect nutrient digestibility, growth performance, and blood parameters in Duroc barrows in the starter phase, from 15 to 30 kg.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Ethics Committee (approval no. 035/2015). The experiment was developed in Recife - PE, Brazil (08º03'14"S, 34º52'52" W, 4 m asl).

The animals were housed in a masonry shed with a ceiling height of 2.10 m, covered with ceramic roof tiles and divided into twenty-five 3.10 × 1.20 m² stalls on concrete floor. Stalls were equipped with trough stainless-steel feeders and nipple-type drinkers.

Ambient temperature and air relative humidity were monitored daily using digital thermohygrometers installed at different locations in the shed, at the height of the back of the animals. During the experimental period, the average minimum and maximum temperature recorded inside the shed were 24.48±1.84ºC and 30.76±1.04ºC, respectively. Air relative humidity remained around 58.42±2.74%.

Fifty barrows of the Duroc breed with an average initial weight of 14.95±3.22 kg were used in a completely randomized experimental design with five treatments and five replicates with two animals were used per experimental unit.

The diets were based on corn and soybean meal and supplemented with vitamins and minerals (Table I).

Treatments consisted of five diets containing different lysine levels, maintaining a fixed ratio with the other digestible amino acids (8.4, 9.4, 10.4, 11.4, and 12.4 g kg^{-1} of diet). The digestible lysine level established by Rostagno et al. (2011) was adopted as the midpoint of the treatments. All diets were isoenergetic.

The diets were supplemented with crystalline amino acids (L-lysine HCl, DL-methionine, L-threonine, and L-tryptophan). To maintain a constant ratio between these limiting amino acids and lysine, in accordance with the concept of ideal protein suggested by Rostagno et al. (2011), the protein level of the diet varied according to the amino acid content. The experiment lasted 28 days, the first seven days

Table I. Centesimal, calculated, and analyzed composition of the experimental diets expressed on an as-fed basis.

Item	Digestible lysine level (g kg^{-1} diet)				
	8.4	**9.4**	**10.4**	**11.4**	**12.4**
Ingredient (kg100 kg^{-1})					
Corn	73.93	71.08	69.04	67.37	65.04
Soybean meal	22.46	25.17	26.95	28.40	30.48
Soybean oil	0.59	0.7	0.75	0.81	0.90
Dicalcium phosphate	1.41	1.40	1.39	1.38	1.37
Limestone	0.75	0.74	0.73	0.73	0.72
Salt	0.43	0.42	0.46	0.42	0.42
Vitamin and mineral premix[1]	0.25	0.25	0.25	0.25	0.25
L-lysine HCl	0.18	0.22	0.30	0.38	0.45
DL-methionine	0.01	0.03	0.07	0.12	0.15
L-threonine	---	0.02	0.06	0.11	0.15
L-tryptophan	---	---	---	0.01	0.02
L-valine	---	---	---	0.03	0.05
Total	100	100	100	100	100
Calculated values (gkg^{-1})					
Crude protein	161.5	172.3	180.0	187.0	196.0
Digestible lysine	8.400	9.400	10.400	11.400	12.400
Digestible methionine + cystine	4.807	5.260	5.820	6.380	6.940
Digestible threonine	5.404	5.920	6.550	7.180	7.810
Digestible tryptophan	1.650	1.790	1.901	2.050	2.230
Digestible valine	6.768	7.208	7.556	7.870	8.065
Total calcium (Ca)	7.33	7.33	7.33	7.33	7.33
Available phosphorus (P)	3.63	3.63	3.63	3.63	3.63
Sodium (Na)	2.00	2.00	2.00	2.00	2.00
Metabolizable energy (Kcal/kg)	3,230	3,230	3,230	3,230	3,230
Analyzed values (gkg^{-1})[2]					
Dry matter	886.2	885.4	888.4	888.1	886.8
Crude protein	166.0	174.9	182.7	189.1	190.6
Total lysine	9.620	10.140	10.710	13.010	13.840
Total methionine+ cystine	5.540	5.740	6.140	7.370	7.730
Total threonine	6.420	6.820	7.180	8.450	9.010

[1]Amount per kg of diet: 15 mg folic acid; 3 mg biotin; 37.5 g choline; 5,000 mg Cu; 2,500 mg Fe; 13 mg I; 334 mg Mn; 5 mg Se; 479 mg niacin; 240 mg pantothenic acid; 48 IU vitamin B_6; 75 mg vitamin B_2; 33 mg vitamin B_1; 643 mg vitamin B_{12}; 150,000 IU vitamin A; 27,000 IU vitaminD_3; 450 IU vitamin E; 14 mg vitamin K; 2,500 mg Zn.
[2] Analyzed by Evonik Industries AG Feed Additives / Animal Nutrition Services.

of which were used as a period of acclimation to the experimental diets and the environment.

To determine the average daily weight gain (ADWG), the animals were weighed at the onset, on the 7th day, and on the 28th day of the experimental period. The diets and water were supplied *ad libitum* throughout the experimental period, and feed leftovers were weighed daily to determine average daily feed intake (ADFI) and feed conversion ratio (FCR). At the end of the experiment, when the animals attained near 30 kg in body weight, their backfat thickness was measured *in vivo* using a Renco Lean-Meater Ultrasonic Back Fat® meter at the P2 point, in the intercostal space between the last and penultimate rib.

To evaluate the apparent digestibility of the diets, the animals initially received the respective experimental feeds containing 0.5% of a source of acid-insoluble ash (Celite®) as a digestibility indicator for 72 h. Partial fecal collection was performed 24 h after the feed with the marker was consumed, starting on the 14th day of the experiment. A partial sample of feces was collected immediately after excretion, so as to prevent contamination with urine or other particles found in the environment.

The collected feces were packed in plastic bags that were then identified and frozen at –20 ºC. Samples were subsequently thawed, homogenized, weighed, and dried in a forced-air oven at 77 ºC for 72 h. Upon being removed from the oven and reaching balance with room temperature, the samples were weighed again and ground for analysis.

Analyses of dry matter (DM) and crude protein (CP) were performed on the samples of feed and feces (Detmann et al. 2012). The total amino acid composition of the diets was analyzed by near-infrared spectroscopy (NIR) by Evonik Industries AG Feed Additives / Animal Nutrition Services. Acid insoluble ash was determined by the methodology described by Van Keulen & Young (1977).

The apparent digestibility coefficients of DM and CP were determined by the partial fecal collection method, using the equation described by Sakomura & Rostagno (2016).

Blood samples were collected at the end of the experimental period by puncturing the retro-orbital sinus of the animals, using hypodermal needles (40 × 1.6 mm), according method described by Melo et al. (2019). Blood was collected before the animals were first weight, after a 12-h fast.

Blood serum samples were collected in 10-mL tubes without anticoagulants. Next, the tubes with samples of collected blood were centrifuged at 3,000 rpm for 15 min to obtain the blood serum. Subsequently, the serum was transferred to previously identified plastic microtubes that were then stored at -20 ºC until laboratory analyses of total proteins, urea, and creatinine.

Blood biochemical parameters were measured using a semi-automatic biochemical analyzer (Doles D250®) and the following DOLES® commercial kits: creatinine and urea 500. Total protein was determined by manual refractometry.

Growth performance, nutrient digestibility and blood analysis data were tested for normality by the Lilliefors test. The data were then subjected to analysis of variance by applying the PROC GLM procedure of SAS software 2012 (Statistical Analysis System, version 9.4.), according to the following mathematical model:

$$Yij = m + Li + eij,$$

in which Yij = dependent variables related to growth performance, digestibility, and blood parameters of animals receiving treatment i (digestible lysine level: 8.4, 9.4, 10.4, 11.4, or 12.4 g kg^{-1}) in replicate j (1, 2, 3, 4, or 5); m = overall mean of the variable; Li = effect of digestible

lysine level i; and eij = random error associated with each observation.

Variables for which significant effects were detected were subjected to regression analyses as a function of lysine levels, adopting a 5% probability level. Initial body weigh was used as covariate for the performance parameters. The PROC REG statistical package of SAS software 2012 (Statistical Analysis System, version 9.4.) was applied to obtain the regression equations and thus estimate the digestible lysine level.

RESULTS

Lysine intake increased linearly, whereas FCR decreased linearly as the lysine levels in the diet were increased (Table II). Conversely, final weight, ADFI, ADWG, and backfat thickness were not significantly influenced by the treatments.

The lysine levels provided a linear increase in the apparent digestibility coefficients of CP (P=0.0005) and DM (P=0.0001) in the pigs (Table III), without, however, altering fecal nitrogen excretion to the environment.

The increasing digestible lysine levels in the diets led to a linear increase in the serum total protein (P=0.0057) and urea (P=0.0251) contents (Table IV), although he creatinine levels were not changed.

DISCUSSION

The increasing concentrations of digestible lysine in the diets provided a significant reduction in feed conversion ratio (FCR). This finding indicates that the pigs efficiently converted feed into bodyweight in the start phase, demonstrating the efficiency of the amino acid and protein levels. Lysine is the limiting amino acid in grain-based diets because, without it, pigs cannot efficiently use the other essential amino acids required for optimal development. In this study, the dietary supplementation of other amino acids allowed better utilization of the protein for animal development.

Some studies have shown that pigs exhibit higher growing rates and better feed efficiency when the dietary lysine levels are elevated. Fraga et al. (2008) evaluated lysine levels in different genetic groups and also observed a linear decrease in FC as the levels were raised. Gandra et al. (2012) also reported a reduction of FCR as they increased the lysine levels in sow diets.

Feed conversion is a parameter that is correlated positively with backfat thickness (0.40 to 0.65) and negatively with loin-eye area (–0.52 to–0.59) (Johnson et al. 1999, Suzuki et al. 2005); i.e., when animals show a lower FCR, they will

Table II. Effect of digestible lysine level on growth performance characteristics of barrows in the starter phase (15 to 30 kg).

Variable	Digestible lysine level (g kg^{-1}diet)					SEM	Probability[1]	
	8.4	9.4	10.4	11.4	12.4		Linear	Quadratic
Average final body weight (kg)	29.01	31.04	30.32	30.39	30.59	1.89	0.3706	0.3866
Average daily feed intake (kg day^{-1})	1.04	1.02	1.00	1.03	1.02	0.05	0.6217	0.4974
Average daily lysine intake (g day^{-1})[2]	8.75	10.93	11.35	12.50	13.24	0.02	0.0009	0.5139
Average daily body weight gain (kg day^{-1})	0.55	0.54	0.58	0.60	0.60	0.07	0.1897	0.9879
Feed conversion ratio[3]	1.94	1.80	1.74	1.75	1.71	0.16	0.0376	0.3407
In vivo backfat thickness (mm²)	5.41	5.68	5.46	5.35	5.28	0.30	0.1769	0.2668

SEM - standard error of the mean.
[1] Significance level at P<0.05.
[2] Y = 0.3782+10.555X (R^2 = 0.94).
[3] Y= 2.4242–0.5780X (R^2 = 0.77).

possibly also have a thinner backfat layer and larger loin-eye area. However, in the present study, backfat thickness was not significantly influenced by digestible lysine levels. This fact may be related to the poor adipose tissue deposition that occurs during the starter phase of growth, in swine.

In the starter phase of piglets is between 10 and 30 kg. This period is characterized by an exponential evolution of their growth curve, when their body weight increases as a function of their age. The same occurs with the protein deposition curve, which is also elevated in that period (Danfaer & Strathe 2012). Another factor that influences the requirements of the animals evaluated in this study is their genetic constitution. Pigs of the Duroc breed have superior or similar productive and carcass characteristics compared to white breeds (Latorre et al. 2003).

However, Duroc pigs will also have their characteristics affected by the nutritional content of their diet. Wood et al. (2004)examined diets with different protein levels—a conventional and a low-protein diet—for Duroc, large White, Berkshire and Tamworth pigs and concluded that, for muscle deposition, the diet effect was more marked in the Duroc breed, for which this variable was approximately 9% lower when they received the low-protein diet. The authors also observed a reduction in growth rate.

During the starter phase, pig production performance is limited by ADFI, as these animals have a low voluntary intake capacity (Whittemore & Kyriazakis 2006), which may limit their ADWG. However, the average ADFI observed in the present study was 1.02±0.02 kg/day, which is lower than the average 1.23 kg/day suggested by Rostagno et al. (2011) for the starter phase. Despite the relatively low voluntary intake, the increasing digestible lysine levels led to a more efficient partial protein digestibility (Table III), which indicates improved utilization of the available amino acids for protein and muscle deposition, corroborating the response shown by FCR.

Nitrogen intake increased with the increasing crude protein and digestible amino acid values; production performance variables were improved; and nitrogen excretion in the feces did not increase (Table IV). As a result of the increasing concentrations of crude protein and digestible amino acids in the diets, the apparent digestibility of CP and DM of the pig diets increased linearly, without changing fecal nitrogen excretion. Nitrogen was likely

Table III. Effect of digestible lysine level on the apparent digestibility of nutrients in diets for barrows in the starter phase (15 to 30 kg).

Variable	Digestible lysine level (g kg^{-1}diet)					SEM	Probability[1]	
	8.4	9.4	10.4	11.4	12.4		Linear	Quadratic
N intake ($gday^{-1}$)[2]	27.68	29.17	29.28	31.28	31.02	1.93	0.0006	0.5404
N Fecal (gkg^{-1})	11.01	12.36	11.39	11.49	11.53	1.30	0.9337	0.4930
ADC_{CP} (gkg^{-1})[3]	923.8	933.7	933.6	957.9	959.1	4.33	0.0005	0.7744
ADC_{DM} (gkg^{-1})[4]	813.9	832.7	846.3	889.0	884.9	1.93	0.0001	0.7790

SEM - standard error of the mean; N -nitrogen; ADC_{CP} - apparent digestibility coefficient crude protein; ADC_{DM} - apparent digestibility coefficient dry matter.
[1] Significance level at P<0.05.
[2] Y = 20.539 + 8.7974X (R^2 = 0.88).
[3] Y = 64.715 + 19.830X (R^2 = 0.88).
[4] Y = 84.311 + 9.4714X (R^2 = 0.91).

excreted in the urine, but this parameter was not evaluated in this study.

The increased apparent digestibility coefficients of CP and DM suggest that the lysine digestibly level can improve nutrient availability. Dietary inclusion of crystalline amino acids allowed the animals to absorb more nutrients, reducing the antinutritional properties present in the feedstuffs, considering that the more digestible a diet is, the higher its digestibility by the animal.

The increased apparent digestibility coefficient of CP may also be associated with the increase in serum urea nitrogen concentration (Table IV), where amino acids are absorbed as ammonia instead of intact amino acids. Ammonia production in the large intestine is mainly generated from dietary or endogenous protein fermentation, and not from urea hydrolysis. Ammonia is quickly absorbed by the large intestine, appearing in portal blood, and most of it is excreted in the urine. The increased serum urea concentration indicates that there was an excess of amino acids circulating in the bloodstream until excretion (Weiner et al. 2015).

Proteins circulating in the bloodstream are synthesized mostly by the liver, and their formation occurs upon the break down and absorption of amino acids by the intestine. The increased serum protein levels (Table IV) indicate that a larger amount of amino acids was provided to the animals, meaning increased protein synthesis, which is verified by the results found for apparent CP digestibility. According to Toledo et al. (2014), amino acids (essential and non-essential) must be supplied in the right amount for protein synthesis to take place. In the present study, the ratio between lysine and essential amino acids was maintained according to the 'ideal protein' concept. The dietary CP level was also allowed to be increased so that the non-essential amino acids could be made available to the animals.

The serum total protein values detected at the lysine levels of 8.4 and 9.4 g $kg^{-1}$are below the reference range for swine (6 to 8 g/dL) (Lopes et al. 2007). Blood urea values, in turn, are within the reference threshold (21.4 to 64.2 mg/dL) (Lopes et al. 2007). Like total proteins, serum urea is synthesized by the liver. Urea has been widely used as a tool to identify the ideal protein level in pig diets, as its concentration in the serum is directly related to the dietary protein content (Coma et al. 1995). Nevertheless, blood urea nitrogen is the best parameter to evaluate the protein quality status.

In the current experiment, the results were higher than those described in the literature. Oliveira et al. (2006) and Carvalho et al. (2010) suggested 1.10% digestible lysine. Rostagno et

Table IV. Effect of digestible lysine level on blood parameters of barrows in the starter phase (15 to 30 kg).

Variable	Digestible lysine level (g kg^{-1}diet)					SEM	Probability[1]	
	8.4	9.4	10.4	11.4	12.4		Linear	Quadratic
Total protein (g dL^{2})	5.66	5.92	6.00	6.10	6.18	0.31	0.0057	0.4768
SUN (mg dL^{-1})[3]	26.14	33.71	31.76	35.56	41.69	10.15	0.0251	0.8607
Creatinine (mgdL^{-1})	0.67	0.66	0.60	0.63	0.61	0.06	0.1258	0.3790

SEM - standard error of the mean; SUN -serum urea nitrogen.
[1] Significance level at P<0.05
[2] L: Y= 4.7032 + 1.2200X (R^2 = 0.92).
[3] L: Y = −0.5004 + 32.955X (R^2 = 0.84).

al. (2011) propose 0.93 g kg^{-1}digestible lysine for barrows in the starter phase. The present results suggest that further research is warranted to investigate the supply of digestible lysine for Duroc pigs in the starter phase.

CONCLUSIONS

The addition of 12.4 g of digestible lysine per kilogram of diet improves on feed conversion ratio and apparent protein digestibility in Duroc barrows in the starter phase.

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