Revista Brasileira de Zootecnia
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ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.40, n.11, p.2388-2393, 2011

Digestible tryptophan levels for 30 to 60 kg pigs

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ABSTRACT - The objective of this study was to evaluate the effects of increasing dietary digestible tryptophan levels on performance and carcass traits of growing pigs. Fifty crossbred castrated male pigs, with average initial and final body weight of 29.0 ± 1.20 kg and 60.4 ± 1.95 kg were allotted in a completely randomized block design, with five treatments (0.125, 0.133, 0.141, 0.149, and 0.157% of digestible tryptophan, corresponding to digestible tryptophan:lysine relations of 15.0, 16.0, 17.0, 18.0, and 19.0%, respectively) and five replicates, with two pigs per experimental unit, which was represented by the pen. Experimental diets and water were supplied *ad libitum* throughout the experimental period. Averages of minimum and maximum temperatures inside the facility were of 24.3 ± 0.87 ºC and 28.0 ± 1.82 ºC, respectively. Feed intake and body weight gain increased linearly with increasing dietary tryptophan levels. However, there was no effect of digestible tryptophan on feed conversion or protein deposition of pigs. The highest tryptophan level evaluated (0,157%), corresponding to a digestible tryptophan:lysine relation of 19.0%, provided the greatest weight gain of 30 to 60 kg castrated male pigs.

Key Words: amino acids, ideal protein, performance, protein deposition

Introduction

The constant genetic selection for increased protein deposition needs constant reassessment of pigs' nutritional requirements, since changes in rates in body tissues deposition affect the daily need of nutrients, particularly amino acids.

Tryptophan is an essential amino acid in the piglets and growing-finishing pigs' nutrition (NRC, 1998) and in diets with corn as the main ingredient tryptophan is the fourth limiting amino acid. Tryptophan is the fourth limiting amino acid after lysine, methionine and threonine. When the level of tryptophan in the diet is limiting compared with other essential amino acids, protein synthesis, body weight gain and feed efficiency are adversely affected (Le Floc'h & Seve, 2007; Jansman, 2010).

Besides its involvement in protein synthesis, tryptophan is the only source of substrate for production of important molecules through different pathways, and still participates in the control of inflammatory and immune response in the body. In the brain and intestine, tryptophan is required as substrate for the synthesis of serotonin, a neurotransmitter that stimulates feed intake (Henry et al., 1992).

Digestible tryptophan requirements for piglets in the nursery phase have been investigated; however, little research has been conducted to determine the requirements of this amino acid in growing and finishing pigs (Guzik et al., 2005). In these studies, the results have been variable both among studies and phases of growth, probably due to differences between genotypes. Animal health, sex, ambient temperature, stage of development, and also diet composition may influence the requirements of tryptophan.

Thus, this study was conducted to evaluate the effect of digestible tryptophan levels and the digestible tryptophan:lysine relation in diets for 30 to 60 kg pigs.

Material and Methods

The experiment was conducted in the nursery of the Setor de Suinocultura of Departamento de Zootecnia, Universidade Federal de Viçosa - UFV, Viçosa, MG, Brazil.

Fifty crossbred commercial castrated males pigs, with initial weight of 29.7 ± 1.20 kg were allotted in a completely randomize block design, with five digestible tryptophan levels (0.125, 0.133, 0.141, 0.149, and 0.157%, corresponding to ratios of 15.0, 16.0, 17.0, 18.0, and 19.0% with digestible

Received December 2, 2010 and accepted March 16, 2011.
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lysine), five replicates, and two pigs per pen, which was considered the experimental unit. Body weight and pigs' kinship were considered as criteria in the blocks formation.

Pigs were housed in suspended metal cages with wired mash floor and sides, equipped with semi-automatic feeders and nipple drinkers, located in concrete building with concrete floor and lowered wooden roof. The temperature inside the facility was measured daily at 8 a.m. through maximum and minimum thermometer, kept in an empty pen in the middle of the room, half-height of the pigs' body.

The experimental isocaloric and isonitrogenous diets (Table 1) were composed primarily of corn and soybean meal to meet the animal requirements for energy, minerals, vitamins and amino acids, according to Rostagno et al. (2000), except for tryptophan. To ensure that no other essential amino acid other than tryptophan was deficient in diets, the ratios of these amino acids with lysine were above those recommended in the ideal protein for animals at the same stage of growth, according to Rostagno et al. (2000).

The treatments, which consisted of different levels of digestible tryptophan, were composed of a basal diet and four other diets obtained by supplementing the basal diet with L-tryptophan in substitution of glutamic acid, proportionally to the concentrations of nitrogen.

Diets and water were supplied *ad libitum* to animals throughout the experimental period. Animals were weighed at the beginning and end of the experiment for determination of weight gain. The experimental diets were weighed when fed to the animals, and leftovers were weighed daily to determine feed intake, feed conversion and digestible tryptophan intake.

At the end of the trial, when animals reached the average weight of 60.4 ± 1.35 kg, they were submitted to a 24-hour fast. After this period, one pig from each experimental unit, weighing closest to 60 kg, was slaughtered by exsanguination, then shaved and eviscerated.

An additional group of five piglets weighing 30.4 ± 1.20 kg were slaughtered following the same procedure used in the

Table 1 - Experimental diets composition

Ingredient	Digestible tryptophan level (%)				
	0.125	0.133	0.141	0.149	0.157
Corn	79.087	79.087	79.087	79.087	79.087
Soybean meal	13.667	13.667	13.667	13.667	13.667
Meat and bone meal	4.500	4.500	4.500	4.500	4.500
Glutamic acid	0.100	0.092	0.077	0.066	0.053
Starch	0.090	0.094	0.097	0.101	0.105
DL – methionine	0.091	0.091	0.091	0.091	0.091
L – isoleucine	0.071	0.071	0.071	0.071	0.071
L – lysine	0.340	0.340	0.340	0.340	0.340
L – threonine	0.131	0.131	0.131	0.131	0.131
L – tryptophan	-	0.008	0.016	0.024	0.033
L – valine	0.003	0.003	0.003	0.003	0.003
Soybean oil	0.746	0.746	0.746	0.746	0.746
Dicalcium phosphate	0.025	0.025	0.025	0.025	0.025
Limestone	0.462	0.462	0.462	0.462	0.462
Vitamin premix[1]	0.200	0.200	0.200	0.200	0.200
Mineral premix[2]	0.200	0.200	0.200	0.200	0.200
Salt	0.267	0.267	0.267	0.267	0.267
Growth promoter	0.010	0.010	0.010	0.010	0.010
β-hydroxytoluene	0.010	0.010	0.010	0.010	0.010
Calculated nutritional composition[3]					
Crude protein (%)	15.668	15.668	15.668	15.668	15.668
Metabolizable energy (Mcal/kg)	3.235	3.235	3.235	3.235	3.235
Digestible lysine (%)	0.830	0.830	0.830	0.830	0.830
Digestible tryptophan (%)	0.125	0.133	0.141	0.149	0.157
Digestible methionine + cystine (%)	0.480	0.480	0.480	0.480	0.480
Digestible threonine (%)	0.581	0.581	0.581	0.581	0.581
Digestible isoleucine (%)	0.491	0.491	0.491	0.491	0.491
Digestible valine (%)	0.861	0.861	0.861	0.861	0.861
Sodium (%)	0.170	0.170	0.170	0.170	0.170
Calcium (%)	0.760	0.760	0.760	0.760	0.760
Available phosphorus (%)	0.360	0.360	0.360	0.360	0.360
Digestible tryptophan:lysine relation (%)	15.00	16.00	17.00	18.00	19.00

[1] Provided per kg of product: vitamin A - 6,000,000 IU, vitamin D3 - 1,500,000 IU, vitamin E - 15,000,000 IU, vitamin B1 - 1.35 g, vitamin B2 - 4 g, vitamin B6 - 2 g, pantothenic acid - 9.35 g, vitamin K3 - 1.5 g, nicotinic acid - 20.0 g, vitamin B12 - 20.0 g, folic acid - 0.6 g, biotin - 0.08 g, iodine - 1.5 g, selenium - 0.3 g, vehicle - 1,000 g.
[2] Provided per kg of product: iron - 100 g, copper - 10 g, cobalt - 1 g, manganese - 40 g, zinc - 100 g, vehicle - 1,000 g.
[3] Values estimated based on the digestibility coefficients of amino acids of ingredients according to Rostagno et al. (2000).

slaughter of pigs in the experiment for determining the initial composition of the carcass.

Whole, eviscerated and blood free carcasses of the slaughtered animals were divided longitudinally and the left half of each carcass (including head and feet) was crushed for 15 minutes in a commercial 30-hp, 1775-rpm cutter. After homogenization of the crushed material, samples were taken from carcasses, then stored in a freezer at -12 °C.

Samples were later thawed at room temperature for a 24-hour period and then submitted to pre-drying process in a forced ventilation oven at 65 °C for 72 hours. Because of the high concentration of fat in the material, a hot pre-defatting was carried out by using Soxhlet extractor for four hours. Pre-dried and pre-defatted samples were ground in a ball mill and placed in identified glasses for further laboratory analyses. Protein analyses of carcass samples were performed at the Laboratório de Nutrição Animal in Departamento de Zootecnia at Unversidade Federal de Viçosa, according to techniques described by Silva (2002).

The deposition of protein in the carcass was evaluated by comparative criteria using carcasses contemporaries animals slaughtered with weight of 15.55 ± 0.75 kg and the animals slaughtered at the end of the experiment - one animal from each experimental unit with weight closest to the average weight of its respective unit, according to the methodology proposed by Donzele et al. (1992).

The parameters evaluated were: average daily gain, feed intake, feed conversion, daily digestible tryptophan intake and daily protein deposition.

Performance variables and deposition of protein were analyzed by using the SAEG (Statistical Analysis System and Genetics) software version 8.0, developed at Universidade Federal de Viçosa (UFV, 2000) , using the procedures for the analysis of variance and regression, according to the following statistical model:

$$Yijk = \mu + Bi + Tj + eijk$$

in which: Yijk = observed characteristic; μ = general trait mean; Bi = effect of block i, i = 1, 2 ... and 6; Tj = effect of level of digestible tryptophan j; j = 1, 2 ... and 5; eijk = random error associated with each observation.

Levels of digestible tryptophan in the diet that provided the best results of performance and protein deposition were determined by linear regression analysis, quadratic and/or discontinuous Linear Response Plateau model (LRP), according to the best data fit for each variable studied.

Results and Discussion

During the experimental period the average minimum and maximum temperatures inside the facility were 24.3 ± 0.87°C and 28.0 ± 1.82°C, respectively. Considering the ideal temperature range for pigs in the growth phase suggested by Coffey et al. (2000) is between 16 and 24°C, it is verified, based on variations in temperature during the experimental period, that pigs were subjected to periods of high temperature.

There was effect ($P<0.01$) of dietary digestible tryptophan levels on daily feed intake (DFI) of pigs (Table 2), which increased linearly according to the equation $\hat{Y} = -705.59 + 9142.51X$ ($r^2 = 0.83$). Similar result was obtained by Henry et al. (1995), who, evaluating the interaction between protein and tryptophan levels for pigs from 38 to 53 kg, found that daily feed intake increased with the concentration of digestible tryptophan in the diet. Linear effect of tryptophan levels on feed intake of female pigs from 22 to 50 kg was also reported by Burgoon et al. (1992) and Guzik et al. (2005), in a study with growing pigs.

Corroborating these results, in more recent studies, Zhang et al. (2007), Strath & Fernadez (2009), and Jansman et al. (2001) also reported significant variation in daily feed intake of piglets due to the increasing level of digestible tryptophan. These results are consistent with reports of Ettle & Roth (2004), that pigs are able to recognize diets containing different concentrations of tryptophan, preferring, along with increases in consumption, the ration with the most appropriate level of this amino acid.

On the other hand, Eder et al. (2003), Rossoni et al. (2003), Susenbeth & Lucanus (2005), Haese et al. (2006) and Pereira et al. (2008) found no increase in the daily feed intake of barrows by increasing the levels of digestible tryptophan in the diet.

Table 2 - Data of performance and protein deposition of 30- to 60-kg pigs fed diets with different levels of digestible tryptophan

	Digestible tryptophan level (%)					CV (%)
Variable	0.125	0.133	0.141	0.149	0.157	
Daily feed intake (g)[1]	1824	1977	1980	2006	2173	7.1
Daily weight gain (g)[1]	683	757	798	825	913	6.8
Feed conversion (g/g)	2.67	2.61	2.49	2.43	2.39	4.0
Protein deposition (g/day)	60.10	80.72	73.43	79.64	83.39	24.2

[1] Linear effect ($P<0.01$).

The linear increase in the daily feed intake of pigs observed in this study may be related to the increase in the ratio between digestible tryptophan and neutral long chain amino acids (NLCAA), phenylalanine, tyrosine, leucine, valine and isoleucine in the diets as a result of digestible tryptophan supplementation. According to Henry et al. (1992), tryptophan competes with NLCAA as they share the same transport system for passage through the blood-brain barrier. In the brain, tryptophan is the main substrate for the synthesis of serotonin, a neurotransmitter that stimulates feed intake. Thus, the relationship between tryptophan and NLCAA in plasma may influence the synthesis of serotonin in the hypothalamus, thus acting as a modulator of feed intake in pigs.

Another factor that may be involved in the stimulatory effect of tryptophan on pigs' feed intake is the plasma increase in the hormone ghrelin. In a study with weaned piglets, Zhang et al. (2007) linked the increase in daily feed intake in response to the level of tryptophan in the diet to the increased concentration of the hormone ghrelin in the blood. According to these authors, tryptophan stimulates the secretion of ghrelin by gastric and duodenal mucosa, which, in turn, acts stimulating the appetite.

In recent studies with weaned piglets, Trevisi et al. (2009) and Jansman et al. (2010) reported that both neurotransmitter serotonin and hormone ghrelin were involved in the stimulatory effect of tryptophan on appetite.

Digestible tryptophan levels affected (P<0.01) average daily gain (ADG) of pigs (Table 2), which increased linearly according to the equation $\hat{Y} = -150.003 + 6716.37X$ ($r^2 = 0.89$). Similar result was obtained by Eder et al. (2003), who, assessing levels of digestible tryptophan in diets for 25- to 50-kg female pigs, observed an increase in ADG GPD of up to 0.141% digestible tryptophan in the diet, corresponding to a ratio of 16.2% with the digestible lysine. The result of ADG obtained in this study is also consistent with that obtained by Burgoon et al. (1992), who observed a linear increase of this variable in pigs from 22 to 50 kg fed diets with varying levels of digestible tryptophan from 0.08 to 0.18%.

Positive effect of tryptophan levels on weight gain of growing pigs was also verified by several authors like Sawadogo et al. (1997), Ferguson & Gous (2002), Pastuzewska et al. (2007), Fernandez & Strath (2009) and Jansman et al. (2010).

Despite the consistency of the findings among the studies regarding the positive effect of increasing concentrations of digestible tryptophan in the diet on the ADG of pigs, the concentrations of this amino acid that resulted in greatest responses for this parameter ranged from 0.22% (Jansman et al., 2010) to 0.26% (Sawadogo et al., 1997).

According to Jansman et al. (2010), the lysine concentration of the experimental diets and the immune system activation of pigs (Le Floc'h et al. 2007; Trevisi et al., 2009) are among the factors that may explain the variation in estimates of pigs' digestible tryptophan requirements observed among studies. Confirming this proposition, Jansman et al. (2010), Eder et al. (2003) and Guzik et al. (2005) found that the weight gain of pigs in the finishing phase was significantly influenced by the levels of tryptophan in the diet, with the best gain response obtained at 0.146 and 0.096% digestible tryptophan, which corresponded to the same relationship with digestible lysine (18%). These results indicated that the requirement of digestible tryptophan may vary with the lysine level of the experimental diets.

With regard to the immune system, Trevisi et al. (2009) found that piglets under challenge with lipopolysaccharide (LPS) had increased the demand for tryptophan in the control group to partially compensate the negative effects of the challenge, increasing feed intake and maintaining an adequate growth rate.

The level of digestible tryptophan of 0.157%, which provided the best response of daily weight gain in this study, was similar to the level of 0.161% recommended by Rostagno et al. (2005), for barrows with high genetic potential and standard performance from 30 to 50 kg, and below the level of 0.20% obtained by Eder et al. (2003), for female pigs from 25 to 50 kg. However, these last authors reported that the level of 0.20% digestible tryptophan may have been overestimated due to the statistical model used.

There was no effect (P>0.05) of digestible tryptophan levels on feed conversion (FC) of pigs (Table 2). However, there was gradual improvement of up to 10.5% in the absolute value of this variable between the extreme levels of tryptophan evaluated (0.125 × 0.157). This would indicate that the level of 0.157% digestible tryptophan, corresponding to a ratio of 19.0% with digestible lysine, was the best fit to the requirements of pigs for better efficiency of feed utilization.

Whereas daily feed intake and daily weight gain of pigs increased linearly, the improvement observed in feed conversion in this study, although in absolute value, can be an indication that the composition of pigs' weight gain may have varied between dietary digestible tryptophan levels, with increased proportion of protein deposited in the carcass. This proposition is confirmed by the results of Latorre et al. (2008), who found that pigs selected for lean deposition have better feed efficiency, showing a positive correlation between these two factors. Moreover, according to Marinho et al. (2007), the increase in protein deposition is the major factor that can justify the increase in weight gain associated with improvement in feed conversion.

There was no effect (P>0.05) of digestible tryptophan levels on daily protein deposition (DPD) of pigs (Table 2). This result differs from that obtained by Ponter et al. (1994), who, assessing levels tryptophan on the performance and the rate of protein deposition in pigs, found an increase in protein synthesis of *longissimus dorsi* and *semitendinosus*, with increasing dietary tryptophan levels.

However, similarly to the results of daily protein deposition observed in this study, Haese et al. (2006) and Pereira et al. (2008) evaluated the effects of digestible tryptophan levels on growth performance and carcass traits of pigs from 60 to 95 kg and from 97 to 125 kg, respectively, and found no effect of tryptophan levels in the yield of lean meat of pigs. Consistent with these results, Henry (1995) worked with pigs from 55 to 88 kg and also found no effect of increasing dietary tryptophan levels in protein deposition of pigs slaughtered at the same body weight. On the other hand, the authors observed an increase in protein deposition in the carcass when pigs were slaughtered at the same age. Based on these results, the fact that the pigs in this study were slaughtered at the same weight may have contributed to the lack of differences in daily protein deposition.

Although protein deposition in the carcass did not vary significantly, it was found that pigs fed diets containing 0.125% digestible tryptophan had lower absolute value of daily protein deposition, which was 32.0% below the average value of deposition observed in the pigs kept in the other treatments. Thus, one can infer that the level of 0.125% digestible tryptophan was insufficient to allow the pigs to express their genetic potential for body protein deposition.

Conclusions

The highest digestible tryptophan level assessed (0.175%), corresponding to a tryptophan:lysine relation of 19%, provides the best result for weight gain in castrated male pigs in the phase of 30 to 60 kg.

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