Digestible tryptophan levels in diets for pigs weighing 15 to 30 kg

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ABSTRACT - In order to evaluate the effects of increasing dietary tryptophan levels on performance and carcass characteristics of growing pigs, a total of 60 crossbred castrated male pigs, with average initial and final body weights of 15.50 ± 1.15 and 30.60 ± 1.35 kg were used. Pigs were allotted in a completely randomized block design, with five dietary treatments (0.149, 0.158, 0.168, 0.177, and 0.187% digestible tryptophan, corresponding to digestible tryptophan:lysine relations of 16.0, 17.0, 18.0, 19.0, and 20.0%), six replicates, and two animals per experimental unit, which was represented by the pen. Experimental diets and water were supplied *ad libitum* throughout the experimental period. The average maximum and minimum temperatures inside the rooms were of 21.5 ± 1.12°C and 26.4 ± 1.91°C, respectively. There was no effect of digestible tryptophan on daily feed intake or protein deposition of pigs. Body weight gain increased quadratically with increasing digestible tryptophan in the diets up to 0.175% maximum response. Increasing digestible tryptophan in the diets linearly improved feed conversion of pigs. The greatest weight gain of 15 to 30 kg castrated male pigs is obtained at 0.175% digestible tryptophan, corresponding to a digestible tryptophan:lysine relation of 18.82% and an estimated daily intake of 2.32 grams of digestible tryptophan.

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

Introduction

Feed formulation based on the values of amino acid digestibility of ingredients and application of the ideal protein concept – understood as the balance of feed amino acids capable of supplying without excesses or deficiencies the requirements of all amino acids necessary for proper maintenance of the growth – has made it possible for nutritionists to obtain diets with lower protein levels, without impairing the performance of pigs. In this concept, lysine, because of its constancy in body protein and its preferential metabolic destination for deposition of lean tissue (Kessler, 1998), has been used as reference for the establishment of the requirements of other essential amino acids, expressed as a relation or percentage of the value of digestible lysine in the diet.

Tryptophan is an essential amino acid in the nutrition of piglets and growing-finishing pigs (NRC, 1998), and in diets in which corn is used as the main ingredient, tryptophan becomes the fourth limiting amino acid, after lysine, methionine and threonine. Limiting levels of tryptophan in the diet, compared with other essential amino acids, influence negatively the synthesis of protein, weight gain and feed efficiency of the animals (Le Floc'h & Seve, 2007; Jansman, 2010).

In addition to its function as an amino acid essential to protein synthesis, tryptophan is the sole source of substrate for the synthesis of important components such as serotonin, a neurotransmitter that stimulates feed intake by animals (Henry et al., 1992). Tryptophan can also be metabolized through the kynurenine pathway, associated with the defense of the body and immune response modulation (Moffett & Namboodiri, 2003; LeFloc'h & Seve, 2007).

Digestible tryptophan requirements for piglets in the nursery phase have been investigated, but few studies have been carried out for the determination of requirements of this amino acid by growing pigs, with results varying for different studies (Guzik et al., 2005). The variations in estimates of digestible tryptophan requirement of growing pigs, reported in the literature, may be caused by the differences among genotypes and sanitary standards of the animals, level of dietary protein and digestibility of the tryptophan of the feed ingredients (Guzik et al., 2005).

The objective of this study was to evaluate the effect of levels of digestible tryptophan and the tryptophan:digestible lysine relation in diets for pigs from 15 to 30 kg.

Material and Methods

The experiment was conducted in a nursery in Setor de Suinocultura in Departamento de Zootecnia at Universidade Federal de Viçosa, Minas Gerais.

The experiment consisted of 60 hybrid commercial barrows, with initial weight of 15.50 ± 1.15 kg, allotted to a completely randomized block design, with five treatments (0.149, 0.158, 0.168, 0.177 and 0.187% digestible tryptophan, corresponding to relationships 16.0, 17.0, 18.0, 19.0 and 20.0% with digestible lysine), six replicates and two animals per pen, which was considered the experimental unit. In the formation of the blocks, initial weight and degree of kinship of the animals were considered.

Animals were housed in suspended metal cages with floor and side screened, equipped with semi-automatic feeders and nipple drinkers, located in masonry buildings with concrete floor and lowered wooden ceiling. Temperature inside the room was measured daily at 8 p.m., through maximum and minimum thermometer, kept in an empty cage in the middle of the room, at half of the animals' height.

Experimental iso caloric and iso nitrogenous 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 relationships 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 other four diets obtained by supplementing the basal diet with L-tryptophan in substitution of glutamic acid, proportionally to the concentrations of nitrogen.

Table 1 - Percentage of calculated compositions of experimental diets

Ingredient	Available tryptophan level (%)				
	0.149	0.158	0.168	0.177	0.187
Corn	74.090	74.090	74.090	74.090	74.090
Soybean meal	18.093	18.093	18.093	18.093	18.093
Bone and meat meal	5.000	5.000	5.000	5.000	5.000
Glutamic acid	0.100	0.087	0.073	0.060	0.045
Starch	0.100	0.104	0.108	0.112	0.117
DL-methionine (99%)	0.062	0.062	0.062	0.062	0.062
L-isoleucine (98%)	0.056	0.056	0.056	0.056	0.056
L-lysine (78%)	0.331	0.331	0.331	0.331	0.331
L-threonine (98%)	0.122	0.122	0.122	0.122	0.122
L-tryptophan (98%)	-	0.009	0.019	0.028	0.038
L-valine (98%)	0.004	0.004	0.004	0.004	0.004
Soybean oil	0.982	0.982	0.982	0.982	0.982
Dicalcium phosphate	0.225	0.225	0.225	0.225	0.225
Limestone	0.325	0.325	0.325	0.325	0.325
Vitamin premix[1]	0.100	0.100	0.100	0.100	0.100
Mineral premix[2]	0.100	0.100	0.100	0.100	0.100
Salt	0.279	0.279	0.279	0.279	0.279
Growth promoter	0.020	0.020	0.020	0.020	0.020
BHT	0.010	0.010	0.010	0.010	0.010
Nutritional calculated composition[3]					
Crude protein (%)	17.458	17.458	17.458	17.458	17.458
ME (kcal/kg)	3222	3222	3222	3222	3222
Digestible lysine (%)	0.930	0.930	0.930	0.930	0.930
Digestible tryptophan (%)	0.149	0.158	0.168	0.177	0.187
Digestible met + cys (%)	0.555	0.555	0.555	0.555	0.555
Digestible threonine (%)	0.632	0.632	0.632	0.632	0.632
Digestible isoleucine (%)	0.566	0.566	0.566	0.566	0.566
Digestible valine (%)	0.656	0.656	0.656	0.656	0.656
Sodium (%)	0.180	0.180	0.180	0.180	0.180
Calcium (%)	0.830	0.830	0.830	0.830	0.830
Available phosphorus (%)	0.430	0.430	0.430	0.430	0.430
Digestible tryptophan:lysine relation	16.00	17.00	18.00	19.00	20.00

[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).

Diets and water were supplied *ad libitum* to animals troughout the experimental period. Animals were weighed at the beginning and end of the experiment for determination of weight gain. Experimental diets were weighed when fed to the animals and the 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 30.60 ± 1.35 kg, they were submitted to a 24-hour feed fast. After this period, one animal from each experimental unit, with weight closest to 30 kg, was slaughtered by exsanguination, then shaved and eviscerated.

An additional group of five piglets weighing 14.9 ± 1.60 kg were slaughtered by following the same procedure used in the slaughter of animals in experiments for determining the initial composition of the carcass.

Whole, eviscerated and blood free carcasses of 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 and 1775-rpm cutter. After homogenization of the crushed material, samples were taken from carcasses, which were stored in a freezer at -12°C.

After that, samples were 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 fat concentration, the samples were submitted to a hot pre-defatting (Soxhlet extractor) during 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 in Laboratório de Nutrição Animal in Departamento de Zootecnia at Universidade Federal de Viçosa, according to techniques described by Silva (2002).

The deposition of protein in the carcass was evaluated by comparative criteria between carcasses of contemporaries animals slaughtered at 15.55 ± 0.75 kg and the animals slaughtered at the end of the experiment,

one animal from each experimental unit with weight closer 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 the deposition of protein were analyzed by 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 which 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 adjustment for each variable studied.

Results and Discussion

During the experimental period, average minimum and maximum temperatures inside the rooms were 21.5 ± 1.12°C and 26.4 ± 1.91°C, respectively. Considering that the ideal temperature range for pigs during growth, suggested by Coffey et al. (2000), is from 18 to 28°C, it was found that the animals were not submitted to heat stress based on temperature variation occurred in this study.

There was no effect (P>0.05) of levels of digestible tryptophan on the daily feed intake of animals (Table 2). Similarly, Susenbeth & Lucanus (2005) also found no effect of increasing levels of tryptophan in the diet on daily feed intake of pigs from 15 to 22 kg.

Table 2 - Data of performance and protein deposition of 15 to 30 kg piglets fed on diets with different levels of digestible tryptophan

Variable	Digestible tryptophan (%)					CV (%)
	0.149	0.158	0.168	0.177	0.187	
Average feed intake (g/d)	1261	1325	1398	1329	1332	8.0
Tryptophan intake (g/d)[1]	1.88	2.09	2.35	2.35	2.49	10.6
Average weight gain (g/d)[2]	686	729	783	776	761	7.1
Feed conversion (g/g)[2]	1.84	1.77	1.78	1.72	1.75	3.0
Protein deposition (g/day)	72.25	82.28	74.18	71.17	75.77	14.2

[1] Linear (P<0.05).
[2] Quadratic (P<0.05).

However, the result of daily feed intake observed in this study is not consistent with those reported by Guzik et al. (2005), who, when evaluating tryptophan levels in diets for piglets from 7 to 15 kg, with tryptophan:digestible lysine relation ranging from 14.5 to 19.5%, found a linear increase in feed consumption of piglets by increasing digestible tryptophan levels in the diets. Similarly, Zhang et al. (2007) investigated the effects of digestible tryptophan varying from 0.12 to 12.26% in diets for weanling pigs and also found positive relationship between levels of digestible tryptophan in the diet and daily feed intake.

In more recent studies, Fernandez & Strathe (2009) and Jansman et al. (2010) also found variation in daily feed intake of piglets by increasing the levels of this amino acid in the diet.

The inconsistency of results noted among studies regarding the effects of digestible tryptophan levels on the daily feed intake may be due, among other factors, to the relationship between digestible tryptophan and neutral long chain amino acids (NLCAA) e.g., leucine, isoleucine, valine, phenylalanine and tyrosine from experimental diets. According to Henry et al. (1992), tryptophan competes with NLCAA for sharing the same transport system for passage through the blood-brain barrier. Thus, a reduced plasma ratio between tryptophan and NLCAA results in decreased availability of tryptophan in the brain, thus reducing the production of serotonin, a neurotransmitter that stimulates feed intake by animals.

In a later study, Henry et al. (1996) confirmed that the reduction in involuntary intake of feed by pigs fed on diets deficient in tryptophan or with excess of protein may be related to lower levels of serotonin in various parts of the brain, and this effect was more pronounced in females when compared with barrows.

Effect (P<0.01) of levels of dietary digestible tryptophan on the daily consumption of digestible tryptophan by the pigs was observed, increasing linearly (Table 2) according to the equation $\hat{Y} = -0.7025 + 17.6492\text{Trp}$ (r² = 0.90). Because daily feed intake of animals did not vary significantly among treatments, the linear increase in digestible tryptophan intake may be explained by its concentration in the feed.

Digestible tryptophan levels affected (P<0.05) daily weight gain (DWG) of animals (Table 2), which increased quadratically up to the estimated level of 0.175%, corresponding to a ratio with digestible lysine of 18.82% (Figure 1). Similar results were obtained by Han et al. (1993), who evaluating levels of digestible tryptophan from 0.08 to 12.15% in diets for pigs from 10.9 to 22.0 kg, also found a linear increase in weight gain by increasing dietary levels

$$\hat{Y} = 3546.98 + 49355.6X - 140744.0X^2$$
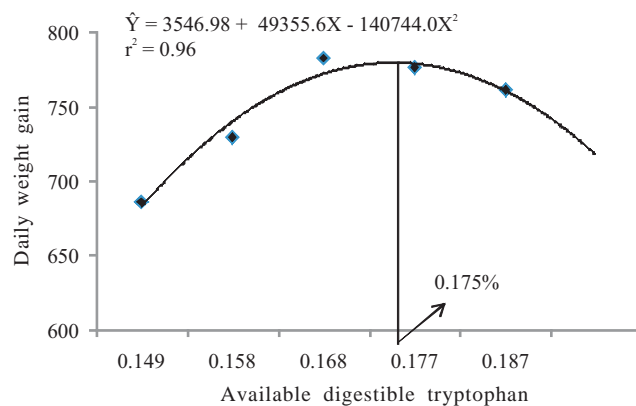$$r^2 = 0.96$$

Figure 1 - Daily weight gain of pigs from 15 to 30 kg fed diets with different levels of available digestible tryptophan.

of tryptophan, with best response for this variable obtained at the level of 0.137% of digestible tryptophan. Despite the similar response pattern as a result of the increased levels of digestible tryptophan on daily weight gain observed in the studies, the level of digestible tryptophan (0.175%) which provided best result of daily weight gain in animals in this study was 28% higher than the one obtained by Han et al. (1993). Positive effect by the addition of tryptophan into diets on weight gain of piglets in the initial growth phase was also observed by many authors (Burgoon et al., 1992; Sawadogo et al., 1997; Ferguson & Gous, 2002; Pastuzewska et al., 2007; Fernandez & Strathe, 2009; Jansman et al., 2010).

Conversely, Susenbeth & Lucanus (2005), in a study with tryptophan supplementation (0.2 to 1.0 g/kg) in diets for barrows from 15 to 22 kg, found no effect of tryptophan levels on weight gain of animals. According to the authors, a digestible tryptophan:lysine relation of 17.0 or 18.0% in diets for growing pigs seems to be sufficient to maximize the performance of animals, provided that the level of digestible lysine is adequate.

The digestible tryptophan:digestible lysine relation (18.82%) observed in this study, in which the best value of DWG was obtained, was higher than that proposed by Chung & Baker (1992) and by NRC (1998), which recommend a relation of 18% between these amino acids for piglets from 10 to 20 and from 20 to 50 kg, respectively.

According to the report by Jansman et al. (2010), the level of digestible lysine used in experimental diets may be one of the factors that justify the variation of results observed among studies. Confirming this proposition, Eder et al. (2003) and Guzik et al. (2005) noted that the daily weight gain of pigs in the finishing phase was significantly influenced by the levels of dietary tryptophan, and the best

responses were obtained at the levels of 0.146 and 0.096% of digestible tryptophan, respectively, which corresponded to the same relationship with digestible lysine of 18%.

The level of digestible tryptophan (0.175%) which provided the best response for weight gain of animals in this study is above the 0.150% level recommended by NRC (1998) for animals from 20 to 50 kg and the recommended (0.168%) by Rostagno et al. (2005) for pigs of average performance at the same growth phase.

Digestible tryptophan levels affected (P<0.05) feed conversion (FC), which improved linearly (Table 2) according to the equation $\hat{Y}$ = 2.1654 – 2.3355 Trp (r^2 = 0.65). This response pattern is consistent with that obtained by Schutte & Van Weerden (1988), who evaluating levels of digestible tryptophan from 0.13 to 0.25% for pigs from 11 to 37 kg, found a linear improvement in feed conversion of the animals.

Positive influence of tryptophan levels on feed conversion of piglets was also observed by Guzik et al. (2002), who evaluating levels of digestible Trp (0.13 to 0.25%) for weaned pigs, found an improvement in feed conversion, although the best response was obtained at the level of 0.18% of tryptophan. The results obtained in this study corroborate those of Guzik et al. (2005) and those of Fernandez & Strathe (2009), who observed a linear increase in feed efficiency of piglets when the tryptophan:digestible lysine relation increased from 14.5 to 19.5% and from 16.0 to 22.0%, respectively.

Conversely, Susenbeth & Lucanus (2005) found no effect of the increase at the concentration of dietary digestible tryptophan on feed conversion of pigs from 15 to 22 kg.

According to data obtained in this study, the requirement of digestible tryptophan by pigs from 15 to 30 kg, for better feed conversion is greater than the necessary to increase the growth rate. However, this observation does not confirm the report of Susenbeth & Lucanus (2005) that the requirement of digestible tryptophan for maximum weight gain is always greater than that needed for a better feed conversion.

There was no effect (P>0.05) of levels of digestible tryptophan on the daily protein deposition of pigs (Table 2). Combining the results of daily protein deposition with the results of feed conversion and daily weight gain obtained in this study, it can be inferred, biologically, that the daily protein deposition of animals observed in this study is inconsistent if considered that the increase in protein deposition in the carcass of pigs is one of the major factors that may explain the result of the increase in weight gain associated with improvement in feed conversion (Krick & Boyd, 1992; Marinho et al., 2007).

Similarly to the result of daily protein deposition obtained in this study, Henry (1995), evaluating the effect of tryptophan on the performance of finishing pigs, also found no effect of tryptophan levels (from 0.10 to 0.13%) on daily protein deposition of the animals when they were slaughtered at the same body weight. However, an increase in protein deposition was observed when animals were slaughtered at the same age. Thus, the fact that animals in this study had been slaughtered at the same body weight contributed to the absence of differences in the carcass parameters evaluated.

Conclusions

The level of 0.175% digestible tryptophan, corresponding to a tryptophan:digestible lysine relation of 18.82% and to an estimated daily consumption of 2.32 g, provides the best result of weight gain of 15 to 30 kg castrated male piglets.

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