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# Digestible lysine levels in diets for pigs from 24 to 50 kg under sanitary segregation[1]

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**ABSTRACT** - This study evaluated the relationship between digestible lysine and metabolizable energy for barrow and gilts from 24 to 50 kg. Performance, digestibility and blood profile were studied. The experimental design was of randomized blocks, with five treatments, eight replicates and two animals per experimental unit in the performance assay and four replicates and one animal per experimental unit in the digestibility assay. The blood profile was chosen at 20 random animals of each sex, with four replicates per treatment and the animal as experimental unit. The treatments were 0.80, 0.90, 1.00, 1.10 and 1.20% digestible lysine. There was a linear effect of lysine levels on weight gain and feed conversion in females and crude protein in both sexes, gross energy excreted in the urine (kcal) and digestible energy (kcal). A quadratic effect of the amino acid studied in the daily consumption of crude protein (g) in both sexes, weight gain and feed conversion of barrows, as well as the nitrogen excreted in the urine (g) and nitrogen retained, and absorbed and retained. The relationship between neutrophils and lymphocytes had quadratic responses with increasing levels of lysine. The maximum performance for the studied genotype was obtained with 0.88 and 0.91% of digestible lysine or 2.60 and 2.67 g of digestible lysine/Mcal of metabolizable energy for females and barrows, respectively, corrected for digestibility trial.

Key Words: barrow, gilt, leucocytes, metabolizable energy, specific genotype

## Introduction

Over the past decades, the nutritional requirements of pigs have been altered depending on the genetic improvement and specific factors, determined by breeding conditions. Moreover, currently, in swine, the important crescent role of consumers seeking healthy foods, produced under conditions that meet animal welfare and respect the environment should also be considered.

In reviews on the nutritional requirements of pigs, all the conditions that influence the genetic expression of animals for meat production should be considered. The amino acid levels present in most diets often follow the recommendations proposed by the tables of requirements that were based on experiments with diets of high protein levels (National Research Council, 1998; Rostagno et al., 2005), making the lysine requirement be above some results presented in the literature, in most cases.

The capacity to accumulate lean tissue in growing pigs is dependent on the fulfillment of the requirements of amino acids, especially lysine, considered the first limiting amino acid in most diets (Zangeronimo et al., 2009). Another important consideration about the protein deposition, determining the efficiency of utilization of dietary nutrients in the process of protein anabolism and pig growth is the corresponding intake of dietary energy. The energy intake should supply the real demands of maintenance and accumulation of body mass; therefore, the energy requirement varies according to the protein level (amino acids) of the diet (Urynek & Buraczewska, 2003).

The challenge of health conditions alters immune responses and the nutritional requirements of pigs (Williams et al., 1997 a,b,c); in accordance, Salak-Johnson & McGlone (2007), in a review, found that, according to some studies, the immune system is also stimulated by stress, and this may modify nutritional requirements.

The objective of this study was to estimate the optimum level of lysine and its relation with the metabolizable energy for pigs from 20 to 50 kg segregated from the production system and with minimal disease, based on the performance variables, apparent digestibility and blood parameters.

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## Material and Methods

The performance assay was conducted between December 17, 2007 and January 18, 2008. Forty gilts and 40 barrows, commercial product of the crossbreeding of P76 males and Näima females (Pen Ar Lan) with initial weight 24.60±0.08 kg and 24.81±0.20 kg and 63 days of age, respectively, were used. The experimental design was of randomized blocks, with five treatments (0.80, 0.90, 1.00, 1.10 and 1.20% of digestible lysine), eight replicates and one animal per experimental unit (stall).

The experimental diets were based on corn, soybean meal, soybean oil, minerals, vitamins and amino acids (Table 1). At the formulation of diets, the levels of digestible lysine were estimated considering a digestibility index and values of total amino acids analyzed in corn and soybean meal. This index was based on the weighted mean of the digestibility coefficients of lysine proposed by Rostagno et al. (2005), considering the inclusion levels of corn and soybean meal in the experimental diets. Subsequently, based on the digestibility assay, the digestible lysine content of diets of 0.70, 0.83, 0.85, 0.93 and 1.06% of digestible lysine was estimated.

In the composition of the diets, minimum ratios between the amino acids were established according to Rostagno et al. (2005), so the amino acids threonine, methionine and tryptophan were added to the diets to supply those ratios.

The animals were housed in stalls of metal structure of 1.00 × 2.00 meters, containing manual-replenishing feeders and nipple-type drinkers. The experimental masonry shed, with a ceiling height of 3.40 meters, had tilt-and-turn side windows to aid in the ventilation control.

The experimental period lasted 32 days, with two controls, one 16 days after the start of the experiment (phase 1) and another in the end, at 32 days of experiment, to collect performance data. Throughout the study, daily temperatures (maximum and minimum) were measured in the morning (8 am) and afternoon (3 pm), using thermometers installed at the floor height. of the stalls. In the first 10 days of the experimental period, animals received medicated feed with antibiotic Tiamulin at 225 g/t of feed and anti helminthic Mebendazole at 450 g/t of feed, as recommended by the manufacturer.

The parameters daily intake of crude protein (kg) and crude protein efficiency were calculated as follows:

Table 1 - Percentage and analyzed composition of experimental diets for pigs in the starter growth phase

| Ingredients (%) | Digestible lysine (%) | | | | |
|---|---|---|---|---|---|
| | 0.80 | 0.90 | 1.00 | 1.10 | 1.20 |
| Ground corn | 72.27 | 72.56 | 72.98 | 73.48 | 74.04 |
| Soybean meal | 23.88 | 23.37 | 22.64 | 21.87 | 21.11 |
| Common salt | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Limestone | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| Dicalcium phosphate | 1.34 | 1.35 | 1.35 | 1.36 | 1.37 |
| Soybean oil | 1.00 | 1.00 | 1.00 | 0.94 | 0.82 |
| Vitamin and mineral supplement[1] | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| L-lysine HCl | 0.005 | 0.147 | 0.295 | 0.445 | 0.590 |
| L-threonine | 0.000 | 0.039 | 0.115 | 0.192 | 0.266 |
| DL-methionine | 0.000 | 0.037 | 0.106 | 0.174 | 0.240 |
| L-tryptophan | 0.000 | 0.000 | 0.012 | 0.034 | 0.056 |
| Total (kg) | 100 | 100 | 100 | 100 | 100 |
| Nutritional values | | | | | |
| Metabolizable energy (kcal/kg)[2] | 3388 | 3392 | 3397 | 3400 | 3400 |
| Crude protein (%) | 16.70 | 16.70 | 16.70 | 16.70 | 16.70 |
| Calcium (%) | 0.60 | 0.63 | 0.63 | 0.70 | 0.68 |
| Total phosphorus (%) | 0.54 | 0.57 | 0.55 | 0.57 | 0.56 |
| Available phosphorus (%) | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Digestible lysine (%)[3] | 0.70 | 0.83 | 0.85 | 0.93 | 1.06 |
| Lysine (g):metabolizable energy (Mcal) | 2.36 | 2.65 | 2.94 | 3.23 | 3.53 |
| Methionine+digestible cystine (%)[3] | 0.51 | 0.52 | 0.59 | 0.62 | 0.66 |
| Digestible threonine (%)[3] | 0.55 | 0.58 | 0.61 | 0.64 | 0.68 |
| Digestible valine (%)[3] | 0.64 | 0.66 | 0.62 | 0.58 | 0.59 |
| Digestible leucine (%)[3] | 1.26 | 1.28 | 1.24 | 1.20 | 1.16 |
| Digestible isoleucine (%)[3] | 0.56 | 0.58 | 0.56 | 0.53 | 0.51 |
| Digestible histidine (%)[3] | 0.39 | 0.38 | 0.38 | 0.35 | 0.35 |
| Digestible phenylalanine (%)[3] | 0.70 | 0.73 | 0.71 | 0.68 | 0.68 |

Lys - lysine; ME - metabolizable energy.
[1] Composition per kg of premix: Fe - 25 g; Cu - 5 g; Mn - 33.33 g; Zn - 66.67 g; Co - 66.67 mg; I - 566.67 mg; Se - 168 mg; vit. A - 2700 IU; vit. D3 - 840 IU; vit. E - 4.2 g; vit. K - 960 mg; thiamine B1 - 480 mg; riboflavin B2 - 2.16 g; pyridoxine B6 - 900 mg; vit. B12 - 14400 mcg; niacin B3 - 9.6 g; pantothenic acid - 4.8 g; folic acid - 420 mg; biotin - 45 mg.
[2] Average value estimated in the digestibility assay.
[3] Value estimated at the digestibility trial considering that the average coefficient of crude protein digestibility was 88.51±0.51 %.

- Daily intake of crude protein (g) = CP (%) in the feed × daily feed intake (g)/100;

- Efficiency of crude protein = daily weight gain/daily intake of crude protein.

At the end of the experiment, blood samples were collected from eight animals (four barrows and four gilts) of each treatment (8 animals × 5 treatments), therefore, 20 barrows and 20 gilts, to determine the number of leukocytes, neutrophils, eosinophils, monocytes and the relation between neutrophils and lymphocytes (absolute number of neutrophils divided by the absolute number of lymphocytes).

The metabolism assay was conducted between January 2 and January 17, 2008. Twenty animals (10 females and 10 barrows) of the same strain and batch were used in the performance assay, with starting weight of 38.545±0.464 kg.

The animals were placed in the unit of study of digestibility and metabolism, in metabolic cages, in a storage shed of masonry, with dimensions of 20 × 6 meters, ceiling height of 3 meters, window of "vitraux" sides, lined with styrofoam and equipped with three units of air conditioners of split system-type.

The duration of the experimental period was ten days: five for animal adaptation to diets, when they were medicated with Mebendazole and Tiamulin, and five for feces and urine collection. In the collection phase, each animal received the same amount of daily diet, on a dry matter basis, per unit metabolic size (body weight $kg^{0.75}$) and other management measures, collection of feces and urine were done through the methods described by Barbosa et al. (1999). In the analysis of metabolism, there was no significant contamination of urine by faeces. So a sieve was used for feces to stay trapped and urine of females was collected in an appropriate e container without using the probe.

In the determination of nitrogen balance, the same diets evaluated on performance trial, with five treatments were used. Since there were no differences between barrows and gilts for the variables studied, four replicates were considered per treatment.

The performance variables, metabolism and leukocytes profile were submitted to regression analyzed by orthogonal polynomial considering the levels of digestible lysine, through the procedure PROC MIXED of SAS (Statistical Analysis System, version 9.1.3), as the model: $Yij = \mu + Ai + Bj + eij$, in which: Yij = constant associated to all observations; μ = overall mean of the variable; Ai = effect of lysine level i, in which i = 1, 2, ... and 5; Bj = effect of block j, in which j = 1, 2, ... and 8; eij = random error associated with each observation.

## Results and Discusssion

During the conduction of the experiment, in the morning and afternoon period mean values of maximum and minimum temperature were 28.97±2.49 °C and 24.00±1.50 °C; and 28.82±2.17 °C and 24.52±1.80 °C, respectively.

There was a linear effect (P<0.05) of dietary lysine level on feed conversion when the animals were in phase 1 (from 24 to 36 kg) and on variables final weight (kg), daily weight gain (g), relative weight gain (%), feed conversion and crude protein efficiency, considering the whole experimental period (from 24 to 50 kg).

There was a quadratic effect (P<0.05) of dietary lysine level on the variables crude protein daily intake (g) and crude protein efficiency, when pigs were in the first phase of the experiment. When the total period of assessment was considered, there was a quadratic effect (P<0.05) on crude protein daily intake (g).

The results obtained in present study (Tables 2 and 3) are in agreement with Fontes et al. (2000b), who worked with crossbred gilts weighing about 30.1 kg and with digestible lysine levels ranging from 1.00 to 1.30% diet and concluded that the maximum genetic potential for weight gain was obtained with 1.10% of digestible lysine. Quadratic effect of variation in dietary lysine up to level of 1.30% on the weight gain in gilts between 21 and 72 kg, selected for deposition of lean meat has been verified by several authors; however, the recommended levels are quite variable (Martinez & Knabe, 1990; Batterham et al., 1990; Bikker et al., 1994; Friesen et al., 1994; James et al., 2002).

The differences in optimal levels of lysine estimated this study could be related to differences in genotype (Stahly et al., 1994; Oliveira et al., 2006b), environment and immune level (Williams et al., 1997a,b,c; Main et al., 2008), feeding system, energy concentration and amino acid profile of experimental diets (Trindade Neto et al., 2005; Main et al., 2008).

The linear effect (P<0.05) of lysine levels on the feed conversion of gilts both in phase 1 and in the total period of this study is in agreement with results observed by Fontes et al. (2000a) and Trindade Neto et al. (2005). On the other hand, Martinez & Knabe (1990), James et al. (2002), Fontes et al. (2005) and Rossoni (2007) found a quadratic effect of digestible lysine level on the feed conversion of gilts in the growth phase, and the recommended levels of amino acid were 0.86, 1.00, 1.16 and 1.08%, respectively.

Based on feed conversion, lower concentrations of lysine have been recommended for gilts in the same stage of development, according to Donzele et al. (1994), Kill et al.

(2003) and Fontes et al. (2005), when recommended 0.80, 0.98 and 1.05% of digestible lysine respectively. Similarly, the NRC (1998) recommends 0.83% of digestible amino acid for growing pigs.

The improvement verified in feed conversion of females at the last level studied, i.e., from 1.20% of digestible lysine, suggests an increase in body protein deposition in relation to fat; according to Trindade Neto et al. (2005), during growth of pigs until 60 kg, approximately, there is greater efficiency of protein deposition and, or, accumulation of muscle mass in the carcass.

Concerning the relative weight gain of females in the total period, the linear effect (P<0.05) observed shows to be consistent and follows the daily weight gain in the total period.

There was no effect (P>0.05) of treatments on daily feed intake of females in the total period of evaluation. Martinez & Knabe (1990), Friesen et al. (1994), Donzele et al. (1994), Fontes et al. (2000b) and Rossoni (2007) also found no variation in feed intake, in function of the lysine level of feed for gilts, while Fontes et al. (2005) found that feed intake ranged quadratically and Fernández-Fígares et al. (2007) found that feed intake decreases linearly with increasing lysine level in diets for piglets during growth.

The results of feed intake obtained confirm the proposition of Edmonds & Baker (1987), that pigs are able to tolerate the excesses of amino acids with no change in voluntary feed intake.

The application the concept of ideal protein is another factor that may have contributed for the lack of variation in feed intake. According to Kill et al. (2003), pigs fed formulated diets keeping the ratio between essential amino acid and lysine are less susceptible to variations in feed intake.

Regarding the daily intake of crude protein and the efficiency of crude protein use by females, the observed effect (P>0.05) of variation of lysine in experimental diets may be related to protein deposition in lean tissue of animals, once the lysine concentration in the diet varied.

When considering the responses of the treatments in these variables, the concentration of 1.07±0.07% of

Table 2 - Performance of gilts from 24 to 50 kg, according to the nutritional levels of digestible lysine (g/kg)

| Variables | Digestible lysine (%) | | | | | SEM | Probability | |
|---|---|---|---|---|---|---|---|---|
| | 0.80 | 0.90 | 1.00 | 1.10 | 1.20 | | Linear | Quadratic |
| | | | Phase 1 - from 24 kg to 36 kg | | | | | |
| Initial weight (kg) | 24.58 | 24.71 | 24.45 | 24.63 | 24.61 | 0.13 | - | - |
| Final weight (kg) | 36.55 | 37.43 | 37.36 | 38.46 | 37.64 | 0.30 | 0.089 | 0.308 |
| Daily weight gain (g) | 748.37 | 798.62 | 807.25 | 865.12 | 814.37 | 16.82 | 0.096 | 0.273 |
| Relative weight gain (%) | 48.85 | 51.92 | 52.88 | 56.27 | 52.83 | 1.12 | 0.118 | 0.251 |
| Daily feed intake (g) | 1549 | 1610 | 1587 | 1616 | 1551 | 24.74 | 0.963 | 0.356 |
| Feed conversion | 2.09 | 2.02 | 1.98 | 1.87 | 1.91 | 0.02 | 0.003 | 0.338 |
| Daily intake of crude protein (g) | 252.62 | 255.12 | 269.00 | 269.13 | 235.75 | 4.41 | 0.494 | 0.017 |
| Crude protein efficiency | 2.60 | 2.77 | 2.64 | 2.85 | 3.05 | 0.03 | <0.001 | 0.037 |
| | | | Total period - from 24 to 50 kg | | | | | |
| Final weight (kg) | 49.88 | 51.74 | 51.96 | 54.10 | 52.18 | 0.45 | 0.008 | 0.063 |
| Daily weight gain (g) | 791.12 | 846.12 | 859.87 | 921.87 | 858.63 | 14.11 | 0.031 | 0.099 |
| Relative weight gain (%) | 103.24 | 110.21 | 112.66 | 119.92 | 111.61 | 2.00 | 0.039 | 0.086 |
| Daily feed intake (g) | 1746 | 1850 | 1792 | 1882 | 1807 | 26.42 | 0.424 | 0.352 |
| Feed conversion | 2.21 | 2.20 | 2.09 | 2.04 | 2.11 | 0.02 | 0.018 | 0.193 |
| Daily intake of crude protein (g) | 284.75 | 293.12 | 303.50 | 313.38 | 274.25 | 4.66 | 0.980 | 0.013 |
| Crude protein efficiency | 2.78 | 2.87 | 2.83 | 2.95 | 3.13 | 0.03 | 0.005 | 0.155 |

SEM - standard error of the mean.

Table 3 - Effect of digestible lysine level on performance of gilts from 24 to 50 kg

| Variables | Regression equation | Digestible lysine estimated (%) | $R^2$ |
|---|---|---|---|
| | Phase 1 - from 24 to 36 kg | | |
| Daily intake of crude protein (g) | $Y = -332.42 + 1401.68X – 610.71X^2$ | 1.15 | 0.68 |
| Crude protein efficiency | $Y = 4.73 – 6.17X + 2.97X^2$ | 1.04 | 0.47 |
| | Total period - from 24 to 50 kg | | |
| Final weight (kg) | $Y = 45.15 + 6.8X$ | - | 0.51 |
| Daily weight gain (g) | $Y = 644.77 + 210.75X$ | - | 0.51 |
| Relative weight gain (%) | $Y = 856.6 + 264.6X$ | - | 0.49 |
| Feed conversion | $Y = 2.48 – 0.36X$ | - | 0.59 |
| Daily intake of crude protein (g) | $Y = -373.95 + 1663.54X – 813.390X^2$ | 1.02 | 0.69 |
| Crude protein efficiency | $Y = 2.15 + 0.77X$ | - | 0.32 |

$R^2$ - correlation coefficient.

digestible lysine was estimated as optimal for the growth of gilts, from 24 to 50 kg.

There was a linear effect (P<0.05) of dietary lysine level in the following variables: final weight (kg), daily weight gain (g), relative weight gain (%) and crude protein efficiency when animals were in phase 1; and relative weight gain (%) and crude protein efficiency, considering the total experimental period (from 24 to 50 kg; Tables 4 and 5).

There was a quadratic effect (P≤0.05) of dietary lysine level on the following variables: feed conversion, for phase 1; and final weight (kg), daily weight gain (kg), feed conversion and daily intake of crude protein (g), for the total period of assessment.

There was no effect (P>0.05) of the digestible lysine levels studied on feed intake both for phase 1 and the total period for barrows. Gasparotto et al. (2001), studying lysine levels that varied from 0.90 to 1.20 % of digestible lysine, with animals selected for fast growth, found a quadratic effect of lysine levels on intake in periods from 0 to 14 and 0 to 28 days of evaluation, but not in the total experimental period. Abreu et al. (2007) also found no effect of lysine on feed intake, like Moretto et al. (2000), Urynek & Buraczewska (2003), Toledo et al. (2005), Oliveira et al. (2006a) and Zangeronimo et al. (2006). According to these studies, maintaining the amino acid profile of the diet does not influence the feed intake of animals. This care was also taken in this study because Partridge et al. (1985) observed that feed intake was altered in an attempt of pigs to supply the deficiency of some amino acid.

For barrows, linear effect (P≤0.05) of lysine level was observed on variables relative weight gain and crude protein efficiency in phase 1 (from 24 to 36 kg); and for total period, linear effect (P≤0.01) of the amino acid level was found on the relative weight gain, suggesting more efficient use of protein for weight gain.

Table 4 - Performance of barrows from 24 to 50 kg, according to the nutritional levels of digestible lysine (g/ kg)

| Variables | Digestible lysine (%) | | | | | SEM | Probability | |
|---|---|---|---|---|---|---|---|---|
| | 0.80 | 0.90 | 1.00 | 1.10 | 1.20 | | Linear | Quadratic |
| Phase 1 - from 24 kg to 36 kg | | | | | | | | |
| Initial weight (kg) | 24.63 | 24.66 | 24.83 | 25.09 | 24.65 | 0.16 | - | - |
| Final weight (kg) | 35.98 | 37.56 | 38.40 | 38.25 | 38.73 | 0.37 | 0.008 | 0.226 |
| Daily weight gain (g) | 709.38 | 806.13 | 893.37 | 822.75 | 879.75 | 19.14 | 0.004 | 0.095 |
| Relative weight gain (%) | 46.14 | 52.27 | 54.70 | 52.69 | 57.25 | 1.45 | 0.020 | 0.492 |
| Daily feed intake (g) | 1497 | 1569 | 1600 | 1549 | 1628 | 32.18 | 0.304 | 0.804 |
| Feed conversion | 2.13 | 1.96 | 1.88 | 1.89 | 1.85 | 0.02 | 0.002 | 0.050 |
| Daily intake of crude protein (g) | 244.13 | 248.75 | 271.12 | 257.75 | 247.62 | 5.34 | 0.673 | 0.155 |
| Crude protein efficiency | 2.90 | 3.23 | 3.19 | 3.18 | 3.57 | 0.04 | <0.001 | 0.621 |
| Total period - from 24 to 50 kg | | | | | | | | |
| Final weight (kg) | 50.53 | 53.40 | 55.13 | 54.38 | 54.45 | 0.49 | 0.050 | 0.025 |
| Daily weight gain (g) | 809.25 | 898.00 | 971.88 | 915.25 | 0.931 | 15.55 | 0.012 | 0.024 |
| Relative weight gain (%) | 105.30 | 116.52 | 120.68 | 117.09 | 121.23 | 2.00 | 0.010 | 0.125 |
| Daily feed intake (g) | 1780 | 1871 | 1945 | 1866 | 1882 | 30.52 | 0.368 | 0.248 |
| Feed conversion | 2.20 | 2.08 | 2.00 | 2.04 | 2.02 | 0.02 | 0.014 | 0.050 |
| Daily intake of crude protein (g) | 290.25 | 296.37 | 329.62 | 310.75 | 286.00 | 5.37 | 0.868 | 0.010 |
| Crude protein efficiency | 2.79 | 3.03 | 2.95 | 2.95 | 3.27 | 0.03 | 0.001 | 0.410 |

SEM - standard error of the mean.

Table 5 - Effect of digestible lysine level on performance of barrows from 24 to 50 kg

| Variables | Regression equation | Digestible lysine estimated (%) | $R^2$ |
|---|---|---|---|
| Phase 1 - from 24 to 36 kg | | | |
| Final weight (kg) | Y = 31.590 + 61.187X | - | 0.79 |
| Daily weight gain (g) | Y = 464.900 + 357.375X | - | 0.59 |
| Relative weight gain (g/kg) | Y = 299.70 + 226.30X | - | 0.75 |
| Feed conversion | $Y = 4.820 – 5.232X + 2.303X^2$ | 1.14 | 0.36 |
| Crude protein efficiency | Y = 1.940 + 1.281X | - | 0.73 |
| Total period - from 24 to 50 kg | | | |
| Final weight (kg) | $Y = -11.770 + 124.182X – 57.678X^2$ | 1.08 | 0.36 |
| Daily weight gain (g) | $Y = -1289.650 + 4207.420X – 1973.210X^2$ | 1.07 | 0.37 |
| Feed conversion | $Y = 4.533 – 4.602X + 2.098X^2$ | 1.10 | 0.23 |
| Daily intake of crude protein (g) | $Y = -500.400 + 1632.660X – 813.390X^2$ | 1.00 | 0.72 |
| Crude protein efficiency | Y = 2.110 + 0.8917X | - | 0.66 |

$R^2$ - correlation coefficient.

In barrows, although there was a linear effect (P<0.05) of the digestible lysine level on the daily weight gain in phase 1, the increase in the level of digestible lysine in the diet determined quadratic effect (P≤0.05) in daily weight gain in the total period and feed conversion in phase 1 and the total period, with the best answers close to the level of 1.10 % of lysine. Trindade Neto et al. (2005), studying levels of metabolizable energy and digestible lysine for barrows and gilts, under conditions of sanitary isolation, observed a linear effect of lysine levels on weight gain and quadratic effect on feed conversion, indicating 1.015% as optimum lysine level. According to these authors, these responses indicate more efficient use of nutrients with increasing dietary lysine concentration and daily intake of amino acid. This observation is confirmed in this study and is similar to the results of Oliveira et al. (2006b), Zangeronimo et al. (2006) and Abreu et al. (2007). Such effects, however, were not observed by Moretto et al. (2000) and Gasparotto et al. (2001).

The results demonstrate greater efficiency in the performance of barrows up to the level 1.08±0.05% of digestible lysine. This value is similar to recommended by Chiaradia (2008), but above those proposed by Trindade Neto et al. (2005), Gasparotto et al. (2001) and Zangeronimo et al. (2006), which were 1.015%, 1.05%, 1.00% of digestible amino acid, respectively, and slightly lower than those recommended by Oliveira et al. (2006b) and Abreu et al. (2007), which was 1.10% of digestible lysine. It is worth remarking that the authors used animals of different genotypes from those utilized in this experiment.

The responses of the treatments in these variables suggest a concentration of 1.08±0.05% of digestible lysine as optimal for barrows growth from 24 to 50 kg.

There was a linear effect (P<0.05) the level of lysine on the variables gross energy excreted in urine (kcal/g) and digestible energy (kcal/g). There was also a quadratic effect (P<0.05) of dietary digestible lysine level on variables crude protein excreted in urine (g), nitrogen excreted in urine (g), retained nitrogen (g), retained nitrogen (%) and retained/absorbed nitrogen (%) (Table 6).

In the digestibility and apparent metabolism assays, no effect (P>0.05) of lysine levels were verified on dry matter intake, dry matter excreted or digestible dry matter (Table 7). A similar result to dry matter intake was observed by Urynek & Buraczewska (2003) and Moreira et al. (2004). Fraga et al. (2008), however, found that there was a decrease in consumption. This effect was expected and confirms results obtained in the performance assay, in which there was no effect of digestible lysine levels on feed intake. As the diets were formulated to contain similar metabolizable energy, no differences were expected between the consumption of diets; furthermore, in the digestibility and metabolism assays, each animal received the same amount of diet daily, on the basis of dry matter, per unit of metabolic size.

Table 6 - Apparent digestibility in barrows and gilts from 38 to 42 kg, according to the nutritional levels of digestible lysine (g/kg)

| Variables[1] | Digestible lysine (%) | | | | | SEM | Probability | |
|---|---|---|---|---|---|---|---|---|
| | 0.80 | 0.90 | 1.00 | 1.10 | 1.20 | | Linear | Quadratic |
| Dry matter of feed (%) | 88.44 | 88.62 | 88.44 | 88.59 | 88.62 | - | - | - |
| Dry matter intake(g) | 5353 | 5257 | 5220 | 5246 | 5277 | 0.56 | 0.691 | 0.512 |
| Dry matter excreted (g) | 565 | 552 | 576 | 540 | 561 | 18.33 | 0.840 | 0.943 |
| Digestible dry matter (%) | 89.45 | 89.40 | 89.05 | 89.77 | 89.36 | 0.23 | 0.897 | 0.868 |
| Crude protein intake (g) | 986 | 939 | 999 | 985 | 904 | 23.47 | 0.132 | 0.128 |
| Crude protein excreted in feces (g) | 108 | 111 | 111 | 114 | 110 | 4.23 | 0.803 | 0.789 |
| Crude protein excreted in urine (g) | 321 | 267 | 241 | 214 | 261 | 12.92 | 0.032 | 0.035 |
| Digestible protein (%) | 89.04 | 88.07 | 89.02 | 88.62 | 87.78 | 0.32 | 0.385 | 0.679 |
| Intake nitrogen (g) | 31.57 | 30.06 | 31.99 | 31.52 | 28.94 | 0.75 | 0.131 | 0.128 |
| Nitrogen excreted in feces (g) | 3.47 | 3.56 | 3.53 | 3.63 | 3.54 | 0.13 | 0.800 | 0.790 |
| Nitrogen excreted in urine (g) | 10.25 | 8.55 | 7.67 | 6.85 | 8.32 | 0.41 | 0.032 | 0.035 |
| Retained nitrogen (g) | 17.83 | 17.95 | 20.75 | 21.04 | 17.06 | 0.67 | 0.651 | 0.020 |
| Absorbed nitrogen (g) | 28.10 | 26.49 | 28.45 | 27.89 | 25.41 | 0.66 | 0.069 | 0.095 |
| Absorbed nitrogen (%) | 89.04 | 88.06 | 89.02 | 88.62 | 87.77 | 0.32 | 0.619 | 0.141 |
| Retained nitrogen (%) | 56.14 | 59.64 | 65.12 | 66.89 | 58.59 | 1.34 | 0.175 | 0.018 |
| Retained/Absorbed N (%) | 63.06 | 67.72 | 73.14 | 75.48 | 66.87 | 1.53 | 0.138 | 0.023 |
| Gross energy intake (kcal/g) | 4798.25 | 4698.00 | 4669.00 | 4678.00 | 4649.50 | 4.82 | 0.392 | 0.676 |
| Gross energy excreted in feces (kcal/g) | 2.14 | 2.10 | 2.20 | 2.12 | 2.13 | 16.91 | 0.997 | 0.874 |
| Gross energy excreted in urine (kcal/g) | 511.25 | 500.25 | 525.50 | 506.00 | 508.50 | 3.81 | 0.031 | 0.138 |
| Digestible energy (kcal/g) | 125.07 | 113.90 | 108.30 | 102.70 | 108.60 | 11.99 | 0.042 | 0.514 |
| Metabolizable energy (kcal/g) | 4044.00 | 3988.75 | 3972.50 | 3980.00 | 3923.00 | 11.68 | 0.124 | 0.360 |
| Energy balance (kcal/g) | 3886.25 | 3880.25 | 3869.00 | 3882.50 | 3819.50 | 5.09 | 0.420 | 0.676 |
| Initial weight (kg) | 39.25 | 38.25 | 38.08 | 38.30 | 38.62 | - | - | - |
| Final weight (kg) | 43.32 | 41.85 | 42.70 | 42.57 | 42.41 | 0.80 | 0.815 | 0.767 |

[1] Results expressed on a dry matter basis.
SEM - standard error of the mean.

There was no effect (P>0.05) of the digestible lysine level on crude protein intake, crude protein excreted in faeces or digestible protein. However, quadratic effect (P<0.05) was observed on the crude protein excreted in the urine, suggesting a higher dietary protein catabolism, due to the increased concentration of digestible lysine in the diet. Similar effects were found by analyzing the nitrogen metabolism.

There was no effect (P>0.05) of digestible amino acid levels on variables nitrogen intake, fecal nitrogen, absorbed nitrogen, or the percentage of absorbed nitrogen. Moreira et al. (2004), Zangeronimo et al. (2009) and Fraga et al. (2008), in contrast, observed linear effect of lysine level on nitrogen intake.

Quadratic effect (P<0.05) was observed on the variables nitrogen excreted in urine, nitrogen retention, percentage of nitrogen retained and percentage of retained/absorbed nitrogen, with greater retention and absorption of nitrogen until the level of 1.00% of digestible lysine; similar to the results found by Bolduan et al. (1992) and Zangeronimo et al. (2009), in the initial phase, and Oliveira (2006a) in the growth phase of pigs. According to Coma et al. (1995), the highest rate of nitrogen retention represents greater protein deposition, and the highest lysine level results in efficient weight gain, i.e., lean meat.

There were no effects (P>0.05) of the level of amino acid on gross energy intake, crude energy excreted in faeces, metabolizable energy or energy balance. This response was expected, since the concentration of metabolizable energy in diets was similar. There was a decreasing linear effect (P<0.05) of the level of lysine on gross energy excreted in urine and in digestible energy, which was probably due to the inclusion of industrial amino acids, which had its inclusion increased as the lysine content in the diets increased. The higher bioavailability of these amino acids would have favored the use of energy, since it was not in excess. In this case, the surplus of amino acids would be removed as described Chiaradia (2008).

The average level of digestible lysine corrected for digestibility assay recommended is 1.04±0.20 % based on formulated diets or 8.80 g digestible lysine per kg of diet in the diets analyzed.

There were no effects (P>0.05) of the lysine level on the production of leukocytes, lymphocytes, eosinophils and monocytes (Tables 8 and 9). However, quadratic effect (P<0.05) was observed on neutrophils and on the neutrophil × lymphocyte ratio.

The results demonstrate that there was higher activation of the innate immune system, due to higher proportion of neutrophils (P<0.05) in pigs subjected to the level of 0.99% of digestible lysine. A similar observation ocurred in the neutrophil × lymphocyte ratio, reflecting greater activation of the first group of cells compared with the second, indicating as optimal level 0.99% of digestible lysine in formulated diets or 0.842% digestible lysine in the analyzed diets.

The level of 0.99% of digestible lysine is below that defined as best to the variables performance, digestibility and apparent metabolism, suggesting no or low interference

Table 7 - Effect of digestible lysine level on digestibility and apparent metabolism in barrows and gilts from 38 to 42 kg

| Variable[1] | Regression equation | Digestible lysine estimated (%) | P value | $R^2$ |
|---|---|---|---|---|
| Crude protein excreted in urine (g) | $Y = 1841.200 – 3044.929X + 1435.714X^2$ | 1.06 | 0.03 | 0.94 |
| Nitrogen excreted in urine (g) | $Y = 58.680 – 96.979X + 45.714X^2$ | 1.06 | 0.03 | 0.94 |
| Retained nitrogen (g) | $Y = -58.260 + 148.146X – 73.036X^2$ | 1.01 | 0.01 | 0.66 |
| Retained nitrogen (%) | $Y = -173.848 + 455.176X – 220.643X^2$ | 1.03 | 0.01 | 0.83 |
| Retained/absorbed nitrogen (%) | $Y = -191.402 + 504.223X – 243.268X^2$ | 1.04 | 0.02 | 0.86 |
| Gross energy excreted in urine (kcal/g) | $Y = 155.865 – 44.150X$ | | 0.03 | 0.68 |
| Digestible energy (kcal/g) | $Y = 4144.400 – 170.750X$ | | 0.04 | 0.77 |

[1] Results expressed on a dry matter basis.

Table 8 - Leukogram of barrows and gilts (in absolute values per micro liter) at the end of the experiment (95 days of age) according to the nutritional levels of digestible lysine (%)

| Variables | Digestible lysine (%) | | | | | Probability | |
|---|---|---|---|---|---|---|---|
| | 0.80 | 0.90 | 1.00 | 1.10 | 1.20 | Linear | Quadratic |
| Leukocytes | 19488± 4795 | 18600±2763 | 19325±3449 | 17813±3255 | 19125±2081 | 0.692 | 0.632 |
| Neutrophils | 4182±1245 | 4583±2400 | 5624±2427 | 4734±2019 | 3819±783 | 0.787 | 0.046 |
| Lymphocytes | 13814±3812 | 12653±2364 | 12298±2836 | 11977±1870 | 13663±1400 | 0.738 | 0.104 |
| Eosinophils | 453±540 | 387±322 | 225±308 | 144±203 | 484±295 | 0.646 | 0.063 |
| Monocytes | 926±796 | 903±579 | 1134±434 | 859±519 | 995±714 | 0.891 | 0.821 |
| Neutr/Lynph | 0.31±0.08 | 0.38±0.24 | 0.48±0.23 | 0.40±0.16 | 0.28±0.05 | 0.809 | 0.016 |

Table 9 - Regression equations showing the effect of digestible lysine level in the leukogram of barrows and gilts from 22 to 50 kg during growth

| Variables | Regression equation | Digestible lysine estimated (%) | $R^2$ |
|---|---|---|---|
| Neutrophils | $Y = -26778 + 64610X - 32592X^2$ | 0.99 | 0.80 |
| Neutr/Lynph | $Y = -3.643 + 8.247X - 4.147X^2$ | 0.99 | 0.80 |

of the immune system activating agent. However, according to Williams et al. (1997a,b,c) and Le Floc'h et al. (2004), it is possible to observe negatives effects of higher activation of the immune system on the process of protein synthesis and accumulation in the pigs during growth, because this would represent utilization of nutritional support for the synthesis of immune cells.

## Conclusions

The performance, apparent digestibility coefficient and blood parameters suggested that the digestible lysine recommendation for gilts and barrows (20 - 50 kg) under conditions of segregation from the productive system was 0.88 and 0.91, respectively. These respective results correspond to 16.49 and 17.20 g digestible lysine/day and 2.01 and 1.92 g digestible lysine/Mcal metabolizable energy.

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