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RESEARCH PAPER

# Evaluating four digestible lysine treatments with three-phase feeding per treatment in piglets from 21 to 63 days of age

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**Abstract**

**One hundred and forty four piglets (68 male and 76 female) selected for high lean gain with initial weight of 6.15 ± 0.56 kg were used in a randomized block design to evaluate four digestible lysine (DL) treatments with three-phase feeding per treatment (12.0–11.0–10.0, 13.25–12.25–11.25, 14.5–13.5–12.5, and 15.75–14.75-13.75 g/kg) during the phases 21 to 35, 36 to 49 and 50 to 63 days of age, respectively. From 21 to 35 days, increasing DL levels had no effect on average daily feed intake and on average daily gain. Feed conversion was linearly improved by increasing DL up to 14.36 g/kg remaining on a plateau. Dietary DL had no effect on piglet's performance and carcass composition from 21 to 63 days. Although piglets required 14.36 g/kg DL from 21 to 35 days, a sequence of diets containing 12.0, 11.0, and 10.0 g/kg DL fed from 21 to 35, 36 to 49 and 50 to 63 days of age, respectively, did not compromise performance and carcass composition.**

**Key words: amino acid, carcass, initial phase, performance**

## Introduction

The current lines of pigs have high potential for protein deposition a feature that most influences the nutritional needs. In this sense, the intensive genetic selection for high lean gain in pigs warrants reassessment of the requirement of dietary nutrients, especially lysine, which is generally the first limiting amino acid for protein accretion in growing-finishing pigs (NRC, 1998), otherwise performance may be compromised.

Conventionally, the lysine requirement of pigs is determined by phases taking in account the level that maximizes performance in each phase (NRC, 1998; ROSTAGNO et al., 2011). However, this kind of methodology may lead to requirements, particularly if quadratic models are used to determine the optimum lysine level (MILLET et al., 2010).

Phase-feeding studies with growing-finishing pigs have shown that the lysine requirements of pigs determined in each phase may be higher than when the evaluation takes into account the overall experimental period (MILLET et al., 2010; KIEFER et al., 2011). Thus, the objective of this study was to determine the lysine requirement to maximize performance and carcass composition of piglets using three-phase feeding with four digestible lysine levels over the entire experimental period (21 to 63 days of age).

## Materials and Methods

One hundred and forty four piglets (68 males and 76 females), selected for high lean gain, with initial weight of 6.15 ± 0.56 kg were used, allotted in a randomized block design (heavy/light) with four levels of digestible lysine (DL) treatments, twelve replicates and three piglets per experimental unit, with three-phase feeding (DL contents of 12.0–11.0-10.0, 13.25–12.25– 11.25, 14.5-13.5–12.5, and 15.75–14.75-13.75 g/kg, during the phases 21 to 35, 36 to 49, and 50 to 63 days of age respectively).

Piglets were housed in suspended metal cages with wired mash floor and sides equipped with semiautomatic feeders and nipple drinkers, located in a concrete building with concrete floor and lowered wooden roof. The thermal environment inside the facility was monitored daily (7, 12, and 17 h) using thermometers to measure the maximum and minimum temperatures, and relative humidity. Thermometers were placed in an empty pen in the middle of the building at half height of the body of pigs. These data were converted to the BGHI index to characterize the environment of the pigs, using the equation proposed by Buffington et al. (1981).

Experimental basal diets for each phase (Table 1) were mainly composed of corn, soybean meal, and supplements of minerals, and vitamins to meet the requirements for all nutrients except DL (ROSTAGNO et al., 2005). The other experimental diets of each phase were obtained by supplementing L-lysine, DL-methionine, L-threonine, L-tryptophan, and L-valine in place of starch in the basal diets. The ideal amino acid ratios to lysine were maintained as recommended by ROSTAGNO et al. (2005).

**Table 1:** Ingredient composition of the basal diets

| Item | Phase, days | | |
|---|---|---|---|
| | 21 - 35 | 36 - 49 | 50 - 63 |
| | Digestible lysine, g/kg | | |
| | 12.0 | 11.0 | 10.00 |
| Corn | 442.07 | 522.74 | 591.69 |
| Soybean meal 45% | 140.00 | 190.00 | 351.00 |
| Whey powder | 133.86 | 127.65 | 0.00 |
| Micronized soybean | 147.00 | 69.80 | 0.00 |
| Whey powder lactose free | 60.00 | 25.00 | 0.00 |
| Blood plasma AP920 | 35.00 | 20.00 | 0.00 |
| Soybean oil | 0.00 | 0.00 | 16.50 |
| Dicalcium phosphate | 8.23 | 15.40 | 14.75 |
| Limestone | 8.19 | 4.67 | 6.50 |
| Salt | 2.60 | 2.63 | 5.20 |
| Aminogut | 8.00 | 6.00 | 0.00 |
| L-lysine HCl 78% | 0.00 | 0.00 | 0.00 |
| DL-methionine 99% | 1.26 | 0.64 | 0.00 |
| L-threonine 98.5% | 0.00 | 0.00 | 0.00 |
| L-valine 96.5% | 0.00 | 0.00 | 0.00 |
| L-tryptophan 99% | 0.00 | 0.00 | 0.00 |
| L-isoleucine 99% | 0.00 | 0.00 | 0.00 |
| Vitamin premix[1] | 1.50 | 1.50 | 1.50 |
| Mineral premix[2] | 1.00 | 1.00 | 1.00 |
| Starch | 12.13 | 1.47 | 10.26 |
| Growth promoter[3] | 1.50 | 1.50 | 1.50 |
| Antioxidant | 0.00 | 0.00 | 0.10 |
| Calculated composition | | | |
| ME, kcal/kg | 3,586 | 3,476 | 3,350 |
| Crude protein, g/kg | 219.00 | 212.46 | 208.00 |
| Digestible lysine, g/kg | 12.00 | 11.00 | 10.00 |
| Digestible tryptophan, g/kg | 2.45 | 2.29 | 2.29 |
| Digestible threonine, g/kg | 7.88 | 7.29 | 6.99 |
| Digestible met + cys, g/kg | 6.72 | 6.17 | 5.97 |
| Digestible isoleucina, g/kg | 8.24 | 8.11 | 8.08 |
| Digestible valine, g/kg | 9.61 | 9.20 | 8.81 |
| Lactose, g/kg | 120.0 | 60.00 | 0.00 |
| Calcium, g/kg | 7.50 | 7.50 | 7.50 |
| Available phosphorus, g/kg | 4.00 | 4.00 | 4.00 |
| Sodium, g/kg | 2.30 | 2.30 | 2.30 |

[1]Provided per kg/product: vitamin A - 10,000,000 IU; vitamin D3 - 2,000,000 IU; vitamin E - 40,000 IU; vitamin K3 - 2,000 mg; vitamin B12 - 20,000 mg; vitamin B2 - 6,000 mg; biotin - 200 mg; niacin - 30 g; pantothenic acid - 16 g; antioxidant - 100 mg; vitamin B1 – 1,500 mg; vitamin B6 - 2,000 mg; folic acid - 600 mg; and vehicle q.s.p. (1,000 g). [2] Provided per kg/ product: iron - 100,000 mg; copper - 10,000 mg; manganese - 40,000 mg; zinc - 100,000 mg; cobalt - 1,000 mg; iodine - 1,500 mg; calcium - 160.68 g; and vehicle q.s.p. - 1,000 g). [3]Colistin sulphate.

Piglets had free access to their respective diet and water throughout the experimental period. Daily feed waste was manually collected and weighed. Piglets were weighed at the beginning and at the end of the experimental period to calculate average daily feed intake (ADFI), average daily gain (ADG), feed conversion (F:G), and daily DL intake (DLI).

At the end of the experiment (63 days), pigs were fasted for 24 h and 1 pig per experimental unit with body weight closest to the mean weight of the cage were selected and were electrically stunned followed by exsanguination. An additional group of six piglets (three males and three females) weighing 6.15 ± 0.35 kg were slaughtered following the same procedure used in the slaughter of pigs in the experiment to determine the initial carcasses composition in fat and protein.

Whole, eviscerated and blood free carcasses were divided longitudinally and the right side of each carcass (including head and feet) was crushed for 20 minutes in a commercial 30-hp, 1775-rpm cutter. After homogenization of the crushed material samples were taken from carcasses and stored at -5°C to determine protein and fat in the carcasses according to the techniques described by DONZELE et al. (1992b).

Analyses of the carcasses were performed according to the techniques described by SILVA (2002). The deposition of fat and protein in the carcass was evaluated by comparative criteria using the carcasses of contemporaries piglets slaughtered at 6.15 ± 0.35 kg and those slaughtered at the end of the experiment - one animal from each experimental unit with body weight closest to the average weight of its respective unit.

Data of performance (ADFI, ADG, and F:G) and carcass composition (fat and protein deposition) were analyzed using the Statistical Analysis System and Genetics (SAEG) software version 8.0, developed at Universidade Federal de Viçosa (UFV, 2000) , using the ANOVA procedures. To determine DL requirements from 21 to 35 days of age, regression analysis using linear, quadratic, or Linear Response Plateau models were performed. Any differences between the means were compared using the Tukey test. For all statistical procedures, differences were considered significant if P<0.05.

## Results and Discussion

The range of thermoneutral zone suggested by COFFEY et al. (2000) for pigs in the initial phase of growth is between 18 and 28°C. ALEBRANTE et al. (2011) in a study with 15 to 30 pigs reported that a BGHI value of 76.3 provides a thermoneutral environment to pigs. From 21 to 63 days of age the average maximum and minimum temperatures observed (28.5 ± 0.93 and 26.3 ± 0.89°C) and the BGHI value of 76.9 calculated during the period indicate that pigs were not exposed to thermal stress. The digestible lysine levels (DL) had no influence (P>0.05) on average daily feed intake (ADFI) of piglets (Table 2).

**Table 2:** Performance and carcass composition of piglets from 21 to 35 and days of age

| Item | Digestible lysine, g/kg (21 to 35 days of age) | | | | P value | CV, % |
|---|---|---|---|---|---|---|
| | 12.00 | 13.25 | 14.50 | 15.75 | | |
| Initial weight, kg | 6.15 | 6.10 | 6.16 | 6.19 | 1.000 | 4.24 |
| Final weight, kg | 9.66 | 9.14 | 9.72 | 9.54 | 0.160 | 6.63 |
| Daily feed intake, g | 321 | 270 | 304 | 290 | 0.111 | 12.69 |
| Daily DL intake, g[1] | 3.76 | 3.50 | 4.31 | 4.48 | 0.001 | 12.79 |
| Daily weight gain, g | 250 | 217 | 254 | 239 | 1.000 | 15.19 |
| Feed conversion[1] | 1.29 | 1.26 | 1.20 | 1.22 | 0.027 | 7.03 |
| Item | Digestible lysine, g/kg (21 to 63 days of age) | | | | P value | CV, % |
| | 12-11-10 | 13.25- 12.25-11.25 | 14.5-13.5-12.5 | 15.75-14.75-13.75 | | |
| Initial weight, kg | 6.15 | 6.10 | 6.16 | 6.19 | 1.000 | 4.24 |
| Final weight, kg | 28.26 | 28.05 | 28.74 | 27.81 | 1.000 | 4.08 |
| Daily feed intake, g | 838 | 808 | 837 | 809 | 0.247 | 5.59 |
| Daily weight gain, g | 526 | 522 | 538 | 515 | 0.358 | 5.74 |
| Feed conversion | 1.59 | 1.55 | 1.56 | 1.57 | 1.000 | 4.53 |
| Carcass composition | | | | | | |
| Protein | 58.1 | 59.2 | 61.0 | 59.7 | 1.000 | 7.03 |
| Fat | 49.1 | 46.3 | 41.8 | 39.6 | 0.075 | 17.9 |

Linear (P<0,05)

Similarly, CALDARA et al. (2003) evaluating the performance of post-weaned piglets from 6 to 11 kg reported the dietary lysine concentration did not affect piglets voluntary feed intake. Similar pattern of response of voluntary feed intake in respect to the dietary level of lysine was also observed by NEMECHEK et al. (2010). However, DEAN et al. (2007) observed variation on ADFI of weaned piglets by increasing the concentration of lysine in the diet.

These divergences of results among studies may be related to differences in the range of growth evaluated. Investigating the influence of dietary lysine on piglets feed intake, ETTLE & ROTH (2009) verified that consumption varied only from the second week after weaning.

The daily digestible lysine intake (DLI) increased ($P<0.05$) linearly by increasing the levels of this amino acid in the diet, according to the equation: $\hat{Y} = 0.7158 + 0.2376X$ ($r^2 = 0.69$). As there was no variation in feed intake of pigs among the treatments, the observed linear increase in the consumption of lysine is directly related to its concentration in the diets.

There was no effect ($P>0.05$) of DL on average daily gain (ADG) of piglets (Table 2). Similar results were obtained by CALDARA et al. (2003), HILL et al. (2007), and NEMECHEK et al. (2010) studying DL digestible lysine in diets for post-weaned piglets. In contrast, DEAN et al. (2007) and NUNES et al. (2008) verified the growth rate of piglets increased linearly by increasing the concentration of digestible lysine in the diet.

Factors such as concentration of metabolizable energy in the diet and piglets health may have contributed to the lack of consistency among studies. Evaluating the effect of lysine:energy ratio on performance of post-weaned piglets, SCHNEIDER et al. (2010) reported that the lysine requirement for greatest ADG varied according to the level of metabolizable energy. With respect to sanitary condition, WILLIAMS et al. (1997) observed that a reduction in the immune challenge followed consequently by a decrease in the immune system activation of 6- to 27-kg pigs, resulted in higher growth rate and lysine requirement.

As ADG of piglets in this study did not change among treatments one can infer the lowest DL level assessed (12.0 g/kg) meets the requirement of piglets with a growth rate of 240 g in the first two weeks after weaning.

The levels of DL influenced ($P<0.05$) the feed conversion (FC), which improved linearly according to the equation: $\hat{Y} = 1.5452 - 0.2192X$ ($r^2 = 0.76$). However, the Linear Response Plateau model was the best fit to the data, estimating at 14.36 g/kg the level of DL from which FC remained at a plateau, according to the equation: $\hat{Y} = 1.7270 - 0.3600X$ ($r^2 = 0.93$).

MARTINEZ & KNABE et al. (1990), DONZELE et al. (1992a), and JUNQUEIRA et al. (1997) reported significant variation in FC of piglets in the initial phase of growth influenced by the concentration of lysine in the diets, which corresponded to 11.6, 11.4, and 12.0 g/kg.

In more recent studies, CALDARA et al. (2003), NUNES et al. (2008), HILL et al. (2007), and DEAN et al. (2007) observed a positive influence of dietary lysine concentration on FC of weaned piglets with greatest results being achieved at 14.2, 14.6, 14.1, 14.0, and 14.3 g/kg digestible lysine. The mean value of the lysine requirement for greatest FC obtained by these authors (14.3 g/kg) was similar to that verified in the present study (14.4 g/kg), while the mean requirement value calculated from the studies of MARTINEZ & KNABE et al. (1990), DONZELE et al. (1992b), and JUNQUEIRA et al. (1997) was 11.7 g/kg.

These differences in the lysine requirements, confirms the report of SUSENBETH (1995) that genetic improvement of pigs for high growth potential and lean gain results in increased needs for essential amino acids, which may explain the increase in the requirement of lysine to maximize FC of piglets observed among the studies in the last two decades.

The response pattern of FC observed in this study shows that although ADG of the piglets had not changed, the composition of the gain may have varied among treatments, with more protein and less fat deposition in the carcass. Studies conducted by MOREIRA et al. (2005) to assess levels of lysine for post-weaned piglets resulted in improvement on FC associated with increased protein deposition. Consistently, CHIBA et al. (1999) reported that the improvements in performance of growing pigs fed high aminacids diets is a result of greater protein accretion and less fat.

The three feeding phase had no influence ($P>0.05$) on ADFI of the piglets, which averaged 823 g/day (Table 2). Similarly, HILL et al. (2007) evaluating nutritional plans based on lysine levels (12.15, 11.25, 10.35 g/kg and 13.95, 13.05, 12.15 g/kg) for pigs from 21 to 56 days of age, also reported no effect on ADFI. Corroborating these results, URYNEK & BURACZEWSKA (2003) in a study with pigs from 10 to 25 kg, respectively, verified no differences on ADFI by increasing the level of dietary lysine.

These results support the proposition of EDMONS AND BAKER (1987), that pigs are able to tolerate a considerable excess of amino acids in the diet, particularly lysine, without significant changes in feed intake.

Another factor that may have contributed to the lack of variation on ADFI was the use of the ideal protein concept in the diets formulation. According to KILL (2002), pigs fed diets formulated maintaining the ratios between the essential amino acids with lysine are less susceptible to variations in feed intake.

There was no effect ($P>0.05$) of the three feeding-phase on average daily gain (ADG) of piglets (Table 2), which was 525 g/day on average. In a similar way NEMECHEK et al. (2010), investigating sequences of lysine levels fed piglets from 21 to 56 days of age in subsequent periods, reported no significant difference on ADG.

From these results one can infer the use of diets with suboptimal levels of lysine for growing pigs during a certain period can influence the response in subsequent periods. There is evidence that piglets can partially compensate the feeding of diets deficient in some nutrients in early growth and subsequently compensate with adequate diets at the end of the growth phase (NEMECHEK et al., 2010) or in the finishing phase (MAIN et al., 2008).

However, HILL et al. (2007) working with post-weaned piglets fed diets with different sequences of lysine (13.5, 12.5, 11.5 g/kg and 15.5, 14.5, 13.5 g/kg) observed improvement in ADG piglets (465g x 441g) by increasing the levels of lysine. Likewise, NAM et al. (1994), WILLIAMS et al. (1997), and KENDALL et al. (2007) also observed changes on ADG of piglets, respectively, from 9 to 26, 6 to 27, and 11 to 27 kg, that increased by increasing the levels of lysine in the diet.

Besides the genetic potential of piglets for lean gain (Stahly et al., 1991), the divergence of results among studies may be related to the thermal environment (Noblet et al., 2001), activation temporary or permanent immune system activation (Williams et al., 1997), and experimental diet composition (MARTINEZ & KNABE, 1990).

In studies with piglets (6 to 27 kg), WILLIAMS et al. (1997) found a decrease in the immune system activation resulted in higher food intake, weight gain, and feed efficiency. As a result, piglets in this study responded positively to higher levels of lysine in the diet compared to those raised in poor sanitary conditions. Concerning to genotype, KENDALL et al. (2002) verified the digestible lysine requirement of pigs with high genetic potential for lean gain were on average 20% higher than the values recommended by the NRC (1998).

The FC was not affected (P>0.05) by the three phase-feeding. However, HILL et al. (2007) and NEMECHEK et al. (2010) reported improvements on FC of piglets that received the nutrition plans with the highest levels of digestible lysine (15.5, 12.5, and 11.5 g/kg).

The positive effect of lysine level on feed efficiency for weight gain of pigs in the early stage of growth was also observed by VAN LUNEN & Cole (1998) and KENDALL et al. (2002).

In this study, the FC was not affected by the treatments, consequently, one can infer the lowest sequence of digestible lysine (12.0, 11.0, and 10.0 g/kg) was sufficient to meet the piglets requirement with a FC averaging 1.57 within the 42 days period. The levels of digestible lysine that resulted in greatest FC in this study, although consistent with the NRC (1998) recommendation which correspond to 13.5 and 11.5 g/kg of total lysine, respectively, for 5 to 10 kg and 10 to 20 kg piglets, are lower than those proposed by ROSTAGNO et al. (2011), corresponding to 14.5, 13.3, and 10.9 g/kg digestible lysine, respectively, for 5.5 to 9, 9.3 to 15, and 15 to 30 kg piglets..

There was no influence (P>0.05) of the three phase-feeding on protein deposition (PD) in the carcass of piglets (Table 2). However, FONTES et al. (2005) and OLIVEIRA et al. (2006), in a study with female and castrated male pigs, respectively, from 15 to 30 kg, observed a linear effect of lysine levels (9.0 to 13.6 g/kg) on the PD.

As in this study the piglets remained in the experiment from 6.15 to 28.2 kg, it can be inferred the difference of results among studies would be evidencing the protein in the feed (lysine) of piglets in the first week post-weaning can influence the pattern of response of piglets to the level of lysine in the subsequent growth period.

The result of PD obtained in our study is consistent with that of FC as deposition of protein in the carcass by adding a high proportion of water usually positively influences the efficiency of feed utilization for weight gain. According to YANG (1999), pigs with high potential for lean gain use the feed more efficiently.

The three phase-feeding had no effect (P>0.05) on fat deposition (FD) of piglets (Table 2). Although no difference was observed, it was found that in absolute values the FD decreased gradually with the increase in the concentrations of lysine, reaching 19.6% (49.1 x 39.6 g/day) reduction.

Considering the phase-feeding with the lowest levels of digestible lysine met the requirements for performance and FD of piglets, and that the level of metabolizable energy of the diets did not vary among treatments, the gradual decrease observed in the FD probably be related to the extra expense of energy involved in the catabolism of amino acids supplied in excess, such as deamination, synthesis and excretion of urea, with the increase in the concentrations of lysine in the diets. According to VAN LUNEN & COLE (1998) lysine concentrations above the optimal level affect performance and nitrogen retention, mainly by reduction of the net energy, as a result of deamination of amino acids and elimination of nitrogen in excess.

Irrespective to superior performance obtained in this study with a high level of digestible lysine (14.36 g/kg) from 21 to 35 days of age, it did not improved the overall piglets' performance and carcass composition (fat and protein deposition) from 21 to 63 days of age. These results indicate the improvements on performance obtained from 21 to 35 days of age by feeding high-amino acid diet were lost in the overall period (21 to 63 days of age). In other words, feeding 12.0 g/kg digestible lysine that is lower than recommended (14.5 g/kg) by ROSTAGNO et al. (2005) had a negative impact on piglets' performance at the end of the first phase (21 to 35 days of age), but not at the end of the whole phase (21 to 63 days of age).

## Conclusions

Although piglets selected for high lean deposition respond to a dietary digestible lysine level of 14.36 g/kg from 21 to 35 days, the phase-feeding containing 12.0, 11.0, and 10.0 g/kg digestible lysine from 21 to 35, 36 to 49 and 50 to 63 days, respectively, meets the lysine requirements for maximum performance and carcass composition. From a practical point of view, reducing amino acid concentration in the diet can reduce production costs and nitrogen excretion whilst maintaining piglets' performance.

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