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Dietary lysine: calorie ratio on performance, blood parameters and apparent nutrient digestibility in pigs from 50 to 70 kg

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Additional keywords

Aminoacids.
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SUMMARY

Three trials occured to estimate the optimal dietary level of lysine (Lys) and its ratio with metabolizable energy (ME) using pigs from 50 to 70 kg BW. The first with 72 barrows (49.75±0.41 kg initial BW) and second with 72 gilts (46.05±0.38 kg intial BW). Dietary levels of Lys were: 7.00, 8.00, 9.00, 10.00 11.00 and 12.00 g/kg, and 14.25 MJ/kg ME. The thirth with 12 barrows and 12 gilts were fed with four diets (7.00, 9.00, 10.00 and 12.00 Lys g/kg) from the performance trial to evaluate apparent nutrient digestibility. In the first trial, there was a linear improve on feed conversion ratio, crude protein intake (g/day) (CPI) and crude protein efficiency (CPE) of the gilts from 0 to 16 days. From 0 to 32 days dietary lysine levels continued to show linear increase in CPI and CPE and a quadratic effect on relative weight gain (RWG) and FCR of the gilts. There was linear increase on CPE for barrows from 0 to 16 days and quadratic effect on CPI from 0 to 32 days. Data from digestibility and metabolism assay did not show interaction between sex as effect of Lys, although N retention (g) was higher in gilts than barrows. Therefore, it was concluded that for gilts and barrows from 50 to 70 kg, the recommended levels were 10.14 and 7.00 g/kg Lys or 0.71 and 0.49 g Lys/MJ ME, respectively.

Lisina dietética: relação calórica no desempenho, parâmetros sanguíneos e digestibilidade aparente de nutrientes em suínos de 50 a 70 kg

Palavras chave adicionais

Aminoácidos.
Atributos de carcaça.
Conversão alimentar.
Nutrição leitões e leitoas.
Leucócitos.

RESUMO

Foram realizados três ensaios para estimar o nível ótimo de lisina (Lis) na dieta e a sua relação com a energia metabolizável (EM) utilizando suinos de 50 a 70 kg de peso vivo. O primeiro com 72 leitões (49,75 ± 0,41 kg de peso inicial) e o segundo com 72 leitoas (46,05 ± 0,38 kg de peso inicial). Os níveis dietéticos de Lis foram: 7,00; 8,00; 9,00; 10,00; 11,00 e 12,00 g / kg, e 14,25 MJ / kg de EM. O terceiro com 12 leitões e 12 leitoas alimentados com (7,00, 9,00, 10,00 e 12,00 Lis g / kg) para avaliar a digestibilidade aparente dos nutrientes. No primeiro ensaio, houve um aumento linear para a conversão alimentar, consumo de proteína bruta (g / dia) (CPI) e eficiência de proteína bruta (CPE) das leitoas de 0 a 16 dias. De 0 a 32 dias, os níveis de lisina na dieta continuaram a apresentar aumento linear de CPI e CPE e um efeito quadrático sobre o ganho de peso relativo (GPR) das marrãs. Houve aumento linear no CPE para leitões de 0 a 16 dias e efeito quadrático no CPI de 0 a 32 dias. Os dados da digestibilidade não mostraram interação entre o sexo e os níveis de Lis, embora a retenção de nitrogênio (g) tenha sido maior nas leitoas do que nos leitões. Assim, concluiu-se que para as leitões e leitões de 50 a 70 kg, os níveis recomendados foram 10,14 e 7,00 g / kg de Lis ou 0,71 e 0,49 g de Lis / MJ EM, respectivamente.

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INTRODUCTION

According to new concepts about human health and increase on protein demand there was significant improvement in carcass quality of pigs to supply market which seeks a pork lean meat. Thus, it should be considered some conditions that impose changes in nutritional requirements of pigs, such as genetic potential for meat production, gender and age for maximal performance.

Amino acid levels in most of diets are recommended from the data reviewed (National Research Council, 1998) and (Rostagno et al. 2011, p.252), whose most of results are based on diets with high levels of crude protein (CP). Thus the lysine requirement may be overestimated. However, lysine is a limiting amino acid in pig diets based on corn and soybean meal and its use to afford the maximum growth should be considered under all conditions that affect the genetic potential for meat deposition (Zangeronimo et al. 2009, p. 1507 and Fix et al. 2010, p.108).

The body protein accretion reflects the efficiency of dietary nutrients utilization on protein anabolism process reflecting on pig growth as well as matches the diet energetic contribution. The energy intake should meet the real demands for maintenance and body mass deposition and the respective requirements change according to protein level (amino acids) of the diet (Urynek & Buraczewska, 2003, p.1227 and Trindade Neto et al. 2008, p.103).

Adipocytes and skeletal muscle cells involved on immune system as flag antigens, through Toll-like receptors under an integrated manner, regulate the growth of mammals (Gabler & Spurlock, 2008, p.64). In midst of this complex growth system and immunological integrity the health and stress challenges may change the immune response (reflecting on WBC difference), therefore affecting the pig nutritional requirements (Williams et al. 1997a, p.2472 and Salak-Johnson & McGlone, 2007, p.81).

Table I. Calculated composition of the experimental diets for pigs from 50 to 70 kg of live weight (Composição das dietas experimentais para suinos de 50 a 70 kg de peso vivo).

Ingredients (g/kg)	Lys					
	7.00	8.00	9.00	10.00	11.00	12.00
Maize	765.00	759.90	747.70	748.30	746.00	747.20
Soybean Meal	200.10	204.20	215.00	212.50	213.00	209.90
Salt	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	6.40	6.40	6.40	6.40	6.40	6.40
Dicalcium Phosphate	10.65	10.62	10.51	10.54	10.54	10.58
Soy Oil	9.43	9.15	8.72	7.75	6.88	5.92
Vitamin and mineral premix[1]	4.50	4.50	4.50	4.50	4.50	4.50
L-Lysine HCl (99% purity)	-	1.13	2.04	3.38	4.61	5.91
L-Threonine (98.5% purity)	-	0.12	0.64	1.33	1.98	2.66
DL-Methionine (99% purity)	-	-	0.52	1.16	1.78	2.39
L-Tryptophan (98% purity)	-	-	-	0.19	0.37	0.56
Total (g)	1000	1000	1000	1000	1000	1000
Nutritional value						
ME	14.25	14.25	14.25	14.25	14.25	14.25
Lys:ME	0.49	0.56	0.63	0.70	0.77	0.84
CP	153.00	155.60	161.30	163.00	165.60	167.00
Calcium (g/kg)	5.70	5.70	5.70	5.70	5.70	5.70
Available Phosphorus	2.90	2.90	2.90	2.90	2.90	2.90
Sodium (g/kg)	1.83	1.83	1.83	1.83	1.83	1.83
Digestible Methionine+Cystine (g/kg)	4.77	4.80	5.40	6.00	6.60	7.17
Digestible Threonine (g/kg)	5.03	5.20	5.85	6.50	7.15	7.70
Digestible Tryptophan (g/kg)	1.54	1.56	1.65	1.80	1.98	2.15
Digestible Valine (g/kg)	6.47	6.54	6.70	6.66	6.66	6.60
Digestible Leucine (g/kg)	14.86	14.96	15.23	15.15	15.14	15.04
Digestible Isoleucine (g/kg)	6.41	6.49	6.68	6.63	6.64	6.57
Digestible Histidine (g/kg)	4.28	4.32	4.43	4.44	4.40	4.36
Digestible Phenilalanine (g/kg)	7.69	7.76	7.97	7.91	7.92	7.85

[1] Composition per diet kg: 112.50 mg Fe; 22.50 mg Cu; 149.99 mg Mn; 300.00 mg Zn; 0.30 mg Co; 2.55 mg I; 0.76 mg Se; 12.15 UI Vit. A; 3.78 UI Vit. D_3; 18.90 mg Vit. E; 4.32 mg Vit. K; 2.16 mg Thiamine B_1; 9.72 mg Riboflavin B_2; 4.05 mg Pyridoxine B_6; 64.80 µg Vit. B_{12}; 43.20 mg Niacin B_3; 21.6 mg Pantothenic Acid; 1.89 mg de Folic Acid; 0.20 mg Biotin.

Thus, the objective of this study was to evaluate the possible effects of digestible lysine level and its ratio with metabolizable energy on pig performance, apparent nutrient digestibility, carcass characteristics and white blood cell counting.

MATERIAL AND METHODS

The experimental protocol was reviewed and approved by the Animal Care and Use Committee (CEUA), Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu. Performance and digestibility trials were carried out in Animal Science Institute of SAA, Sao Paulo, Brazil.

Animals, design and diets

A total of 144 crossbred (P76 x Näima P76 - Pen Ar Lan) were used in two trials as follow: 72 gilts (46.05±0.38 kg of BW) and 72 barrows (49.75±0.41 kg of BW), both with an average of 91 d. Pigs were blocked based on BW and then assigned randomly to fed five dietary Lys levels as follow: 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 g/kg. Each dietary treatment was assigned to six replicate pens and pigs had unlimited access to feed and water. The experimental unit was two pigs by pen.

Pigs were housed in solid concrete floors (2x1m) separated by steel grates equipped and the experimental facility had windows on the sides to control ventilation. Daily environmental temperatures (maximum and minimum) were recorded 8am and 3pm.

Experimental diets were based on corn, soybean meal, soybean oil, minerals, vitamins and industrial amino acids **(Table I)**. Total Lys levels analysed in corn and soybean meal were used to estimate digestible amino acid considering 88% as average rate of standardized digestibility whose index was based on weighted mean of lysine digestibility coefficients proposed by Rostagno et al. (2005), according to dietary inclusion levels of corn and soybean meal.

During first 10 days of initial experimental period the diets were supplied with Tiamulin (225g/ton) and Mebendazole (450g/ton).

Performance and ultrasound evaluation

Pigs were weighed at 16th and 32nd days of evaluation to calculate body weight (BW), average daily gain (ADG), relative daily gain (RDG), feed intake (FI), feed conversion ratio (FCR), crude protein intake (CPI) and crude protein efficiency (CPE) according to Gandra et al. (2012, p. 2039). All pigs were subjected to backfat thickness (BT) and loin area (LA) measurements by ultrasound on the last day of the experiment, around 70 kg of BW and 123 d age. Backfat thickness was measured 4 to 5 cm from midline on the right side at the 10th rib using a real-time ultrasonic Vetko Plus Brand.

Blood parameters

On day 32nd day were sampled 5 mL blood from 4 gilts and 4 barrows by treatment via jugular venipuncture through heparinized syringes and placed on ice immediately after collection. Into the 20 min blood samples were centrifugated at 2,500 × g for 15 minutes. Plasma aliquots were refrigerated and sent to laboratory for white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS), monocytes (MON) count. Lymphocytes and neutrophils ratio (NEU/LYM) was calculated to clarify if WBC increase was due to acute immune reaction (higher NEU) or chronic (higher LYM).

Digestibility assay

A balance trial was conducted to evaluate the N retention, apparent digestibility and metabolizable energy of the diets used on performance evaluation. Environmental temperatures in the digestibility room were controlled by windows ventilation or "Split System" air conditioners.

A total of 24 crossbred pigs similar in ancestry with an initial BW of 49.075 ± 4.34 kg were randomly assigned as follow: 12 gilts and 12 barrows, individually housed in adjustable metabolism crates (1.4 x 0.6 m) to fed 4 diets used in performance evaluation, according to 4 x 2 factorial with the factors being Lys levels and sex, respectively. Diets were formulated to four Lys levels (7.00, 9.00, 10.00 and 12.00 g/kg) defined as two higher and intermediate levels used in pig performance trials. The experimental period lasted 10 d and first 5 d were for diet and cage adaptation, when also were fed with antibiotic and anthelmintic, as supplied in performance trials, and subsequent 5 d were for collection of feces and urine to determine N, DE and ME.

For collection phase pigs were fed their respective diets based on $BW^{0.75}$ d^{-1}. Pigs were fed twice daily at 8a.m. and 3p.m. and had free access to water. Feces and urine collections were performed according to Barbosa et al. (1999). Gilts urine collection occurred without probe use.

Nitrogen balance was determined by measuring nitrogen intake and nitrogen excretion in feces and urine. Variables evaluated were digestible dry matter (g/kg) (DDM), digestible nitrogen (DN) (g/kg), absorbed nitrogen (g/kg) (AN), retained nitrogen (g/kg) (RN), digestible energy (MJ/kg) (DE) and metabolizable energy (MJ/kg) (ME).

Statistical Analyses

Performance, carcass ultrasound measurements and WBC variables were submitted to regression analysis through orthogonal polynomial considering the digestible lysine levels, using PROC MIXED of SAS

Table II. Gilts performance according to Lys nutritional levels (g/kg) (Desempenho de leitoas de acordo com os níveis de lisina (g/kg))

Variable	Lysine (g/kg)						SME	P value	
	7.00	8.00	9.00	10.00	11.00	12.00		L	Q
Initial weight (kg)	46.07	46.16	46.37	45.36	45.93	46.39	0.45	-	-
BW (kg)									
At 16 days	57.62	59.32	59.50	57.50	58.67	60.34	0.67	0.236	0.665
At 32 days	70.95	73.53	74.02	71.59	73.26	73.55	0.48	0.320	0.525
BWG (g/day)									
0 to 16 days	770	878	875	809	849	930	20.89	0.078	0.930
0 to 32 days	810	887	895	875	879	874	12.95	0.233	0.102
RWG (%)									
0 to 16 days	24.97	28.44	28.25	26.88	27.72	30.02	0.58	0.073	0.893
0 to 32 days	52.25	57.39	57.51	58.15	57.52	56.52	0.71	0.168	0.049
FI (g/day)									
0 to 16 days	2243	2197	2215	2042	1994	2202	35.94	0.150	0.180
0 to 32 days	2259	2227	2311	2126	2071	2255	34.66	0.345	0.499
FCR									
0 to 16 days	2.97	2.54	2.55	2.52	2.36	2.39	0.06	0.003	0.170
0 to 32 days	2.82	2.52	2.58	2.43	2.36	2.57	0.04	0.073	0.043
CPI (g/day)									
0 to 16 days	355	342	350	320	325	300	7.39	0.002	0.327
0 to 32 days	338	370	376	340	316	331	7.33	0.019	0.747
CPE									
0 to 16 days	2.28	2.41	2.41	2.70	2.80	2.87	0.06	0.002	0.322
0 to 32 days	2.39	2.41	2.38	2.66	2.78	2.84	0.06	0.008	0.062

(Version 9.1.3, SAS Institute, Cary, NC 2004), according to the model: $Y_{ij}= \mu + A_i + B_j + e_{ij}$,, where:

Y_{ij} : constant associated to all observations;

μ: overall average of the variable;

A_i: effect of lysine level i, being i = 1, 2, ... and 6;

B_j: effect of block j, being j = 1, 2, ... and 6;

e_{ij}: random error associated to each observation.

Variables from N balance, DE and ME were submitted to regression analysis through orthogonal polynomial considering the digestible lysine levels, using PROC MIXED of SAS (Version 9.1.3, SAS Institute, Cary, NC 2004), according to the model: $Y_{ij} = \mu + A_i + Sj + Ai(Sj) + e_{ij}$, where:

Y_{ij}: constant associated to all observations;

μ: overall average of the variable;

A_i: effect of lysine level i, being i = 1, 2, 3 and 4;

Sj: effect of sex j, being j = 1 and 2;

Ai(Sj): effect of interaction;

e_{ij}: random error associated to each observation.

Considered effects for treatment was $P < 0.05$ and Lys level estimated as optimal was obtained from quadratic equation derived.

RESULTS

Performance and ultrasound evaluation (gilts)

Mean values of maximum and minimum temperature were 25.17±2.08ºC and 20.08±2.97ºC in the morning and 25.58±1.93ºC and 21.42±1.62ºC in the afternoon.

Table III. Backfat thickness (BT) and loin eye area (LEA) measured through ultrassound in gilts with 72.82 kg average body weight (Espessura de gordura e area de olho de lombo mensurados através de ultrassonografia em leitoas com 72,82 kg de peso vivo).

Variable	Lys (g/kg)						SEM	P value	
	7.00	8.00	9.00	10.00	11.00	12.00		Linear	Quadratic
BT (mm)	10.90	10.93	10.98	13.11	10.73	12.66	0.27	0.020	0.386
LEA (cm^2)	50.32	52.87	53.53	55.99	51.61	52.97	0.60	0.593	0.030

The performance of gilts is shown in **Table II.** There was no effect of Lys levels on BW, ADG and FI for any studied periods and on RDG from 0 to 16 days of experiment. According to evaluated periods (P<0.05) from d 0 to 16, FCR was increase linearly (Y=3.495-0.098X. R^2=0.70). CPI decreased from d 0 to 16 (Y=417.590-9.561X, R^2=0.68) and from d 0 to 32 (Y=414.820-8.332X, R^2=0.67). In CPE was linear (Y=1.350+0.135X, R^2=0.57) from d 0 to 16 and from d 0 to 32 d (Y=1.784 +0.088X, R^2=0.70).

Quadratic improvements (P<0.05) from d 0 to 32 were observed with increasing dietary Lys in RDG (Y=-1.980+12.056X-0.601X^2, R^2=0.56) and FCR (Y=6.200-0.738X+0.036X^2, R^2=0.60). Both equations estimated 10.14 g/kg Lys as optimal for growth of gilts from 46 to 73 kg.

Results from ultrasound measurements in gilts at the 72 kg **(Table III)** showed linear increase (P<0.05) of Lys in BT (Y=8.741+0.296X, R2=0.50) and quadratic response (P<0.05) to LA (Y=8.881+9.208X-0.467X2, R2=0.42) whose estimating was 9.86g/kg Lys as optimal level.

Blood parametrs (gilts)

Results of WBC by regression analysis **(Table IV)** did not show effect (P>0.05) of Lys in WBC, NEU, LYM, EOS, MON and NEU/LYM.

The performance of barrows **(Table V)** presented (P<0.05) linear increase in CPE (Y=1.830+0.079X, R^2=0.46) from d 0 to 16 and quadratic response (Y=97.730+63.327X-3.472X^2, R^2=0.67) from d 0 to 32. The most important results in performance evaluation shown that lower studied level of Lys (7.00g/kg) was sufficient to meet FCR and ADG.

Performance and ultrasound evaluation (barrows)

On BT and LA evaluations there was no effect of dietary Lys: ME ratio in barrows during experiment when pigs reached 75 kg BW **(Table VI)**. However the BT increased linearly in gilts as response to dietary Lys: ME increase as well as there was quadratic effect on LA and estimated level was 9.86 g/kg Lys by MJ ME.

Blood parametrs (barrows)

As dietary Lys increase, WBC results **(Table VII)** was unchanged (P>0.05); however, MON had quadratic (P<0.05) variation (Y=6942.780-1309.630X+66.620X^2, R^2=0.26) and lower number of these cells was observed at least 9.83 g of Lys/kg of feed.

Digestibility assay

The analysed gross energy (GE) of diets **(Table VIII)** containing 7.00, 9.00, 10.00 and 12.00g of Lys/kg of feed were:, 18.39, 18.49, 18.41 and 18.38 MJ/kg, respectively. There was no interaction between sex and Lys level for most of studied variables, however, gilts shown higher RN (P<0.05). There was a linear increase (P<0.05) in AN (Y=13.781+0.445X, R^2=0.18) and RN (Y=-1.956+1.300X, R^2=0.45), and quadratic effect (P<0.05) on DE (Y=4.872+2.186X-0.108X^2, R^2=0.61) and ME (Y=4.396+2.149X-0.105X^2, R^2=0.63), as dietary Lys increased. The estimated level of amino acid for improve energetic metabolism was 10.17g/kg of Lys.

DISCUSSION

The linear improvement (P<0.05) on FCR and CPE observed from d 0 to 16 for gilts and CPI reduction from d 0 to 16 and d 0 to 32 are in agreement with other similar studies (Main et al. 2008, p.2190 and Fix et al. 2010, p.108). The lysine level increase, relative to metabolizable energy, by adding synthetic amino acid DL-methionine and keeping the proportions of other amino acids to lysine for pigs have been reported by Main et al. (2008, p.2190). The present estimate of 10.14 g/kg Lys and 0.71 g/MJ Lys:ME is similar to the value 10.10 g/kg Lys or 0.67 g/MJ Lys:ME suggested by Main et al. (008) for pigs to meet maximum growth and feed efficiency. However, recommended levels by Rostagno et al. (2011, p.252) are lower (9.45 g/kg Lys or 0.77 g/MJ Lys:MJ) indicating that Lys level changes according to dietary energy. Furthermore, Fix et al. (2010, p.108) demonstrated that a modern feeding program for pigs using supplementation with synthetic amino acids results in better performance and carcass independently of genotype.

Observed ratio between BT and LA remained constant, around 0.21 to 9.86 g/kg Lys. De la Lata et al. (2002) also observed linear increase on BT, according to Lys increase but not found effect on LA for barrows. On the other hand, Main et al. (2008, p.2190) verified linear decrease on LA by increasing of total Lys that changed from 7.1 to 12.2 g/kg. As discussed by Schinckel et al. (2002, p.1419), backfat thickness and longissimus muscle obtained by ultrasound method are appropriated measurements, despite not represent similar values those collected directly in carcass after slaughter. Changes on fat and lean meat in carcass are due to nutritional adjustment of diet and genotype (Rosenvold & Andersen, 2003, p.2129) and probably the changes observed in present study are due to genotype and their intrinsic characteristics.

Innate immunity is always present under different degrees and may be strengthened or weakened by several factors such as: injuries, dehydration, nutritional status, genotype and stress (Carroll & Forsberg, 2007, p.105).

Table IV. White blood cell count of gilts (absolut values by microliter±standard deviation) at the end of the trial (123 days of age) according to Lys nutritional levels (g/kg) (Contagem de leucócitos de leitoas (valores absolutos micolitros±desvio padrão) no final do experimento (123 dias de idade) de acordo com os níveis de lisina).

			Variables					
Lys (g/kg)			LEU	NEU	LYM	EOS	MON	NEU/LYM
7.00			14975±3791	2185±1384	11862±3457	509±262	381±303	0.19± 0.11
8.00			24000±8044	4483±1839	17526±6133	1020±907	819±545	0.27±0.24
9.00			18450±1461	3713±668	12893±1553	1184±1103	658±286	0.29±0.07
10.00			17475±2905	3929±724	12191±2361	521±421	695±572	0.33±0.07
11.00			23575±9123	5770±2523	15756±7078	909±417	1001±302	0.38± 0.14
12.00			19625±4697	4161±1595	14434±4388	364±224	540±191	0.30± 0.15
Reference values*			11000 - 22000	3080 - 10340	4300 - 13640	0 – 2400	200 – 2200	-
P Value	Lysine	Linear	0.390	0.088	0.726	0.532	0.414	0.145
		Quadratic	0.493	0.299	0.920	0.156	0.162	0.317

*Jain & Schalm´s (1986)

The authors reported that the immune system under good working protect body against most of pathogenic organisms and respective diseases propitiating physical barriers such as: skin, gastrointestinal mucosa, stomach acid and non-specific cells (WBC) that detect and immediately eliminate these agents.

Lys is directly involved in some defence functions of animal physical integrity as the regulation of nitric oxide synthesis, in antiviral activity (fight against herpes), protein methylation (e.g. trimethyl-lysine in calmodulin), among others, and, indirectly, in the structure of hydroxy-lysine molecule and collagen function (Wu, 2009, p.1).

Therefore, without evidence for any type of disease and no statistical effect in WBC of gilts due to experimental treatments, it was observed that genetic

Table V. Performance of barrows according to Lys nutritional levels (g/kg) (Desempenho de leitões de acordo com os níveis de lisina (g/kg)).

Variable	Lys (g/kg)						SEM	P value	
	7.00	8.00	9.00	10.00	11.00	12.00		L	Q
Initial weight (kg)	50.25	49.65	49.60	49.10	50.12	49.80	0.52	-	-
BW (kg)									
At 16 days	63.00	63.83	63.97	63.27	64.52	64.35	0.52	0.347	0.948
At 32 days	73.97	75.25	75.27	74.90	75.91	75.65	0.47	0.315	0.728
BWG (g/day)									
0 to 16 days	850	945	958	945	960	969	15.64	0.091	0.281
0 to 32 days	931	997	999	1009	1005	1005	12.02	0.148	0.231
RWG (%)									
0 to 16 days	25.31	28.52	29.13	28.86	28.80	29.39	0.49	0.068	0.191
0 to 32 days	56.68	60.80	61.41	62.73	60.79	61.33	0.84	0.133	0.117
FI (g/day)									
0 to 16 days	2411	2268	2500	2474	2307	2469	34.04	0.625	0.930
0 to 32 days	2366	2403	2589	2546	2461	2497	29.10	0.219	0.132
FCR									
0 to 16 days	2.85	2.41	2.61	2.64	2.41	2.57	0.05	0.287	0.371
0 to 32 days	2.54	2.42	2.59	2.52	2.45	2.50	0.03	0.839	0.921
CPI (g/day)									
0 to 16 days	361	377	407	326	352	363	6.36	0.257	0.765
0 to 32 days	354	399	421	335	376	367	6.20	0.352	0.043
CPE									
0 to 16 days	2.34	2.54	2.35	2.89	2.73	2.68	0.05	0.046	0.410
0 to 32 days	2.63	2.51	2.37	3.01	2.67	2.74	0.04	0.139	0.902

Table VI. Backfat thickness (BT) and loin eye area (LEA) measured through ultrassound in barrows with 75.16 kg average body weight (Espessura de gordura e area de olho de lombo mensurados através de ultrassonografia em leitões com 75,16 kg de peso vivo).

Variable	Lys (g/kg)						SEM	P value	
	7.00	8.00	9.00	10.00	11.00	12.00		Linear	Quadratic
BT (mm)	12.81	12.28	14.16	13.96	12.80	13.35	0.34	0.465	0.263
LEA (cm^2)	53.53	52.01	56.20	54.30	54.06	55.91	0.60	0.313	0.961

Table VII. White blood cell count of barrows (absolut values by microliter±standard deviation) at the end of the trial (123 days of age) according to Lys nutritional levels (g/kg) (Contagem de leucócitos de leitõs (valores absolutos micolitros±desvio padrão) no final do experimento (123 dias de idade) de acordo com os níveis de lisina).

		Variables					
Lys (g/kg)		LEU	NEU	LYM	EOS	MON	NEU/LYM
7.00		19875±3320	4474±1207	13947±2602	279±232	1031±331	0.32± 0.03
8.00		8.00	4546±1839	13896±3624	632±461	689±657	0.34±0.15
9.00		10.00	4219±2799	12849±1530	342±181	674±349	0.31±0.21
10.00		15875±2625	3092±1533	11950±1906	365±251	467±116	0.26±0.13
11.00		18475±1951	5198±1164	11995±1411	613±283	516±290	0.44±0.13
12.00		18550±2257	5560±2038	11619±2141	321±090	869±265	0.50±0.26
Reference values*		11000 - 22000	3080 - 10340	4300 - 13640	0 – 2400	200 - 2200	-
P value Lysine	Linear	0.377	0.429	0.077	0.882	0.338	0.118
	Quadratic	0.283	0.203	0.799	0.404	0.042	0.215

*Jain & Schalm´s (1986)

potential for growth was characterized, even with some white blood cell changes, whose values were above or below the reference postulated by Jain & Schalm's, (1986, p.240).

For barrows, the Lys effects were only observed on crude protein efficiency showing linear increase and on daily crude protein intake characterized by quadratic effect and estimation of 10.9g/kg Lys as optimal level. However, according to the other results for variables of higher interest, that showed similarity among treatments, it was recommending 7.00 g Lys/kg for barrows from 46 to 72 kg of BW. Unlike happened with gilts, the estimated Lys level for barrows is below those suggested by Rostagno et al. (2011, p.252) who suggested 8.83 g/kg of Lys or 0.65 g Lys/MJ ME for pigs from 50-70 kg BW.

Fix et al. (2010, p.108) compared two pig genotypes and two feeding programs (from 80's to current decade) and did not observe differences in backfat thickness using same feed formulation. However, there was an increase in rib eye area regardless the genetics. In contrast, Main et al. (2008, p.2190) observed a linear reduction in backfat thickness and quadratic effect on loin eye area, when increased from 7.9 to 14.0 g/kg of dietary Lys and obtained 7.95 g/kg of Lys or 0.53 g/MJ of ME, as optimal for barrows. Monocytes are immature macrophages that participate in phagocytosis (Guyton & Hall, 2006, p.1264) and in innate immunity through nonspecific defence mechanisms that serve as the first defence line against infectious organisms and occur quickly, preceding the emergence of antigens in the body (Salak-Johnson & McGlone, 2007, p.81). The monocyte numbers of barrows were lower under the level 9.83 g/kg of Lys, showing that these pigs, probably, behaved better against any aggressive agent present in the experimental facilities. Although some values of WBC have been shown above or below those postulated by Jain & Schalm's (1986, p.240), the pigs did not show any disease clinical signs during the trial period.

Regarding to protein metabolism, differences between genders have been confirmed during digestibility trial, when gilts presented higher efficiency in nitrogen retention. The present study occurred under similar conditions to Trindade Neto et al. (2005, p.1980) who evaluated ME and Lys levels for a specific genotype and also observed that gilts are more efficient in nutrient use to meet performance needs, compared with castrated male pigs. Thus, generally the gilts have higher nutrient requirement than the barrows (Ekstrom, 1991, p.415) and from 30 kg of BW is possible verify effects of sex on pig performance until the end of finish phase.

As observed the maximum DE and ME were obtained at the levels of 10.12 and 10.23 g/kg of Lys, respectively and the mean value 10.17 g/kg of Lys,

Table VIII. Apparent digestibility of barrows and gilts, with average body weight of 53.5 kg, according to Lys nutritional levels (g/kg) (Digestibilidade aparente de leitões e leitoas com média de peso vivo de 53,5 kg de acordo com os níveis de lisina (g/kg)).

			Variables[1]					
Sex	Lys (g/kg)		DDM (g/kg)	DP (g/kg)	AN (g)	RN (g)	DE (MJ/kg)	ME (MJ/kg)
Factors Means								
Sex								
	Males		822.00	779.00	17.24	7.12[a]	15.59	14.98
	Females		814.56	754.92	17.03	8.64[b]	15.45	14.91
Lys (g/kg)								
	7.00		812.50	759.33	16.36	5.67	14.96	14.36
	9.00		806.17	752.83	16.81	7.09	15.32	14.73
	10.00		818.33	773.83	18.41	10.06	16.37	15.80
	12.00		836.33	782.33	16.96	8.70	15.43	14.90
P Value	Sex		0.457	0.104	0.617	0.030	0.485	0.746
	Lysine	L	0.075	0.172	0.020	0.002	0.012	0.005
		Q	0.234	0.587	0.752	0.857	0.004	0.003
	Interaction		0.650	0.767	0.275	0.174	0.699	0.584
SEM			4.57	6.97	0.24	0.48	0.13	0.14

[1]Results expressed based on dry matter, obtained accoring to analysis performed at Bromatology Laboratory of Animal Nutrition and Production Department /FMVZ/USP.

while N absorbed and retained increased linearly in response to Lys levels. Thus, it was confirmed that protein deposition in growing pigs is limited by energy intake (Bikker et al., 1994, p.1744) and there is an equilibrium or adjustment among ingestion, absorption and retention (Resende et al., 2006, p.1101).

CONCLUSIONS

Suggested Lys levels for gilts and barrows from 50 to 70 kg live weight are: 10.14 and 7.00 g/kg Lys or 0.71 and 0.49 g/MJ Lys:ME, respectively, according to experimental conditions this study.

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