Digestible threonine-to-lysine ratio for piglets under health challenge

Relações entre treonina e lisina digestíveis para leitões mantidos sob condições de desafio sanitário

Relaciones entre treonina y lisina digestible para lechones mantenidos en condiciones de riesgo para la salud

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Abstract

Aiming to determine the ration between digestible lysine and digestible threonine (Thr/Lys) for weaned piglets at 27 days, kept in the nursery under sanitary challenge conditions, 105 hybrid piglets with initial weight of 7.2 ± 0.41 kg, were distributed in randomized block design inside of five treatments (Thr/Lys ratios of 0.56; 0.63; 0.70; 0.77 and 0.84), seven replicates and three piglets per experimental unit. The Thr/Lys ratios did not affect (P>0.05) piglet performance. There was quadratic effect (P=0.02) of the Thr/Lys ratio on the protein/fat ratio and the estimated point of the bigger protein/fat ratio occurred with the Thr/Lys ratio of 0.68. The Thr/Lys ratio did not affect (P>0.05) the carcass parameters and serum urea levels, however, the liver and kidney weights raised with the increase (P=0.01) in Thr/Lys rate. The Thr/Lys ratio had a quadratic effect (P=0.01) on rate of plasma haptoglobin, Thr/Lys ratio was estimated at 0.74. It was concluded that the ratio between digestible threonine with digestible lysine for piglets from 27 to 48 days of age raised under sanitary challenge conditions is 0.74.

Keywords: Amino acids; Haptoglobin; Protein deposition; Immune system; Swine.

Resumo

Com o objetivo de determinar a relação entre treonina digestível com lisina digestível (Tre/Lis) para leitões desmamados aos 27 dias, mantidos na creche em condições de desafio sanitário, 105 leitões híbridos com peso inicial de 7,2 ± 0,41 kg foram distribuídos em delineamento com blocos ao acaso com cinco tratamentos (relações Tre/Lis de 0,56; 0,63; 0,70; 0,77 e 0,84), sete repetições e três leitões por unidade experimental. As relações Tre/Lis não afetaram (P>0,05) o desempenho dos leitões. Houve efeito quadrático (P=0,02) da relação Tre/Lis sobre a taxa proteína/gordura e o ponto estimado de maior taxa proteína/gordura se deu com a relação Tre/Lis de 0,68. A relação Tre/Lis não afetou os parâmetros de carcaça e teores de uréia no soro, entretanto, os pesos do fígado e do rim aumentaram linearmente (P=0,01) com o aumento da taxa de Tre/Lis. A relação Tre/Lis teve efeito quadrático sobre a taxa de haptoglobina no plasma, estimou-se a relação Tre/Lis de 0,74. Concluiu-se que a relação entre treonina digestível com lisina digestível para leitões dos 27 dias aos 48 dias de idade criados sob condições de desafio sanitário é de 0,74.

Palavras-chave: Aminoácidos; Haptoglobina; Deposição de proteína; Sistema imune; Suínos.

Resumen

Con el fin de determinar la relación entre treonina digestible y lisina digestible (Tre/Lis) para lechones destetados a los 27 días, mantenidos en vivero en condiciones de desafío sanitario, se utilizaron 105 lechones híbridos con un peso pro medio de 7,2 ± 0,41 kg. Los animales fueron distribuidos en un diseño experimental completamente al azar con cinco tratamientos (razones Tre/Lis de 0,56; 0,63; 0,70; 0,77 y 0,84), siete repeticiones y tres lechones por unidad experimental. Las proporciones Tre/Lis no afectaron (P> 0,05) el rendimiento de los lechones. Hubo un efecto cuadrático (P=0,02) de la relación Tre/Lis en la relación proteína/grasa y el punto estimado de la relación proteína/grasa más alta fue con la relación Tre/Lis de 0,68. La relación Tre/Lis no afectó los parámetros de la canal y los niveles de urea sérica, sin embargo, los pesos del hígado y riñón aumentaron linealmente (P=0,01) con el aumento de la tasa Tre/Lis. La relación Tre/Lis tuvo un efecto cuadrático sobre la tasa de haptoglobina plasmática, se estimó que la relación Tre/Lis era 0,74. Se concluyó que la relación entre treonina digestible y lisina digestible para lechones de 27 a 48 días de edad criados en condiciones de desafío para la salud es de 0,74.

Palabras clave: Aminoácidos; Haptoglobina; Deposición de proteínas; Sistema inmunológico; Cerdos.

1. Introduction

The social rupture with the mother and factors such as environment change, adaptation to new facilities and diet change (liquid to solid) may increase the stress conditions of piglets at weaning (Moeser et al., 2017), leading to marked changes in the physiology, microbiology, and immunology of their gastrointestinal tract (Heo et al., 2013). The interaction of those factors may result in decreased immunity and feed intake, facilitating the manifestation of opportunistic diseases (Turpin et al., 2017).

As a result, the nutritional requirements can be increased in function of the competition between body growth and defense (Le Floc'h et al., 2009). Haptoglobin production can be altered in response to the immune stress caused by the weaning process, this acute-phase protein is synthesized in the liver in response to activation of inflammatory cytokines, such as IL-1 and IL-6 (Wassell, 2000). Thus, it has been used as an indicator of inflammation (Melchior et al., 2004; Le Floc'h et al., 2006 and 2009) and infections (Knura-Deszczk et al., 2002).

The decreased feed intake, the health challenges, the environment, and other nutritional factors, besides weaning age, can also influence the optimum ratio between amino acids (Guzik et al., 2002; Susenbeth 2006; Le Floc’h et al., 2009). It has been reported that, in addition to being present in sufficient quantities for piglets, essential amino acids must be available to them by maintaining an adequate ratio with the available lysine (Van Milgen and Dourmand 2015). Therefore, as the third limiting amino acid in corn- and soybean meal-based diets, threonine should be maintained in the diets at an adequate ratio with digestible lysine.

Threonine participates in intestinal mucus production and, as such, it can increase the immunity of piglets (Ren et al., 2014). In this way, threonine may become the first limiting amino acid in terms of immunity of piglets reared in an environment with a greater health challenge (Munasinghe et al., 2017).

Some research has shown that the ratio between digestible threonine and digestible lysine for piglets is 0.63 (Rostagno et al., 2011), agreeing with that value, Jayaraman et al. (2016) recommended a threonine-to-lysine ratio of 0.65 to optimize the feed efficiency of piglets weaned in health-challenging conditions. However, Pinheiro et al. (2014) concluded that the ratio of 0.55 meets the requirements of piglets from 6 to 16 kg.

Considering the information presented, the study analyzed the hypothesis that piglets submitted to sanitary challenge conditions, may have their amino acids requirement altered, and for this, affecting the threonine/lysine ratio. In this scenario, the study was undertaken to examine the ratios between digestible threonine and digestible lysine for piglets weaned at 27 days of age reared in health-challenging conditions.

2. Methodology

The study was approval by the Ethics Committee in Animal Experimentation (CEUA/UFV) (approval no. 73/2011).

2.1 Experimental Design, Animals and Treatments

The experiment was carried out in the Pig Farming Section at the Department of Animal Science, Center for Agricultural Sciences, Federal University of Viçosa (UFV), which is located in Viçosa - MG, Brazil. The municipality is situated at the following geographic coordinates: 20º 45’ 45” S and 42º 52’ 04” W, at an altitude of 657 m.

A total of 105 male and female hybrid piglets (Landrace×Large White) weaned at 27.0 days of age, with an initial weight of 7.2±0.41 kg, were assigned to five treatments in a randomized-block design with seven replicates per treatment and three pigs per pen. The piglets’ weight was adopted as the criterion for forming the blocks, as recommended by the Sakomura and Rostagno (2016). Experimental treatments were represented by the digestible threonine-to-digestible lysine ratios (Thr/Lys) of 0.56, 0.63, 0.70, 0.77, and 0.84.

Experimental diets were formulated to meet the requirements of piglets in the starter phase according to the recommendations the Rostagno et al. (2011), except for the studied amino acid and lysine (Table 1). A sub-optimum digestible lysine level (1.197%) was adopted. Inert was used in all diets to ensure the variation in the inclusion of amino acids. Piglets had free access to water and to the experimental diets.

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Table 1 - Percentages and calculated component in the experimental diets (on an as-fed basis)

Ingredientes	Digestible threonine-to-lysine ratio				
	0.56	0.63	0.70	0.77	0.84
Corn meal	53.20	53.20	53.20	53.20	53.20
Soybean meal	18.00	18.00	18.00	18.00	18.00
Micronized Soybean	6.160	6.160	6.160	6.160	6.160
Dry whole milk	14.00	14.00	14.00	14.00	14.00
Whey Powder	4.50	4.50	4.50	4.50	4.50
Vegetable oil	0.39	0.39	0.39	0.39	0.39
Dicalcium phosphate	1.23	1.23	1.23	1.23	1.23
Limestone	0.64	0.64	0.64	0.64	0.64
Salt	0.31	0.31	0.31	0.31	0.31
L- Lysine HCl (78%)	0.26	0.26	0.26	0.26	0.26
DL- Methionine (98%)	0.17	0.17	0.17	0.17	0.17
L- Tryptophan (99%)	0.05	0.05	0.05	0.05	0.05
L- Threonine (99%)	-	0.08	0.17	0.25	0.34
Mineral Supplement[1]	0.10	0.10	0.10	0.10	0.10
Vitamin Supplement[2]	0.13	0.13	0.13	0.13	0.13
Antioxidant[3]	0.02	0.02	0.02	0.02	0.02
Inert	0.80	0.71	0.63	0.54	0.46
	Calculated compositions				
ME, MJ/kg	13,92	13,92	13,92	13,92	13,92
Crude protein (%)	18.74	18.74	18.74	18.74	18.74
Digestible Lysine (%)	1.19	1.19	1.19	1.19	1.19
Dig. Methionine+Cystine (%)	0.73	0.73	0.73	0.73	0.73
Digestible Tryptophan (%)	0.26	0.26	0.26	0.26	0.26
Digestible Threonine (%)	0.67	0.75	0.83	0.92	1.00
Digestible Valine (%)	0.85	0.85	0.85	0.85	0.85
Digestible Arginine (%)	1.07	1.07	1.07	1.07	1.07
Digestible Phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Calcium (%)	0.82	0.82	0.82	0.82	0.82
Sodium (%)	0.23	0.23	0.23	0.23	0.23
Lactose (%)	8.19	8.19	8.19	8.19	8.19

[1]Provided per ton of feed: 7,800,000 IU of Vitamin A; 2,150,000 IU of Vitamin D3; 25,760 IU of Vitamin E; 4,000 mg of Vitamin K3; 3,000 mg of thiamine; 7,320 mg of riboflavin; 1,560 mg of pyridoxine; 30.000 mcg of vitamin B12; 19.700 mg of calcium pantothenate; 35,130 mg of niacin; 43 mg of biotin, 765 mg of folic acid and 5,000 mg of BHT. [2]Provided per ton of feed: 1,000 mg of Co; 80 g of Fe; 40 g of Mn; 100 g of Zn; 12 g of Cu; 1,000 mg of I and 0.3 mg of Se. [3]Butylated hydroxytoluene (BHT): 100 g per ton of feed.
Source: Authors

After weaning, the animals were transferred to suspended metal cages with mesh floor and sides inside masonry nurseries with concrete floors, lowered wooden ceiling, and tilting windows on the sides. The environment was characterized as of health challenge, once installations were not cleaned or disinfected (Oliveira Jr. et al., 2016). In addition, the piglets did not receive antibiotics in their diets. However, the animals were vaccinated at day of weaning with a dose of vaccine against *Mycoplasma* and another against *Pasteurella*. According to the manufacturer's instructions, the vaccines contained the following microbial agents: *Mycoplasma hyopneumoniae, Bordetella bronchiseptica, Pasteurella multocida* types A and D, and *Erysipelothrix rhusiopathiae*.

The temperature inside the nurseries was measured daily at 1700 h using maximum-minimum thermometers placed at a height of 1.5 m. Air relative humidity was measured at 0700 h, 1200 h, and 1700 h, based on temperature data collected with a dry- and wet-bulb thermometer placed in the center of the room.

2.2 Performance and Body Composition

Piglet performance and the other traits were evaluated from weaning (at 27 days) until 48 days of age. For this, the animals, supplied feed, and orts were weighed whenever necessary.

Body composition was performed at the beginning and at the end of the experiment. At the beginning eight piglets with an average weight of 8.0 kg were slaughtered. At the end of the experiment, 12 piglets for treatments (the lightest and heaviest of each replicate) were also slaughtered (Oliveira Jr. et al., 2016). Piglets were slaughtered after 24-h fast, following the norms of humane slaughter, after being stunned by an electric shock, as recommended by the Ministry of Agriculture, Livestock and Food Supply (Brasil 2000).

The liver and kidneys were collected from each carcass. After being cleaned, they were hung in the shade for approximately 20 min to drain the water and blood. Once dry, the organs were weighed.

Carcasses were sawn lengthwise and the left half was ground through a 30-HP commercial cutter at a speed of 1,775 rpm. The protein and fat deposition rates were also determined based on the balance between the carcasses at the start and end of the experiment, following the basic protocol described by García-Valverde et al. (2008). The procedures were followed except for pre-drying, which was performed in a forced-air oven at approximately 60 ºC for 72 h, and the pre-defatting of the samples, which were kept in a Soxhlet extractor for 4 h. The fat and crude protein contents in the carcass samples were analyzed following the techniques described by AOAC (1990).

2.3 Blood Analyzes

Blood samples (±10 mL) were harvested by jugular venipuncture in two piglets (the heaviest and the lightest) per experimental unit on the 41st day of age. Blood was collected according to the following protocol: 1 - piglets were feed-deprived from 1800 h of one day until

0700 h of the following day; 2 - at 0700 h, they received feed ad libitum for two hours; and 3 - after another feed deprivation period of four hours, blood was collected.

The harvested blood was centrifuged for 20 min at 7000 rpm to obtain ±1.5 mL of serum. The serum was transferred to appropriate bottles, which were then frozen at –18 ºC.

Serum urea analyses were carried out by the UV enzymatic technique, using commercial kits (K 056, Bioclin®) in accordance with the procedures described by Lee et al. (2019).

The serum haptoglobin concentrations were determined in a nephelometry assay using the N Antisera to Human Transferrin and Haptoglobin (Siemens®) commercial kit. Sensitivity was determined as 8 mg/dL haptoglobin.

2.4 Statistical Analyzes

Thus, the analyses were realized at the SAS software. The Thr/Lys ratios were evaluated based on the performance results. Fat and protein deposition rates, serum urea content, and serum haptoglobin content were evaluated using linear or quadratic models according to the best fit for each one of the parameters. The lowest feed conversion of the Thr/Lys ratio was considered 100% (standard), and the other ratios were analyzed in percentage terms relative to the standard ratio. Variations higher or lower than 5.0% were considered significant.

The quantitative method was adopted in this study (Sakomura and Rostagno, 2016; Pereira et al., 2018).

3. Results and Discussions

The maximum and minimum temperatures observed during the experimental periods were 31.1±1.1ºC and 27.1±1.0ºC, respectively. Air relative humidity averaged 72.6±5.6%.

Results for piglet performance and protein and fat deposition as a function of the treatments are shown in Table 2. No differences were observed between the treatments ($P>0.05$) for performance parameters or protein deposition.

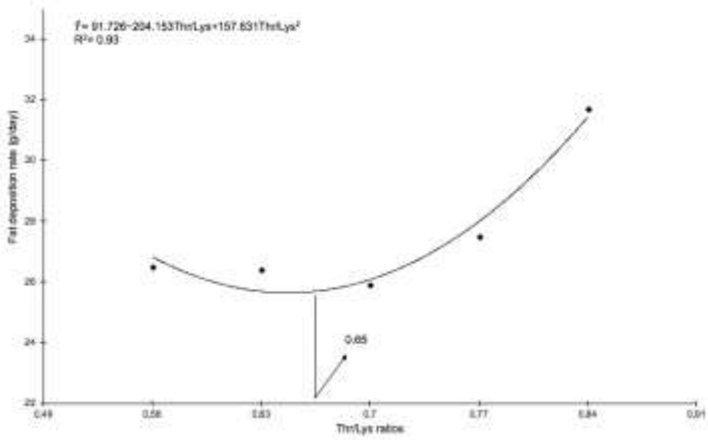
Table 2 – Piglet performance, digestible threonine intake, protein and fat deposition in the carcass as a function of the digestible threonine to lysine ratios[1]

Variables	Digestible threonine-to-lysine ratio					Statistics		
	0.56	0.63	0.70	0.77	0.84	L	Q	CV (%)
Initial BW, kg	7.3	7.4	7.2	7.3	7.3	-	-	-
BW at 48 day, kg	13.6	13.6	13.1	13.9	14.0	-	-	-
ADG, g/d	310.9	310.3	293.6	318.1	332.3	ns	ns	14.3
ADFI, g/d	460.3	476.8	440.3	472.0	472.1	ns	ns	9.4
Threonine intake, g/d	3.1	3.6	3.7	4.4	4.8	0.01	ns	9.3
F:G (g/g)	1.51	1.55	1.51	1.49	1.44	ns	ns	-
Percentage ratio of F:G	100.0	102.6	100.0	98.7	95.4	ns	ns	-
Protein deposition, g/d	21.0	24.2	21.8	23.5	24.8	ns	ns	27.7
Fat deposition, g/d	26.5	26.4	25.9	27.5	31.7	ns	0.04	25.2
P:F ratio	0.79	0.93	0.86	0.85	0.78	ns	0.02	20.8

[1]Data were calculated with 7 replicates/treatment. P-values for the comparisons: L = linear; Q = quadratic. NS = not significant.
Source: Authors.

The treatments led to a linear increase (P<0.01) in digestible threonine intake. The linearity equation for this effect was Y=5.827Thr:Lys – 0.177 (R^2=98.0%). The linearity equation based on the Linear Response Plateau model was Y=5.529Thr:Lys + 0.006.
Fat deposition (P=0.04) and protein-to-fat ratio (P=0.02) responded quadratically to the Thr/Lys ratios. The graphic representations of these effects are shown in Figures 1 and 2.

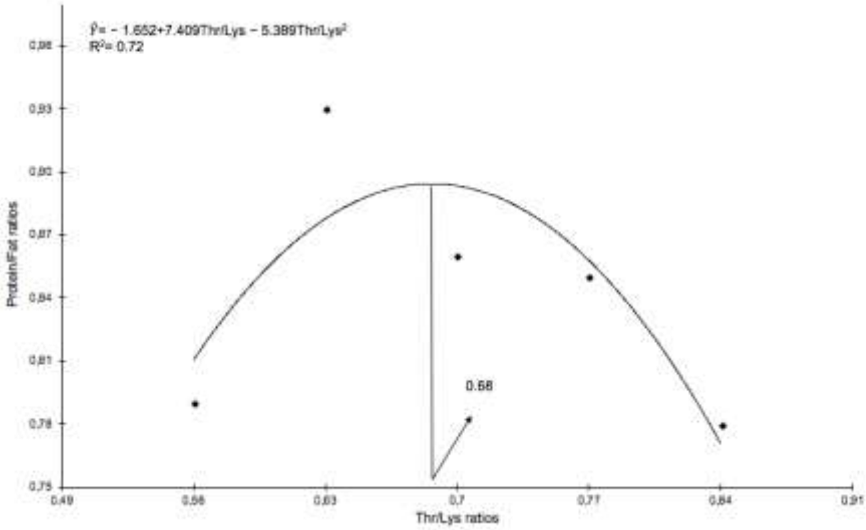
Figure 1 - Representation of the effects of Thr/Lys ratios on fat deposition rates



Source: Authors.

The Thr/Lys ratios estimated by the quadratic model for lowest fat deposition and highest protein-to-fat ratio were 0.65 and 0.68.

Figure 2 - Representation of the effects of Thr/Lys ratios on protein/fat



Source: Authors.

Table 3 shows the results pertaining to carcass parameters, organ weights, and blood parameters of the piglets according to the treatments. Hot carcass weight and carcass yield (both post-fast) were not influenced (P>0.05) by the Thr/Lys ratios.

Table 3 - Weight, carcass yield, absolute and relative weights of organs and blood parameters as functions of the ratio of digestible threonine to lysine[1]

Variables	Digestible threonine-to-lysine ratio					Statistics	
	0.56	0.63	0.70	0.77	0.84	L	Q
Fasting weight, kg	12.4	12.6	12.0	12.7	12.8	ns	ns
Carcass, kg	8.7	8.7	8.6	8.4	9.0	ns	ns
Carcass yield, %	70.2	69.9	70.6	66.6	71.8	ns	ns
			Absolute Weight, g				
Liver	308.4	331.5	320.6	337.7	337.3	0.01	ns
Kidney	62.9	61.4	58.8	66.8	66.5	0.01	ns
			Relative Weight, %				
Liver	3.6	3.8	3.7	4.0	3.8	ns	ns
Kidneys	0.7	0.7	0.7	0.8	0.7	ns	ns
			Blood Parameters, mg/dL				
Haptoglobin	30.3	16.7	12.9	16.2	16.8	ns	0.01
Urea	25.9	25.5	25.1	26.9	25.5	ns	ns

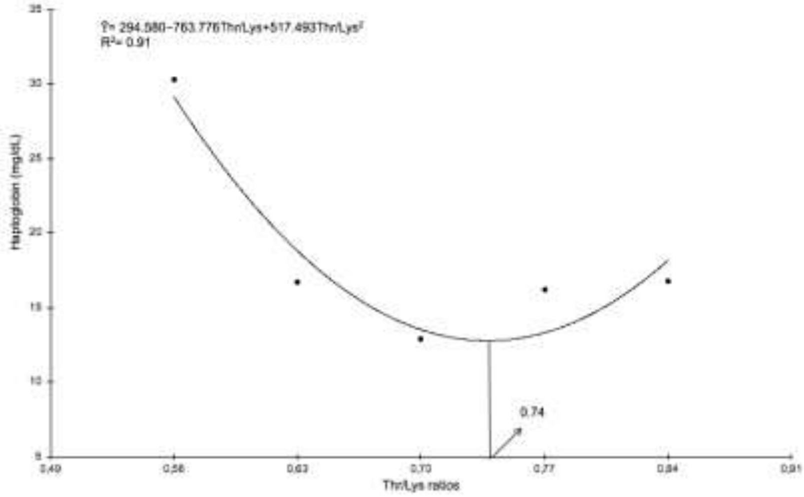
[1]Data were calculated for 12 piglets/treatment (the lightest and the heaviest); 6 replicates/treatment. P-values for the comparisons: L = linear; Q = quadratic. Ns = not significant.
Source: Authors.

There was a linear increase in the absolute weights of liver and kidneys (P=0.01) as the Thr/Lys ratio was elevated. The respective linearity equations for the absolute weights of liver and kidneys were Y=0.263 + 0.091Thr:Lys (R^2=0.82) and Y= 0.051 + 0.018Thr:Lys (R^2=0.88).

Based on the Linear Response Plateau model, the estimated Thr/Lys ratio for the absolute weight of the liver is 0.63.

The increasing Thr/Lys ratios elicited a quadratic response (P=0.01) from haptoglobin content. The graphic representation of these effects can be visualized in Figure 3. The serum urea content was not influenced (P>0.05) by the Thr/Lys ratios.

Figure 3 - Representation of the effects of Thr/Lys ratios on the haptoglobin content



Source: Authors.

Average maximum and minimum temperatures as well as air relative humidity remained within the thermoneutral zone for piglets in a nursery (Liu et al., 2017). Thus, the environmental conditions must not have interfered with the observed variations in the parameters.

The Thr/Lys ratios did not affect piglet performance, but the observed feed intake can be considered low, considering the genetics and weaning age of the piglets used in this study. Berto et al. (2002) investigated piglets weaned at 21 days of age and challenged with vaccines and also did not find effects of threonine on their performance, however observed a 17.0% higher feed intake in the animals weaned at 21 days in comparison with those in the present study, which were weaned at 28 days. The reduction in feed intake led to considerable differences in daily threonine intake. The average threonine intake (4.8 g/day) of the piglets used in the present study was lower than the 5.1 g/day found in challenged piglets by Berto et al. (2002). Results similar of those obtained in the current experiment were reported by Pinheiro et al. (2014) in piglets weaned at 21 days of age that were also vaccinated.

The health challenge that the animals were submitted in the current study may have caused the effects in the Thr/Lys ratios and on feed intake ultimately affecting muscle deposition. In addition, may have altered the requirements of other aromatic amino acids such as tryptophan. Pinheiro et al. (2014) also described the importance of threonine to feed intake and to the immune system. Ning and Qian (2008) also reported that decreasing feed intake affected the production performance and health status of piglets. Therefore, a reduction of feed intake might have been the main cause of the worse performance and decreased deposition of muscle and fat in the carcasses of the animals evaluated in the current study.

It is possible that up to the Thr/Lys ratio of 0.65 there was no excess threonine in the diets, since fat deposition was lower and the protein-to-fat ratio was higher up to that level. Because the piglets from all treatments consumed similar amounts of feed, the amino acid ratios were also similar. Saraiva et al. (2006 and 2007) reported analogous results in an experiment with piglets weighing between 15 and 30 kg in thermoneutral environments with elevated temperatures. Pinheiro et al. (2014) evaluated piglets weaned at 21 days of age and also observed that their fat content rose as the digestible threonine level was increased, but no change was observed in protein deposition.

Alterations in the production of acute-phase protein such as haptoglobin and immune activation may alter nutrient digestibility in the liver and kidneys, and these organs may change in size and weight (Munasinghe et al., 2017). However, although the absolute weights of those organs were different, no such effects were seen in the present study, with relative weight remaining constant. The experimental period was likely not sufficient for these effects to be noticeable.

It is also possible that the mobilization of amino acids absorbed in quantities larger than necessary and/or the quantities mobilized due to deficiencies were not sufficient to have an impact on the organs, since no differences were detected in plasma urea content. Pozza et al. (2000), evaluated the threonine requirements of piglets from 15 to 30 kg and observed that their plasma urea content was influenced by the increasing concentration of threonine in the diets.

The increasing Thr/Lys ratios influenced the haptoglobin content in the piglets up to the estimated ratio of 0.74; demonstrating the threonine requirements, in situations of greater sanitary challenge, can be altered, due to increased of intestinal mucus production and increased production of acute-phase proteins; e.g., haptoglobin. Detection of acute phase proteins can be used to monitor stress, inflammatory and infectious processes in the pig (Lee et al., 2019).

In view of the decreased haptoglobin production, alterations in fat deposition rate and protein-to-fat ratio in the carcass, and low feed intake observed in piglets weaned at 28 days of age and housed in nurseries with health challenges, the adequate Thr/Lys ratio.

4. Conclusion

The ratio between digestible threonine and digestible lysine for piglets from 27 to 48 days of age reared in health-challenging conditions is 0.74.

Therefore, the greater health challenge increases the Thr/Lys ratio, affecting the nutritional requirements of piglets after weaning.

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