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Metabolizable and net energy requirements for entire and immunocastrated male pigs and digestible lysine for immunocastrated males

Joyce Barcellos
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2025

JOYCE BARCELLOS

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Melissa Izabel Hannas

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Assent:

Joyce Barcellos
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Melissa Izabel Hannas
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Dedico este momento aos meus pais, por acreditarem nos meus sonhos, e a Deus por me permitir conquistar muito além do que eu poderia imaginar.

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ABSTRACT

BARCELLOS, Joyce, D.Sc., Universidade Federal de Viçosa, July, 2025. **Metabolizable and net energy requirements for entire and immunocastrated male pigs and digestible lysine for immunocastrated males**. Adviser: Melissa Izabel Hannas.

In the first chapter, the objective was to determine the metabolizable and net energy requirements for maintenance and production of entire males and immunocastrated males from 70 to 130 kg of body weight, using the factorial method. Sixty entire male pigs were randomly assigned to a performance trial with four treatments (ad libitum, 75%, 50%, and 25% of the ad libitum feed), with 12 replicates and one pig per pen. The parameters of the energy model were established using the comparative method for protein and fat deposition through the Dual-Energy X-ray Absorptiometry (DEXA) approach. Energy requirements for weight gain were determined based on body energy content and the efficiency of energy utilization for weight gain. Metabolizable energy (ME) and net energy (NE) requirements for maintenance (MEm and NEm) were 160.33 and 134.52 kcal/kg $BW^{0.75}$ per day, respectively, for entire males. The efficiency of utilization of ME for NE was 0.84, estimated from the slope of the linear regression equation of retained energy as a function of metabolizable energy (ME) intake. The ratio of lean to fat mass decreased during the growth period and was more pronounced in immunocastrated males, indicating that fat retention becomes more energy-efficient as body weight increases. Entire male pigs are less demanding in net energy for gain (NEg) than immunocastrated males. The ME and NE requirements for production (MEp and NEp) were 4.498 and 3.779 kcal per kilogram of body weight for entire males, and 5.298 and 4.451 kcal for immunocastrated males, respectively. The second chapter evaluated digestible lysine (DLys) levels in the diet of immunocastrated male pigs during the finishing phase regarding performance, in vivo body composition, carcass traits, and meat quality, as well as to determine DLys requirements. Sixty immunocastrated male pigs were randomly allotted to five treatments with DLys levels of 0.55, 0.61, 0.68, 0.75, and 0.82%, respectively, in 12 replicates. Overall, performance improved in response to increasing DLys levels. Carcass yield and loin weight increased linearly ($P < 0.05$) with increasing DLys levels, whereas loin area (LA) showed a quadratic increase ($P < 0.05$). Meat quality was affected, showing a linear increase in sarcomere length, drip loss ($P < 0.10$), and color values *a** and *b** ($P < 0.05$), as well as changes in the chemical composition of loin chops, with a quadratic decrease for intramuscular fat and crude protein in dry matter (P

< 0.05). Body composition was affected, with a linear increase in fat mass gain and a quadratic increase in bone mineral content (BMC) gain ($P < 0.05$); however, only total body fat gain increased significantly. The DLys requirement was estimated at 0.74% for carcass parameters (carcass yield and loin weight) using the quadratic model, and for ADG using the linear-plateau model. A daily intake of 33 g DLys/day for 1.79 kg/day of ADG, or 17.90 g DLys per kg of body weight gain, is recommended for immunocastrated male pigs housed individually during the finishing phase.

Keywords: nutritional requirements; body composition; DEXA; factorial method.

RESUMO

BARCELLOS, Joyce, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Exigências de energia metabolizável e líquida para suínos machos inteiros e imunocastrados e lisina digestível para machos imunocastrados**. Orientadora: Melissa Izabel Hannas.

No primeiro capítulo, o objetivo foi determinar as exigências de energia metabolizável e líquida para mantença e produção para machos inteiros e machos imunocastrados de 70 a 130 kg de peso vivo, utilizando o método fatorial. Sessenta suínos machos inteiros foram distribuídos aleatoriamente em um ensaio de desempenho com 4 tratamentos (*ad libitum*, 75%, 50% e 25% da ração *ad libitum*), com 12 repetições e 1 suíno por baia. Os parâmetros do modelo de energia foram estabelecidos utilizando o método comparativo de deposição de proteína e gordura por meio da abordagem do *Dual-Energy X-ray Absorptiometry* (DEXA). As exigências de energia para ganho de peso foram determinadas com base no conteúdo energético corporal e na eficiência da utilização de energia para ganho de peso. Foram obtidos valores de EM de mantença (EMm) e EL de mantença (ELm) de 160,33 e 134,52 Kcal/kg $PC^{0,75}$ por dia, respectivamente, para machos inteiros. A eficiência de uso da EM para EL foi de 0,84, utilizando o slope da equação de regressão linear de energia retida em função da EM ingerida. A relação entre massa magra e massa gorda diminuiu durante o período de crescimento e foi acentuada em suínos machos imunocastrados, indicando que a retenção de gordura é mais eficiente energeticamente com o aumento do peso corporal. Os suínos machos inteiros são menos exigentes em energia líquida para ganho (ELg) do que os suínos machos imunocastrados. As exigências de EM e EL para produção, EMp e ELp, respectivamente, foram de 4.498 e 3.779 kcal por quilograma de PC para machos inteiros e 5.298 e 4.451 kcal para machos imunocastrados, respectivamente. O segundo capítulo avaliou níveis de lisina digestível (DLis) na dieta de suínos machos imunocastrados em fase de terminação, quanto ao desempenho, composição corporal in vivo, características da carcaça e qualidade da carne, além de determinar a exigência de DLis. Sessenta suínos machos imunocastrados foram distribuídos aleatoriamente em cinco tratamentos com níveis de DLis variando de 0,55, 0,61, 0,68, 0,75 e 0,82%, respectivamente, em 12 repetições. De modo geral, o desempenho foi melhorado em resposta aos níveis de DLis. O rendimento da carcaça e o peso do lombo aumentaram linearmente ($P < 0,05$) em resposta aos níveis de DLis, enquanto a área do lombo (AL) apresentou uma resposta crescente quadrática ($P < 0,05$). A qualidade da carne foi afetada, com aumento linear do comprimento do sarcômero, perda por gotejamento (P < 0,10) e valores de cor *a** e *b** (P < 0,05), mudanças na composição química dos bifes de lombo com resposta quadrática decrescente para gordura intramuscular e proteína bruta na matéria seca (P < 0,05). A composição corporal foi afetada, com um aumento linear no ganho de massa gorda e um aumento quadrático no ganho de conteúdo mineral ósseo (BMC) (P < 0,05); no entanto, apenas o ganho total de gordura corporal aumentou. A exigência de DLis foi estimada em 0,74% para os parâmetros de carcaça (peso da carcaça resfriada, rendimento de carcaça e lombo) utilizando o modelo quadrático, e para o GPD utilizando o modelo linear-platô. Recomenda-se um consumo diário de ração de 33 g de DLis/dia para 1,79 kg/dia de GPD, ou 17,90 g de DLis por kg de ganho de peso corporal, para suínos machos imunocastrados, alojados individualmente e em fase de terminação.

Palavras-chave: exigências nutricionais; composição corporal; DEXA; método fatorial

SUMÁRIO

INTRODUCTION

Maximization of pig performance has traditionally been the goal of swine producers and nutritionists (NRC, 2012). At same time, the efficiency of pig production using nutrients has increased over the years. Still, better efficiency of nutrient utilization can be achieved by feeding pigs with diets adjusted to their estimated requirements (POMAR, ANDRETTA & REMUS, 2021). Energy, amino acids, minerals, vitamins, and water are essential nutrients needed by animals to live (maintenance), grow, and produce (NRC, 2012). Special attention should be paid to lysine contents and energy levels. Lysine and energy are usually considered the most important components in pig production costs (LÉTOURNEAU-MONTMINY ET AL., 2011), and they also considerably influence pig performance.

Energy is the most expensive and complex component of the diet and is required for all biological processes in pigs, thus, an adequate supply of energy in addition to the supply of nutrients is a prerequisite for optimal pig production (KIM; KILL; STEIN, 2013). Furthermore, swine growth is dependent on the energy concentration of the diets; the feed intake is adjusted according to the energy levels of the diet (BARBOSA ET AL., 2002; KIM; KILL; STEIN, 2013; HANNAS ET AL., 2024), who reinforces its importance.

Lysine is the first limiting amino acid for pigs fed corn-soybean meal diets (SONG ET AL., 2022). It is important to supply pigs with sufficient lysine to support muscle growth and development while minimizing excess that will be excreted into the environment (GAFFIELD ET AL., 2022). Given these conditions, evaluating lysine requirements of the current high lean pig genotypes is essential for generating cost-effective diets for growing-finishing pigs and reducing nitrogen excretion (ALEBRANT ET AL., 2015). At same time, by optimizing SID Lys (standardized ileal digestible lysine) recommendations, the industry can maximize both lean muscle deposition and economic returns as growth performance and meat quality attributes improve (GAFFIELD ET AL., 2022).

Numerous studies have shown that the nutritional requirements of pigs vary according to their age, gender, weight, breed, production potential, physiological state, and housing environment (CLOUTIER ET AL., 2015; KAHINDI ET AL., 2017; AYMERICH ET AL., 2021). Increased social pressure in some countries to stop castration surgery in male pigs will increase the relative production of boars in the coming years (AYMERICH ET AL., 2021) and, consequently, immunocastrated males (SKRLEP ET AL., 2020). Considering these aspects, raising entire male pigs and immunocastrates poses various challenges on how the nutrient requirements of these growth-efficient animals can be met (BEE ET AL., 2020). Moreover, nutritional requirements of heavy immunocastrated male pigs and therefore appropriate feeding strategies have not yet been determined (BATOREK-LUKAC ET AL., 2021).

Therefore, defining precise nutritional strategies, especially regarding dietary energy and digestible lysine requirements, is critical to support the growth potential of entire males and immunocastrated pigs while optimizing carcass composition, production efficiency, and environmental sustainability.

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CAPÍTULO I

Energy Requirements and Energy Efficiency of Utilization for Entire and Immunocastrated Male Pigs

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ABSTRACT

The study aimed determine the energy requirements for maintenance and production, and the efficiency of energy utilization in entire and immunocastrated male pigs using the factorial method. Sixty entire male pigs were randomly allotted in a performance assay with 4 treatments (*ad libitum*, 75%, 50% and 25% of *ad libitum* feed), with 12 replicates and 1 one pig per pen. The parameters of energy method were establish using comparative protein and fat deposition method through of Dual-Energy X-ray Absorptiometry (DEXA) approach. Energy requirements for weight gain were determined based on the body energy content and efficiency of energy utilization for weight gain. Maintenance metabolizable energy (MEm) and maintenance net energy (NEm) values of 160.33 and 134.52 Kcal/kg $BW^{0.75}$ d were obtained, respectively, for entire male pigs. The efficiency of metabolizable energy use for NE was 0.84, using the slope of the linear regression equation of retained energy as a function of metabolizable energy intake. Entire male pigs require less net energy for gain (NEg) than immunocastrated male pigs. The metabolizable and net energy requirements for production, MEp and NEp, respectively, were 4.498 and 3.779 kcal/kilogram of BW for entire and 5.298 and 4.451 kcal for immunocastrated male pigs, respectively.

Key words: DEXA, energy model, factorial method, metabolizable energy requirement, net energy requirement, maintenance.

1. INTRODUCTION

An adequate supply of energy in addition to the supply of nutrients is a prerequisite for optimal pig production [1]. Not all gross energy (GE) that is consumed will be retained by the animal, because there are losses in the feces and urine, and as gases and heat. Based on these losses in the process of energy utilization, different energy values and energy systems have been defined: digestible energy (DE); metabolizable energy (ME), and net energy (NE) [2]. Regardless of the energy system adopted, pigs require energy for maintenance and productive purposes, and an accurate amount of available energy in feeds should be provided according to their energy requirement.

Pigs prioritize the use of dietary energy to meet maintenance requirements, and surplus energy intake may be retained as body protein and lipid, contributing to productive growth [3-5]. The factorial approach estimates energy and nutrient requirements by summing the biological demands for maintenance and growth [6]. This method is widely applied in pig nutrition modeling and has been incorporated into established predictive systems [7- 11].

Studies employing the factorial method to determine energy and nutrient requirements remain scarce in the literature, with the empirical dose–response method being more commonly used [12]. One explanation for the limited application of the factorial approach is that accurately evaluating the efficiency of energy and nutrient utilization, or determining their requirements, requires precise measurements of body composition or whole-body nutrient content [13], which are often time-consuming and costly. However, with the advent of Dual-Energy X-ray

Absorptiometry (DEXA) technology, it is now possible to rapidly and cost-effectively determine protein and fat deposition parameters using the same animal, thereby enabling accurate estimates of nutrient retention that were previously labor-intensive and expensive [14-15], with high precision and minimal random error [16-17].

The goals of this study were to determine the metabolizable and net energy requirements for maintenance and production in entire male and immunocastrated pigs; to establish the utilization coefficients of metabolizable and net energy for maintenance and production; and, consequently, to develop mathematical and predictive models for estimating energy requirements for entire male and immunocastrated pigs.

2. MATERIAL AND METHODS

Two experiments were conducted in the Unit of Teaching, Research, and Extension in Production and Nutrition of Pigs of the Department of Animal Science of the Center of Agrarian Sciences of the Federal University of Viçosa - campus Viçosa. The first experiment was conducted to determine the energy requirements for maintenance and production, and the efficiency of energy utilization in entire and immunocastrated male pigs. The second one, was conducted to determine the metabolizable energy intake.

2.1. EXPERIMENT 1

2.1.1. Animals, experimental design and diets

Thirty-two crossbred entire male pigs from Camborough x AGPIC337 (PIC Agroceres, Patos de Minas) were used with an initial BW of 70.43 ± 1.45 kg. The pigs were housed individually in pens (2.29 x 2.17 m) equipped with a nipple and a 33 x 65 cm brick feeder, and reared at 23.1 °C (± 0.92 °C). In this study the pigs were submitted to immunocastration. The pigs received the first dose at the beginning of the experiment, and received the second dose of the immunizer (Vivax, Zoetis) at 36 days after the first. Pigs were classified as entire males until administration of the second immunization dose, after which they were considered immunocastrated males. The second dose was administered when pigs reached approximately 100 kg BW, corresponding to the transition between the growth phases (70–100 and 100–140 kg BW).

Animals were distributed in a completely randomized design consisting of four treatments, eight replicates per treatment, and one animal per replicate, considered an experimental unit. The four feed intake treatments consisted of *ad libitum*, 75%, 50%, and 25% of *ad libitum* intake. The feed intake in the *ad libitum* group were measure for each two days, in order to correct the amount of feed delivery in other treatments. The diet utilized for feeding the pigs was formulated according to [10] recommendations, with 3,291 kcal ME/kg, 15.18% CP and, 0.90% standardized digestible lysine using ideal protein profile (Table 1).

TABLE 1. Ingredients and the calculated composition of energy and nutrients

Ingredients	Experimental diet, %
Corn, 7.88 %	76.59
Soybean meal, 45 %	19.10
Soybean oil	1.01
Vitamin supplement [1]	0.30
Mineral supplement [2]	0.20
Antioxidant	0.01
Dicalcium phospate	1.09
Limestone	0.60
Salt	0.39
L-lysine HCl	0.36
DL-methionine	0.09
L-threonine	0.11
L-tryptophan	0.03
Choline chloride, 60%	0.03
Inert	0.10
Calculated composition of energy and nutrients	
Metabolizable energy, Kcal/kg	3,291.80
Net energy, Kcal/kg	2,570.00
Digestible protein, %	13.08
Calcium, %	0.57
Total phosphorus, %	0.49
Lysine, %	0.99
Digestible lysine, %	0.90
Digestible methionine, %	0.31
Digestible met + cys, %	0.54
Digestible threonine, %	0.58
Digestible tryptophan, %	0.18

[1] Provided per kilogram of supplement: Zn 25 g, Cu 40 g, Fe 15 g, Mn 13 g, I 350 mg.

[2] Provided per kilogram of supplement: Se 75 mg, Folic acid 125 mg; Niacin 825 mg, Biotin 12.5 mg, Pantothenic acid 4,000 mg; Vitamin A 2,100,000 UI, Vitamin B1 250 mg, Vitamin B12 6,000 mcg, Vitamin B2 1,350 mg, Vitamin B6 250 mg, Vitamin D3 350,000 UI, Vitamin E 5000 UI, Vitamin K3 850 mg.

2.1.2. Energy Requirements for Maintenance

In order to determine the metabolizable energy for maintenance (MEm) requirements, the energy balance (EB) parameters must be found. The EB refers to the amount of energy retained (RE), and the RE may be split into energy retained as protein and energy retained as fat, and it's were determined by DEXA (Dual-Energy X-ray Absorptiometry) technology. All pigs in the trial were submitted to DEXA measurements at the beginning of the feeding study and at the end of the controlled feeding trial in each growth phase (70–100 and 100–140 kg BW).

The measurements were performed in the Laboratory of Body Composition and Densitometry at Animal Science Department at Universidade Federal de Viçosa, being performed in eight pigs of each treatment (08 x 04). Before the procedure, the DEXA was configured in the "Small Animal" mode of the GE Healthcare encore software, version 18, and the BW (kg) information and the dimensions of each pig (width x length x height) were inserted.

The pigs were prepared for the analysis with prior fasting of 08h due to sedation protocol (acepran: 0.1 mg/kg; xylazine: 1.5 mg/kg; and ketamine: 15 mg/kg; via IM). The measurement through DEXA provided the results of fat mass (kg), lean mass (kg), total mass (kg), and bone mass content (BMC) (g) for each pig.

The individual data for each pig's fat mass, lean mass, and bone mass were adjusted for both initial and final body weights from the maintenance assay. These adjusted data were then processed using equations [15] to determine the quantities of protein and fat retained in body and, then the ER as protein and fat.

Heat production (HP) was determined by the difference between the ME intake (MEI) and RE. The logarithmic relationship between HP and MEI provided the maintenance net energy requirement (NEm), as being the HP at zero ME intake [18].

The maintenance ME requirement (MEm) was determined by two procedures. The first procedure for determining the MEm requirement was the linear relationship between RE and MEI that provided the MEm at the intercept with x-axis being ME intake at zero energy retained [19]. The MEm requirement was also determined by a second procedure according to the following model:

$$MEm = MEI - (RE/kg)$$

where MEm = maintenance ME (kcal/kg$^{0.75}$ per d), MEI = ME intake (kcal/ kg$^{0.75}$ per d), RE = retained energy (kcal/ kg$^{0.75}$ per d), and kg = efficiency of energy utilization for retention [20].

2.1.3. Energy Requirement for Weight Gain

The data (growth, protein retained, and fat retained) obtained with entire male pigs submitted to *ad libitum* feed levels treatment during 21-d and with immunocastrated pigs submitted to ad libitum feed levels treatment during additional 21-d were used to determine energy content in total body and calculated total energy retained. The procedures utilized to prepare animals, submitted to DEXA and body composition analysis were the same as described for the experiment to determine the MEm.

Net energy requirement for weight gain (NEg) was obtained by linear regression between body energy content (BE) and BW. The slope of the equation provided the net energy requirement per gram of weight gain. The ME requirements for weight gain (MEg) were determined considering the NEg and kg.

2.1.4. Efficiencies of Energy Utilization

The efficiency of metabolizable energy utilization for gain (kg) was determined using two methodologies. In the first approach, kg was calculated directly from the energy balance equation:

$$\mathrm{Kg} = \mathrm{RE}/\,\mathrm{MEI} - \mathrm{MEm}$$

where RE represents retained energy, MEI is metabolizable energy intake, and MEm is the metabolizable energy requirement for maintenance. In the second approach, kg was estimated as the slope of the linear regression between retained energy (RE) and metabolizable energy intake (MEI).

The efficiencies of metabolizable energy utilization for protein (kp) and fat (kf) deposition were determined using multiple linear regression. In this approach, metabolizable energy intake (MEI) was modeled as a function of retained energy as protein (REp) and retained energy as fat (REf) [20]. The metabolizable energy requirement for maintenance (MEm) was estimated as the intercept of the regression equation:

$$\mathrm{MEI} = \mathrm{MEm} + 1/\mathrm{kp}\ \mathrm{REp} + 1/\mathrm{kf}\ \mathrm{REf}.$$

2.2. EXPERIMENT 2

2.2.1. Animals and experimental design

This experiment was using to determine the correct metabolizable energy intake for entire male pigs submitted to the four feed intake treatments and using to determine metabolizable an net energy and metabolizable energy requirements.

Twenty-four entire male pigs (Camborough x AGPIC337, Agroceres PIC) with an initial body weight (BW) of 70.96 ± 0.833 kg were allocated in a completely randomized design with four dietary levels (*ad libitum*, 75%, 50%, and 25% of *ad libitum*), six replicates and one animal per experimental unit.

2.2.2. Apparent metabolizable energy corrected for nitrogen (AMEn)

Pigs were individually housed in adjustable metabolism cages (1.27 m × 0.56 m × 0.75 m) with individual stainless feeders, that was used to provide water before and after meals. The trial lasted 12 d, consisting of a 7-d adaptation period to the metabolic cages and experimental diets, followed by a 5-d collection period. During the collection phase, total feces and urine were collected to determine energy intake and excretion following the total collection methodology described by [12].

The same experimental diet was used in both trials. The daily feed allowance at each feeding level was identical to that provided to animals in Experiment 1. During the adaptation and collection phases, mash diets were offered twice daily (0700 and 1600 h), and water was available *ad libitum*.

Fecal collection was carried out once a day for four consecutive days, with samples being weighed daily and stored in plastic bags at −20 °C. At the end of the collection period, all fecal samples were thawed and homogenized, and subsamples were lyophilized and stored for further analysis. The total urine volume per pig was collected over 5 d in buckets containing 7.5 to 10 mL of HCl and positioned below the funnel coupled to each cage. After each 24-h period of urine collection, samples of 10% of the urine volume were taken and stored at −20 °C to capture a homogenous and representative urine sample per pig over the 5-d collection period.

Subsamples of diets, feces, and urine were analyzed at the Animal Nutrition Laboratory of the Universidade Federal de Viçosa (Viçosa, Minas Gerais, Brazil). Prior to chemical analysis, feces and diet samples were ground using a ball mill (Micro Spray Mill, R-TE 350, TECNAL, São Paulo, Brazil). Gross energy (GE) of the diet and feces was measured by a C500 adiabatic bomb calorimeter (IKA-Werke GmbH & Co. KG, Staufen, Germany). Nitrogen content of the diets, feces, and urine was analyzed using a distiller (TE-036/1, TECNAL, São Paulo, Brazil) following the Kjeldahl method (determination of N content and calculation of crude protein content, method 988.05 [21]). A factor of 6.25 was used to convert the N content to crude protein (CP). Urine energy content was analyzed at the CBO Laboratory (Valinhos, SP, Brazil).

Triplicate analysis was performed to determine the dry matter (DM) content in the diets and feces (dry matter content, method 930.15 [21]). N determination in the diets, feces, and urine samples was analyzed in duplicate; repetitions were performed for a sample coefficient of variation above 5%.

Energy and nitrogen contents of the diet, feces, and urine were used to calculate the apparent metabolizable energy corrected for nitrogen (AMEn) of the diet, which was subsequently used to estimate metabolizable energy intake (MEI).

2.3. Statistical analysis

The data were analyzed using the general linear model procedure PROC in SAS 9.0 [22] a completely randomized design. Individual pigs served as the experimental unit for all outcomes. Polynomial orthogonal contrasts were used to evaluate the linear and quadratic effect of feed levels (*ad libitum*, 25%, 50% and 75% of *ad libitum*).

3. RESULTS AND DISCUSSION

The ME intake was calculated based on feed intake and the AMEn of diet determined: 3,291; 3,353; 3,357; and 3,346 kcal/kg of diet fed according to the feeding levels: *ad libitum*, 75%, 50%, and 25% of *ad libitum*, respectively. The AMEn of diet does not differ (P = 5643).

The RE and HP increase when the feed level increases, as expected (Table 2). [23] have found the same behavior, where the metabolizable energy intake and HP increased with feeding level, and therefore the total energy retained. The feed intake can increase the HP through thermogenesis. In this process, an energetic cost is associated with transport of nutrients through the gastrointestinal tract, transport of nutrients via the blood and into tissues, and energy loss associated with anabolic processes like synthesis of fat and protein for retention [24]. Therefore, the reduction in feed intake, and consequently in energy and nutrient consumption, led to decreased heat production (HP) and a reduced capacity for energy retention in pigs.

TABLE 2. Means of ME intake, retained energy, and heat production for entire male pigs in finishing phase.

Feeding level	**ME intake**	**Retained Energy**	**Heat Production**
	Kcal/kg $BW^{0.75}$ per d		
Ad libitum	323±7.9	141±6.2	181±7.3
75% *Ad libitum*	275±4.5	90±9.8	185±11.8
50% *Ad libitum*	199±2.8	31±6.2	167±6.0
25% *Ad libitum*	111±3.0	-41±9.5	151±10.6

[1] The ME intake was calculated based on feed intake and the AMEn of diet determined: 3,291; 3,353; 3,357; and 3,346 kcal/kg of diet fed according to the feeding levels: *ad libitum,* 75%, 50%, and 25% of *ad libitum*, respectively. AMEn of diet does not differ between treatments (P = 0,5643).

[2] The retained energy was calculated considering the initial BW of 63.30; 62.70; 63.54 and 63.23kg, and total body energy of 2.644; 2.589; 2.746, and 2.534 kcal/kg respectively in *ad libitum,* 75%, 50% and 25% *ad libitum*, respectively.

3.1. Maintenance Energy Requirements

The regression of ER and heat production as a function of MEI, EMm, NEm, efficiency of energy utilization for gain (kg), and maintenance (km) for entire male pigs from 70 to 100 kg are presented in Table 3.

TABLE 3. Regression of retained energy (RE) and heat production (HP) as a function of ME intake (MEI), values of maintenance ME (MEm), maintenance net energy (NEm), efficiency of energy utilization for gain (kg), and maintenance (km) for entire male pigs from 70 to 100 kg.

Regressions[1,2]	R^2	Requirements	Efficiencies
		Kcal/kg $BW^{0.75}$ per d	
RE = -134.52+0.8392MEI	0.98	MEm= 160.33	$Kg^3 = 0.84$
HP = 134.52+0.1607MEI	0.66	NEm= 134.52	$Km^4 = 0.84$
HP = 136.81 $e^{0.0010EMI}$	0.65	NEm= 136.81	$Km^4 = 0.85$

[1] Regressions were significantly different from zero ($P < 0.01$).

[2] e = 2.718, is the base of the natural logarithm, when MEI is zero, $e^0 = 1$ and the HP is the intercept of equation.

[3] kg = slope of linear regression of RE as a function of ME.

[4] km = NEm/MEm.

The data of MEm, NEm and km for pigs are limited, and reported values are conflicting. Studies carried out before 2000 estimated the MEm for pigs at 106 kcal ME/kg $BW^{0.75}$ [25] and 109 kcal ME/kg $BW^{0.75}$ [26]. Most recently, [23] determined the EMm at 220 kcal/kg $BW^{0.6}$ d^{-1} for barrows with 60 to 100 kg BW. When we compare the EMm estimated in previous studies over time, we can realize that EMm has increased. Considering the average body weight of entire male pigs in this study (86 kg BW), the metabolizable energy requirement for maintenance (MEm, kcal/d) reported in the literature varies considerably, ranging from 2,993 [25] to 3,184 [23], and between 2,692 and 3,474 [27]. However, the difference increases when compared to our study, with an MEm of 4,528 kcal. Some authors have suggested that entire male pigs may have a higher metabolizable energy requirement for maintenance (MEm) than castrated males [28-30]. However, [31] did not observe a sex effect when comparing entire males, females, and castrated pigs. Theoretically, EMm can be higher for entire male pigs. Entire male pigs have greater protein accretion based in the elevated androgenic potential and, consequently, in protein deposition [32]. No recent studies have been reported in the scientific literature comparing the metabolizable energy requirement for maintenance (MEm) among entire, castrated, and immunocastrated male pigs. In this context, the contrast between authors might have been caused by a lack of standardization of methods used to determine energy balance. Additionally, the genetic, BW, environmental temperature, energy levels of diets and feed intake could affect the data variability for HP and energy utilization [33, 31, 27]. Therefore, assuming a constant requirement across gender, and lines, may not be appropriated because it's may represent an oversimplification of the biological reality.

The value of HP at 0 (zero) was obtained in the regression method (may be used as an estimate of NEm). Estimates of NEm for entire male pigs were obtained by linear and exponential equations and no differences was observed (134.52 x 136.81 kcal/kg $BW^{0.75}$). The value for HP0 in this experiment (134.52 Kcal/kg $BW^{0.75}$ per d) represent 3,799 kcal/kg based in pigs with 86 kg BW. This contrasting with 2,519 kcal (174 kcal/kg $BW^{0.6}\cdot d^{-1}$) obtained by [23] 2,707 kcal (187 kcal/kg $BW^{0.6}\cdot d^{-1}$) determined by [5] and 1,942.2 kcal/kg (560.75 kJ/kg $BW^{0.6}\cdot d^{-1}$) by [34].

3.2. Energy requirement for growth

DEXA is a valuable tool enables accurate assessment of body composition and, when combined with predictive equations, allows for the estimation of whole-body energy content and its partitioning into energy retained as fat and protein [14-16]. Nevertheless, the DEXA readout does not provide information on water content. The water content in the fat-free mass (sum of CP, ash, and water), needs to be determine by chemical methods [14]. The data obtained by DEXA were presented in Table 4.

TABLE 4. Body weight (g), protein and fat compositions of entire males and immunocastrated pigs from 70 until 130 kg.[1]

Body Weight, Kg	Lean Mass, Kg	Fat Mass, Kg	Fat Body, kg[3]	Protein Body, kg[4]	Energy, kcal/kg
70.73±1.01	62.8±2.49	7.9±1.77	10.8±1.782	12.2±0.537	2.408±0.217
94.28 ±1.64	78.8±1.64	15.5±2.30	18.4±2.223	15.7±0.678	2.773±0.206
125.3±2.38	97.5±3.40	27.9±2.52	31.0±2.543	19.7±0.734	3.204±0.170

[1] Pigs receive the second dose for imunnocastration at 36 days after the first.

[2] Mean ± SEM.

[3] Fat content in empty body weight estimate using Pomar et al. (2017).

[4] Protein content in empty body weight estimate using Pomar et al. (2017).

In entire male pigs, body composition undergoes significant changes throughout the growth period, with a progressive reduction in the lean-to-fat mass ratio as body weight increases. Specifically, in our study, the ratio declined from 7.95:1 at 70.73 kg to 5.08:1 at 94.28 kg in entire male pigs indicating a relatively greater accumulation of fat with increasing body weight. According to [14]), in entire male pigs at 20 kg of body weight, the lean-to-fat mass ratio was 12.25:1, indicating a predominance of lean tissue relative to fat; but as animals reached 60 kg, this ratio decreased to 8.21:1, and further declined to 6.20:1 at 100 kg, reflecting a relative increase in fat deposition as the animals matured. In this study, at 100 kg BW, pigs received a second dose of immunization against gonadotropin-releasing hormone. Following the administration of the second dose, i.e., in immunocastrated male pigs, this ratio further decreased to 3.49:1. After the immunization, immunocastrated male pigs rapidly change their metabolism to castrate-like, with increased feed consumption and fat deposition [35]. A study of [36] revealed that, after effective immunization, the immunocastrated increase fat tissue deposition at the expense of lower heat production, while protein deposition remains like entire males and different from surgical castrates, which deposit fat instead of protein (i.e., muscles).

Energy retention, expressed in kcal/kg, increased linearly with increasing BW. The energy values obtained of 2,773 kcal/kg and 3,204 kcal/kg represent increases of approximately 15.15% and 33.06%, respectively, relative to 2,408 kcal/kg. In absolute terms, these increments correspond to an additional 0.365 kcal/kg and 0.796 kcal/kg, respectively. The proportion of energy retained as fat and as protein also changes according to the growth curve and the deposition of protein and fat tissues. From 70 to 100 kg of BW the entire male pigs increase protein and fat deposition rates by 28.7% and 70.3%, respectively, and from 100 to 140 kg of BW the immunocastrated male pigs increased this deposition rates by 25.7% and 68.5%, respectively.

The ME requirements for growth were determined for entire and immunocastrated male pigs. The linear regression among body energy and BW, ME, and NE requirements for growth are presented in Table 5. The ME requirements determined for growth were 4,724 to 4,498 kcal/kg for entire male consider the efficiency of energy used by 80 and 84%, respectively and, 6,270 to 5,298 for immunocastrated male pigs with energy efficiency between 71 and 84%, using the procedure 1 and 2, respectively. The ME required for growth is also largely affected by the efficiency of energy retention [33], but regardless of the procedure used to estimated efficiency, the energy requirement is higher for immunocastrated male pigs compared to entire. The efficiency of energy utilization for gain (kg) by procedure 2 appears to be most appropriated, consider the slope of equation as energy retained as function of metabolizable energy intake, and trough method 2 the efficiency for entire and immunocastrated male pigs was the same. The value for Kg was about 20% greater than 64% and 66% estimated by [23] and [34], even using a similar BW. A number of factors like the chemical composition of the diet, the physical form of the diet and environmental temperature, significantly influence the utilization of energy in pigs [24] and mainly genetic selection allow the last decade.

TABLE 5. Linear regression of body energy (BE) as a function of BW, net energy requirements for gain (NEg), efficiency for gain (kg), and ME requirements for gain (MEg) of entire male pigs from 70 to 100 and immunocastrated pigs from 100 to 140 kg.

	Regression[1]	R^2	NEg (Kcal/kg)	Efficiency (%)	MEg (Kcal/kg)
Entire male pigs	BE = -95.95+3,779 BW	0.87	3,779	80%[2]	4,724
				84%[3]	4,498
Immunocastrated male pigs	BE = -157.37+4,451 BW	0.92	4,451	71%[2]	6,270
				84%[3]	5,298

[1] Regressions were significantly different from zero ($P < 0.01$).

[2] kg = Retained energy/ (ME intake - ME for maintenance).

[3] kg = Slope of regression (Retained Energy = -134.526 + 0.8392 ME intake, $R^2 = 0.98$).

The efficiency of RE as protein and fat and the MEm requirement are presented in Table 6. The results of MEm were similar to those determined by other procedures. Table 6 shows that fat retention is more energetically efficient than protein retention, with less energy lost as heat during the process. This is partly because fat has a higher energy density than protein, providing approximately 9 kcal/g compared to 4 kcal/g for protein. According to [24] as pigs grow, their efficiency in retaining energy improves. This increase in energetic efficiency can be favored by a high fat retention (less heat is lost when fat is retained), and by a high live weight (heat production is constant per $kg^{0.75}$). In our study, such differences, although numerically small, may have significant implications when scaled to the overall energy requirements or feed formulations in animal nutrition studies, highlighting the importance of precise energy estimation for optimizing performance and efficiency. The efficiencies of energy utilization for protein (Kp) and fat (Kf) retention were 35% and 117%, respectively. The kp and kf values presented in the literature can be varied because of the differences in genetic, sex, age, diets, and methodologies utilized for determining RE [33, 24]. For pigs, fat retention occurs with an efficiency of approximately 80%, while protein retention occurs with an efficiency of approximately 60% [24]. However, [31] observed that the Kg depended on the composition of growth because the efficiencies of using ME for protein (Kp) or fat deposition (Kf) were quite different.

TABLE 6. Linear regression of retained energy as protein (REp) and retained energy as fat (REf) in function of ME intake (MEI), maintenance requirements (MEm) and efficiencies for protein retention (kp) and fat retention (kf) of entire male pigs from 70 to 100 kg.

Regression[1]	R^2	Kp[3] (%)	Kf[4] (%)	MEm (kcal/kg $BW^{0.75}$ per d)
MEI = 150.24+2.85REp+0,86REf	0.98	35	116	150.24

[1] MEI, REp, and REf are expressed in kilocalories per $kilogram^{0.75}$ per day.

[2] Regressions were significantly different from zero ($P < 0.01$).

[3] kp = 1/2.85

[4] kf = 1/0.86

Determining the metabolizable and net energy requirements of pigs is essential for establishing diets that meet nutritional needs while maintaining production efficiency. However, experiments designed to estimate these requirements are complex, resource-intensive, and demand precise methodological control, resulting in few published studies, particularly for finishing pigs. The lack of data is even more critical when considering different sex categories, such as entire and immunocastrated males, whose physiological differences affect energy metabolism and, consequently, nutrient requirements. Therefore, studies addressing these variations are fundamental to improve the accuracy of feeding recommendations and to advance the understanding of energy utilization in pigs under diverse physiological conditions.

The integration of values can be used to describe a model to estimate the ME and NE, respectively, as ME kcal/d = 160,33 $(BW)^{0.75}$ + 4,498 (BWG) and NE kcal/d = 134,52 $(BW)^{0.75}$ + 3,777 (BWG) for entire male pigs and ME kcal/day= 160,33 $(BW)^{0.75}$ + 5,298 (BWG) and NE kcal/d = 134,52 $(BW)^{0.75}$ + 4,451 (BWG) for immunocastrated male pigs, as WG = average of body weight gain in kilograms per day.

The application of the factorial method can be used for estimating energy requirements for maintenance and production and the efficiency of energy utilization in male pigs with distinct physiological conditions. By integrating detailed body composition data obtained via DEXA, this study reinforces the relevance of partitioning energy requirements into maintenance and production components. Furthermore, the findings underscore the critical impact of sexual status on energy metabolism. Immunocastrated male pigs exhibited different energy demands and deposition patterns compared to entire male pigs, particularly with greater fat deposition efficiency and higher net energy requirements for gain.

Consider the values established for ME and NE for maintenance and gain for entire male pigs and immunocastrated male pigs from 70 to 140 kg of BW, its possible development a model to estimate metabolizable and net energy requirements for entire male pigs and immunocastrated male pigs to support precise nutrition. Future validation need to be development to evaluated the coefficients and values defined in this research.

4. CONCLUSION

In the present study, the metabolizable and net energy requirements for maintenance were estimated at 160.33 and 134.52 kcal/kg $BW^{0.75}$ d for entire and immunocastrated male pigs, respectively. The metabolizable energy requirements for growth differed between sexes, being estimated at 4.498 and 5.298 kcal/kg $BW^{0.75}$ d for entire and immunocastrated males, respectively. The efficiency of metabolizable energy utilization for net energy was estimated to be 0.84 for both sexes. These results provide a basis for sex-specific predictive models and challenge the common practice of using a single equation to estimate energy requirements across sexes.

Credit authorship contribution statement

Joyce Barcellos: Data curation, Methodology, Writing - original draft. Bruno Teixeira Ramos: Methodology. Mariana Anastácio do Nascimento: Methodology. Gabriel Ribeiro Braga: Methodology. Lucimauro da Fonseca: Methodology. Maria Rogervânia Silva de Farias: Methodology. Erica Beatriz Schultz: Methodology, Writing - review & editing. Melissa Izabel Hannas: Conceptualization, Methodology, Supervision, Writing - review & editing.

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Institutional Review Board Statement:

Animal care and management were in accordance with Brazilian legislation on Animal Experimentation and Welfare, and the experimental protocol was approved by the Ethics

Committee on the Use of Production Animals (CEUAP-UFV) of the Federal University of Viçosa (protocol 102/2023).

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request**.**

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CAPITULO II

Dietary digestible lysine levels for immunocastrated male pigs on performance, carcass parameters, and meat quality

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ABSTRACT

This study aimed to evaluate dietary digestible lysine (DLys) levels for finishing immunocastrated male pigs on performance, *in vivo* body composition, carcass characteristics, and meat quality, in addition to determining the requirement for DLys. Sixty immunocastrated male pigs were randomly allotted in 05 treatments (0.55, 0.61, 0.68, 0.75, and 0.82% DLys), with 12 replicates. Regression analyses were performed. Overall, performance was improved in response to DLys levels. Carcass yield and loin weight increased linearly in response to DLys levels, while loin area (LA) showed a quadratic increasing response. Meat quality was influenced by a linear increase in sarcomere length, drip loss, and the color parameters *a** and *b**. Loin intramuscular fat and crude protein decreased quadratically with increasing DLys levels. Fat mass gain increased linearly, while bone mineral content (BMC) gain exhibited a quadratic response; however, only total body fat gain was increased. The DLys requirement was estimated at 0.74% for carcass parameters (carcass yield, and loin) using quadratic model, and for ADG using a linear plateau. A daily feed intake of 33 g of DLys/day for 1.79 kg/day of

ADG, or 17.90 of DLys/kg of body weight gain is recommended for individually housed, finishing immunocastrated male pigs.

Keywords: body composition, DXA, intramuscular fat.

1. INTRODUCTION

Increased social pressure in some countries to stop castration surgery in male pigs will increase the relative production of boars in the coming years (Aymerich et al., 2021) and, consequently, immunocastrated males (Skrlep et al., 2020). In comparison, surgically castrated, boars, and immunocastrated pigs have distinct metabolic characteristics. While surgically castrated males undergo early castration, late castration is performed in immunocastrated males, leading to rapid metabolic changes, resulting in higher feed intake and rapid growth (Dunshea et al., 2001, Batorek-Lukacˇ et al., 2016).

Differences in meat performance and quality observed between castrated, boars, and immunocastrated male pigs (Batorek et al., 2012; Candek-Potokar et al., 2017; Skrlep et al., 2020) may indicate differences in nutritional requirements for each sex. Theoretically, the elevated androgenic potential preceding the second vaccination and, consequently, in protein deposition of boars (Claus et al., 1994), may increase the requirement for digestible lysine to maximize their weight gain (Aymerich et al., 2020). Lysine is an essential amino acid and the most abundant amino acid in the body protein gain of growing and finishing pigs (7.1 g lysine per 100 g protein), and usually the first limiting amino acid in grain and cereal-based diets (NRC, 2012). Due to the progress of genetic improvement, it became necessary to frequently evaluate the requirement of digestible lysine (Gaffield et al., 2022). It is attributed that genetic improvement in pigs has resulted in a higher rate of protein deposition, leading to changes in nutritional requirements (O'Connell et al., 2006; van der Peet-Schwering & Bikker, 2018), especially lysine. Thus, the accurate estimate of the digestible lysine requirement of the current genotypes is essential to generate economic diets for growing and finishing pigs, reduce nitrogen excretion (Alebrante et al., 2015), and explore its potential gain.

Studies indicate that immunocastrated male pigs have a higher requirement of digestible lysine (%) during the growth and finishing phase than surgically castrated males, especially after the second dose of immunization (Elsbernd et al., 2017; Muniz et al., 2019). On the other hand, when compared to boars, the requirement for digestible lysine g SID Lys/MJ DE appears to be lower when the first 2 wk after the second immunization are completed (Moore et al., 2016). However, the evaluation of digestible lysine requirement for growing and finishing immunocastrated male pigs still needs to be explored. In addition, although the requirement of digestible lysine in finishing pigs is comparatively lower in percentage than in the previous phases, its supplementation can influence the quantitative and qualitative characteristics of carcasses at slaughter (Wang et al., 2015).

In this sense, by optimizing the recommendation of digestible lysine, the industry can maximize both lean mass deposition and economic return as there is improvement in performance and meat quality attributes (Gaffield et al., 2022). Factors such as sex and slaughter weight affect the content of the main chemical constituents of the carcass and should be considered for the production of carcasses, cuts, or meat products to meet the desired characteristics (Zomeño et al., 2023). Thus, evaluating the effect of different levels of dietary digestible lysine on carcass parameters and meat quality for immunocastrated males in the final finishing phase is necessary to align performance with a higher-quality swine carcass.

The objective of this study was to evaluate the effects of dietary digestible lysine on performance, body composition *in vivo*, carcass characteristics, and meat quality in immunocastrated male pigs in the finishing phase and determine the requirement for digestible lysine.

2. MATERIALS AND METHODS

The experiment was conducted in the Unit of Teaching, Research, and Extension in Production and Nutrition of Pigs of the Department of Animal Science of the Center of Agrarian Sciences of the Federal University of Viçosa - campus Viçosa. Animal care and management were in accordance with Brazilian legislation on Animal Experimentation and Welfare, and the experimental protocol was approved by the Ethics Committee on the Use of Production Animals (CEUAP-UFV) of the Federal University of Viçosa (protocol 102/2023).

2.1. Animals, experimental design and diets

Sixty immunocastrated male pigs from Camborough x AGPIC337 (PIC Agroceres, Patos de Minas) were used with an initial BW of 98.7 ± 1.94 kg. The pigs received the second dose of the immunizer (Vivax, Zoetis) eight days before the beginning of the experiment and 36 days between doses. The pigs were distributed in a completely randomized design consisting of five levels of dietary digestible lysine, twelve replicates per treatment, and one animal per replicate, considered an experimental unit. The experimental period comprised the phase of 100 to 140 kg, with a duration of 19 days, for the evaluation of the response variables and later determination of the requirement of digestible lysine of immunocastrated male pigs.

The pigs were housed individually in pens (2.29 x 2.17 m) equipped with a nipple and a 33 x 65 cm brick feeder. The experimental diets were prepared using the dilution technique (Fisher & Morris, 1970), in which a diet containing a low level of digestible lysine (0.55%) was gradually mixed with a diet containing a high level of digestible lysine (0.82%). These diets were formulated based on corn and soybean meal, with mixing ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 between the low and high lysine diets. From these proportions, the final dietary treatments were obtained (Table 1), with digestible lysine levels of 0.55, 0.62, 0.68, 0.75, and 0.82%, corresponding to 80, 90, 100, 110, and 120% of the digestible lysine recommended by Rostagno et al. (2017). The feed supply was performed twice a day, in the morning and afternoon, to guarantee 20-30% feeder coverage; pigs were provided with access to water and feed *ad libitum*.

2.2. Performance

The pigs were weighed individually at the beginning and end of the experimental period, with an interval of 19 days. During the experimental period, the individual feed intake (feed offered minus leftovers) was measured. The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion (FC) were calculated per pig during the experimental period.

2.3. Assessment of body composition in vivo

The assessment of body composition and densitometry was performed through DXA (Dual-Energy X-ray Absorptiometry) in the Laboratory of Body Composition and Densitometry of the Department of Animal Science of the Federal University of Viçosa, being performed in eight pigs of each treatment (08 x 05) before the beginning of the experimental period and its end. Before the procedure, the DXA was configured in the "Small Animal" mode of the GE Healthcare encore software, version 18, and the BW (kg) information and the dimensions of each pig (width x length x height) were inserted.

The pigs were prepared for the analysis with prior fasting of 08h due to sedation protocol (acepran: 0.1 mg/kg; xylazine: 1.5 mg/kg; and ketamine: 15 mg/kg; via IM). The measurement through DXA provided the results of fat mass (kg), lean mass (kg), total mass (kg), and bone mass content (BMC) (g) for each pig.

The individual data for each pig's fat mass, lean mass, and bone mass were adjusted for both initial and final body weights from the performance test. These adjusted data were then processed using the equations from Kasper et al. (2021) to determine the quantities of protein, fat, and body ash.

2.4. Slaughter and carcass parameters

The pigs were slaughtered at the Teaching Slaughterhouse (Universidade Federal de Viçosa, campus Viçosa, Minas Gerais, Brazil) on three consecutive days, including the same number of pigs per treatment each day. All pigs were kept in a 12-hour fasting. On the day of slaughter, the pigs were weighed while in a fasting state (n = 3 for each treatment on the first and second day and n = 2 for each treatment on the third day of slaughter) and transported to the slaughterhouse together (5min) and kept in the same waiting pen. The slaughter was carried out through electronarcosis followed by bleeding, following the current regulations applied to the slaughter of pigs.

After slaughter, the hot carcass was weighed (removing the digestive, reproductive, and respiratory tract, and perirenal fat). The carcass yield was calculated by dividing the hot carcass weight by the final BW. After 24h of cooling at 4º C, the weight of the cooled carcass was obtained, and the right side was cut in the region of the 10th rib to evaluate the backfat thickness (BT) and loin area (LA). The BT was assessed using a digital caliper. At the same time, the muscular area of the *Longissimus lumborum* between the 10th and 11th vertebrae was outlined on transparent paper with a fine-tip permanent marker to determine the loin area (LA). The area within the contour was calculated using ImageJ software (version 1.51, National Institutes of Health, Bethesda, MD, USA). After the measurements of the BT and LA, the weights cuts (ham, shoulder, boston butt, loin, belly, and ribs) were obtained. The pH and temperature measurements were performed using a portable pH/temperature measuring device (Testo SE & Co., Lenzkirch, FR, Germany) in the cooled carcass (24h). The pH meter was calibrated using standard solutions of pH 4.01 and 7.01 in accordance with the manufacturer's instructions (Hanna Instruments). Calibration was performed before each measurement session to ensure accuracy in analysis. Furthermore, the automatic temperature compensation function of the pH meter was used, adjusting the pH readings according to the temperature variations of the samples.

2.5. Meat quality analysis

From the right half carcass, a 20 cm segment of the *Longissimus lumborum* was collected and stored in vacuum bags and kept at -20º C. Subsequently, it was sectioned to obtain two steaks (~ 2.54 cm) vacuum-packed individually and frozen without the fat cover, but keeping the epimysium layer. One steak was evaluated for its bromatological composition. The second was submitted to an instrumental color evaluation, estimation of losses by thawing and cooking, determination of Warner-Bratzler shear force (WBSF), and sarcomere length. All quality analyses were conducted at the Laboratory of Meat Science – (LCC) of the Department of Animal Science of the Federal University of Viçosa.

2.5.1. Assessment of steak composition

One steak of each replicate (n = 8 per treatment) was used to determine the dry matter content, crude protein, ether extract, and ash via chemical analysis. The steaks were weighed on a semi-analytical scale and then chopped and placed for freeze-drying (Liobras, model LP510, São Carlos, SP, Brazil). The lyophilized samples were weighed and ground using a stainless ball mill (Micro spray Mill, R-TE 350, TECNAL® - São Paulo, Brazil), and the subsamples submitted to dry matter analysis (INCT-CA, G-003/1, 2021), crude protein Kjeldahl N x 6.25 (INCT-001, 2021), ethereal extract (AOCS, Am 5-04, 2009) and ash (INCT-CA, M-001/2, 2021).

2.5.2. Assessment of meat color

The steaks were thawed overnight at 4º C, removed from vacuum packs, and exposed to oxygen 30 minutes before measurements. Instrumental color readings were obtained using a Hunter MiniScan EZ (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA) and calibrated immediately before data collection. The mean values of $L*$ (luminosity), $a*$ (redness), and $b*$ (yellow) of each steak were determined from five readings on the surface of the steak using illuminant D65, a 31.8 mm door size, and a standard observer of 10º.

2.5.3. Warner-Bratzler shear force and loss measurements

The WBSF was measured in the steaks (2.54 cm thick) thawed and cooked as suggested by AMSA (1995). In summary, steaks were thawed at 4º C for 24h and grilled in an electric oven (model: Luxo Inox, Layr, São Paulo, SP, Brazil) preheated to 150º C. The internal temperatures of the steak were monitored approximately at the geometric center of each steak. The steak was turned over when the interior temperature reached 35º C and allowed to reach an internal temperature of 71º C before removing it from the oven. The steaks were weighed before and after thawing and cooking to obtain the losses, which are expressed as a percentage of the weight of the steak before each procedure. The cooked steaks were cooled for 24h at 4º C before removing six cores (1.27 cm in diameter) of each steak parallel to the longitudinal orientation of the muscle fibers. Each core was cut once, perpendicular to the longitudinal orientation of the muscle fibers, using a Warner-Bratzler shear machine (G-R Electrical Manufacturing Company, Manhattan, KS, USA). The Warner-Bratzler shear force was expressed in Newton (N).

2.5.4. Sarcomere length

Cube samples (3.0 x 3.0 x 2.0 cm) were placed in scintillation vials and fixed in glutaraldehyde (5% glutaraldehyde in 0.1M $NaHPO_4$ buffer at pH 7.2) for four h at 4º C. The cubes were placed in a second vial with a sucrose buffer solution (0.2M of sucrose in 0.1M $NaHPO_4$ buffer at pH 7.2) and stored overnight at 4º C. Six fiber beams were obtained from each sample. Sarcomere length was measured using helium and neon laser diffraction (Model 05-LHR-021, Melles Griot, Carlsbad, CA) and calculated according to Cross, West, & Dutson (1981).

2.5.5. Drip loss

For drip loss assessment, a sample of the *Longissimus lumborum* (thickness of ~ 2.0 cm) was immediately collected after obtaining the cuts and weighed on a semi-analytical scale. The sample was stored in plastic pots containing a net, where it remained suspended, allowing it to drip without contact with the tissue with the liquid exuded by the meat. The samples remained for 48h under refrigeration at 4º C and, after this period, were dried gently on a paper towel and weighed.

2.6. Statistical analysis

The data were analyzed using the general linear model procedure PROC in SAS 9.0 (SAS Institute Inc., Cary, NC, USA) in a completely randomized design for performance parameters. The day of slaughter was considered as a block for analysis of the carcass characteristics and meat quality. The animal was used as the experimental unit for the response variables in the model, which included digestible lysine levels in the diet as the main effect, and the initial weight was used as a covariate for the analysis of ADG, ADFI, and FC. Polynomial orthogonal contrasts were used to evaluate the linear and quadratic effect of digestible lysine levels on the performance, carcass parameters, and meat quality of immunocastrated pigs.

The evaluation of the requirement was performed considering performance and carcass parameters. The ADG, ADFI, and FC were selected as responses of interest to estimate the ideal level of digestible lysine to maximize the responses of immunocastrated male pigs and submitted to orthogonal contrasts for linear and quadratic response. In the presence of linear response ($P < 0.10$), the adjustment to the Linear Broken-Line (LBL) model was evaluated. The LBL model was estimated by nonlinear method, being expressed as $Y = \beta_0 + \beta_1 (\beta_2\text{-}X)$, where $(\beta_2\text{-}X) = 0$ for $X > \beta_2$, Y is the dependent variable, X is the lysine dietary concentration, $\beta 0$ is the value in the plateau, β_1 is the slope and β_2 is the concentration of digestible lysine at the breaking point. For carcass parameters, the linear and quadratic effects were used to define the optimal level of dietary digestible lysine.

3. RESULTS AND DISCUSSION

The final BW, ADG, and ADFI increased linearly in response to dietary digestible lysine concentration ($P < 0.10$), while FC was not affected ($P > 0.10$) (Table 2). The increase in final BW and ADG with increasing levels of dietary digestible lysine was expected since lysine has a vital role in muscle development and, therefore, in animal performance. Lysine is a substrate for synthesizing body proteins, peptides, and other molecules, and excess is catabolized as a source of energy. In this sense, there are indications that the increase in muscle growth of monogastric animals, such as pigs, through supplementation of digestible lysine in the diet seems to be more related to increased protein synthesis than to decreased protein degradation (Liao et al., 2015).

The performance results obtained in this study are similar to Lima et al. (2023), where it was observed the linear increase of final body weight, average daily gain, and feed efficiency of immunocastrated male and female pigs (102 to 127 kg), with digestible lysine intake of 24.7 g/kg gain and 0.56 to 0.84% digestible lysine. Moore et al. (2016) also observed an increase in the final average daily gain and feed efficiency of immunocastrated pigs (60 to 107 kg) using 0.32 to 0.75% of digestible lysine. The improvement in the performance of finishing pigs can be observed in response to high levels of digestible lysine, optimizing final body weight, average daily gain, and feed efficiency (Royall et al., 2022; Belloch et al., 2015).

On the other hand, in some studies, the effect on the performance of finishing pigs in response to digestible lysine concentration in the diet is not observed. Song et al. (2022) suggest that digestible lysine restriction may affect performance in the final finishing phase less than in previous phases. According to van der Peet-Schwering & Bikker (2018), a 10% reduction in digestible lysine can reduce average daily gain by 3 to 4% and increase feed conversion by 2 to 3% of growing and finishing pigs. The inconsistencies in the results found in the literature regarding dietary lysine supplementation of finishing pigs' performance may be related to different factors such as the level of crude protein and digestible lysine in the diet, sex, body weight range, and the experimental conditions of each study.

Another significant consideration is that as growth rates increase and feed efficiency is improved through genetic improvements, the recommended dietary intake of digestible lysine is projected to be increased to meet the nutritional demands of pigs (PIC, Nutrition and Feeding Guidelines, 2021). According to van der Peet-Schwering & Bikker (2018), the daily requirement of digestible lysine (g/d) for future genetics is higher than that observed for current genetics due to more significant weight gain and protein deposition capacity. Comparatively, Rostagno et al. (2017) suggest that high genetic potential growing castrated pigs with 133 kg have an estimated average daily gain of 0.95 kg/d with an average daily feed intake of 3.34 kg/d. In our study, pigs had an average daily gain of 1.73 kg/d with an average daily feed intake of 4.44 kg/d, leading to an increase in 0.78 kg/d of average daily gain and 1.1 kg/d in average daily feed intake. Pigs with high genetic index were used in this study, confirming that constant analysis of nutrient requirements to support gains is necessary. Furthermore, the pigs were raised in individual pens and this may have influenced some of the improvements in weight gain and feed intake and this should be considered.

Digestible lysine levels affected the pig carcass in this study (Table 2). Carcass yield and loin weight increased linearly in response to digestible lysine levels. At the same time, LA had a quadratic increasing response ($P < 0.10$), with a maximum point obtained in 0.71% digestible lysine. The weight of the hot carcass, the BT, the weight of the main cuts, pH 24h, and temperature 24h were not affected ($P > 0.10$).

The results obtained are similar to those of Moore et al. (2016), where the increase in the concentration of dietary digestible lysine, from 0.32 to 0.75%, promoted a quadratic response for hot carcass weight and carcass yield of immunocastrated pigs slaughtered at 107.5

kg. Similar to Wang et al. (2015), where the increase in dietary digestible lysine concentration, from 0.43 to 0.98%, increased carcass percentage yield, ham weight, and total weight of lean cuts of pigs slaughtered at 140 kg.

Distinct responses were observed for carcass parameters and meat quality when pigs above 100 kg were submitted to increasing levels of digestible lysine for females (Witte et al., 2000; Belloch et al., 2015), castrated males (Cho et al., 2012; Tous et al., 2014; Belloch et al., 2015), mixed gender of females and castrated males (Soto et al., 2018; Aymerich et al., 2020; Becker et al., 2022), immunocastrated males (Moore et al., 2016; Alebrant et al., 2015) or evaluating the effect of digestible lysine deficiency for castrated males (Granados et al., 2019) and mixed gender (Jin et al., 2010). However, carcass improvements can be observed in this study concerning carcass yield, loin weight, and LA in pigs fed with higher digestible lysine levels than lower or deficient levels. Studies that evaluate the effect of digestible lysine levels in the diet on carcass parameters of finishing pigs are scarce or have a simplified evaluation, making it difficult to compare responses and determine optimal levels for carcass improvements.

As for meat quality (Table 3), it was observed in this study that sarcomere length, drip loss and values for color a^* and b^* responded quadratically ($P < 0.10$), while the WBSF, drip loss, thawing, cooking loss, and color value L^* were not affected ($P > 0.10$). The composition of the *Longissimus lumborum* muscle was affected in response to dietary digestible lysine levels. Intramuscular fat, determined as ether extract in the loin, and crude protein in dry matter showed a significant decreasing quadratic response ($P < 0.05$), reaching minimum values at 0.71% and 0.70% digestible lysine, respectively.

The variation in meat color, drip loss, sarcomere length, and composition found in the *Longissimus lumborum* muscle in response to dietary digestible lysine differ from findings in the literature where dietary digestible lysine has not demonstrated an effect on this muscle (Cho et al., 2012; Tous et al., 2014; Belloch et al., 2015). Sarcomere length represents the contractile unit of the muscle and has been related to meat tenderness, where sarcomere shortening is observed for dry and tough meat. Little is known about the effects of lysine supplementation on sarcomere length, but our study shows that sarcomere length can be positively affected. According to Barkley et al. (2022), the use of redness as an indicator of pork stability, as is commonly applied for beef, is not well supported. However, in his study, redder pork loins were associated with greater color stability. On the other hand, the reduction of yellowing is associated with greater color stability, being more critical for the characterization of the stability of pork than for standard assessments of loin color. The drip loss was affected by lysine levels, and in all levels the value observed was higher than average expected for this parameter. According to Fischer (2007), pork with higher drip loss was predominantly combined with a pH less than 6.2, an electrical conductivity 24 h p.m. higher than 5.0 and a loin area higher than 56 cm^2, similar to behavior observed in our study.

Changes in the composition of *Longissimus lumborum* are relevant since the amount and composition of intramuscular fat affect the organoleptic characteristics of meat (Wood et al., 2008), and there is a positive relationship between the acceptability or tenderness of pork and its intramuscular fat content (Fonti-Furnols et al., 2012). The quadratic response observed for intramuscular fat, determined as ether extract in the loin, was unexpected, as higher dietary lysine is generally associated with increased protein deposition rather than fat accumulation.

The body composition of pigs *in vivo* (Table 4) was affected in response to digestible lysine levels, with a linear increase in final fat mass and fat mass gain ($P < 0.05$). In contrast, bone mass gain obtained an increasing quadratic ($P < 0.10$). Total body fat gain increased linearly in response to digestible lysine levels ($P < 0.05$), however, no effect was observed on protein and body ash ($P > 0.10$). The higher concentration of digestible lysine in the diet led to increased body fat deposition *in vivo*, but without affecting the traits of the muscles of pigs.

In the literature, however, the evaluation of the effect of digestible lysine level on body composition is often performed indirectly by evaluating the percentage of lean carcass, loin area and backfat thickness for finishing pigs (Witte et al., 2000; Belloch et al., 2015; Soto et al., 2018; Aymerich et al., 2020) and or associated with the evaluation of chemical composition in young animals (Cho et al., 2012; Nieto et al., 2015). DXA is a valuable tool that provides precise, repeatable body composition measurements of live pigs and their carcasses (Pomar et al., 2017; Kipper et al., 2019; Kasper et al., 2021).

The DXA assessment revealed a linear effect of digestible lysine on fat mass and body fat, which was not reflected in carcass backfat thickness. This discrepancy may be attributed to the inability of DXA to distinguish lipid content from the gastrointestinal tract, organs, visceral adipose tissue, and intramuscular fat, among other components (Kasper et al., 2021). Furthermore, animals in this study exhibited higher feed intake, which can be associated with an increase in gastrointestinal tract weight. Similarly, Zomeño et al. (2022) reported that immunocastrated male pigs increased both feed intake and gastrointestinal tract weight after the second vaccine dose, highlighting the link between feed intake and digestive tract development. In addition to DEXA limitations in assessing body fat, this tool also failed to detect differences in protein gain, which were nevertheless reflected in the higher carcass yield observed in the animals. Overall, the comparison between protein and fat gains estimated by DEXA and those determined through direct carcass evaluation highlights the need for further research to fully understand the limitations of this technique.

The digestible lysine requirement for maximum ADG in this study was 0.74% and 0.82%, using the linear plateau model and linear regression, respectively (Table 2). Regarding carcass parameters, the requirement of digestible lysine for the higher weight of the cooled carcass, carcass yield, and the loin was 0.82%, and for LA was 0.71%, estimated by quadratic regression analysis.

The values found in this study are similar to Lima et al. (2023), where the level of digestible lysine estimated for immunocastrated females and males from 102 to 127 kg ranged from 0.68 to 0.76% for maximum average daily gain, depending on the statistical model. Considering lighter immunocastrated males, from 60 to 108 kg, Moore et al. (2016) estimated the requirement of digestible lysine for an average daily gain of 0.40 to 0.64%, lower than estimated in this study. On the other hand, Gonçalves et al. (2017) observed similar results when evaluating castrated male and female pigs in the same body weight range, estimating the requirement of digestible lysine for average daily gain at 0.70 to 0.75%.

The contrast in estimating the requirement of digestible lysine among the studies reinforces the need to constantly update digestible lysine levels, which, despite its nutritional relevance, needs more studies, especially in immunocastrated males. The variation in digestible lysine requirement between studies can be attributed to differences in genetic capacity for protein deposition, amino acid digestibility, or stress conditions (Kendall et al., 2007). Considering the digestible lysine consumption of 33.18 g/d and the maximum absolute response of ADG 1.79 kg/d, obtained at the dietary level of 0.74% of digestible lysine, using the models cited by Hannas et al. (2024) estimates of digestible lysine requirement of maintenance and production were established at 1.27 and 31.91 g/d, respectively, and dietary digestible lysine requirement was 17.86 g/kg of body weight gain. Muniz et al. (2019) evaluated the effect of three factorial methods (Rostagno et al., 2017; van Milgen et al., 2008; NRC, 2012) applied to performance data, obtaining a variation in the maintenance digestible lysine requirement of 1.3 to 2.2 g/d and for production of 19.7 to 28.1 g/d for immunocastrated male pigs from 105 to 130 kg. The difference in the requirement of digestible lysine for production in this study can be attributed to the superior performance in the phase evaluated as a function of the genetic potential of the pigs.

4. CONCLUSION

The digestible lysine in the diet impacted production performance and body composition in finishing immunocastrated pigs. The changes also affected carcass characteristics and meat quality. Higher digestible lysine levels and intake can improve performance, carcass characteristics, and body fat deposition. Therefore, an accurate recommendation of digestible lysine in the diet is necessary to obtain a carcass and or a specific cut with the required characteristics for processing most efficiently. The level of 0.74% of digestible lysine is recommended to maximize the responses of weight gain and carcass characteristics of individually housed immunocastrated pigs from 100 to 140 kg. Considering the response to maximum weight gain, an intake of 33.18 g/d of digestible lysine or 17.89 g of digestible lysine per kg of body weight gain for immunocastrated male pigs with high genetic potential is estimated.

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Credit authorship contribution statement

Joyce Barcellos: Data curation, Methodology, Writing –original draft. Davi Pimentel Cardoso: Methodology. Gabriel Ribeiro Braga: Methodology. Jenifer Maira Lima Ramos: Methodology. Erica Beatriz Schultz: Methodology, Writing – review & editing. Mario Luiz

Chizzotti: Methodology, Writing – review & editing. Melissa Izabel Hannas: Conceptualization, Supervision, Writing – review & editing.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request**.**

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Table 1 - Ingredients and the calculated composition of energy and nutrients†

Ingredients	0.55%	0.82%
Corn (7.88%)	82.58	82.01
Soybean meal (45%)	15.20	15.20
Limestone	0.74	0.74
Dicalcium phosphate	0.72	0.72
Salt	0.36	0.36
Vitamin premix‡	0.20	0.20
Mineral premix§	0.15	0.15
Choline chloride (60%)	0.03	0.03
Antioxidant	0.01	0.01
L-lysine HCl	0.02	0.37
L-threonine	-	0.11
DL-methionine	-	0.08
L-tryptophan	-	0.04
L-valine	-	0.01
Calculated composition of energy and nutrients		
Metabolizable energy, (ME, Kcal/kg)	3,259.04	3,266.56
Net energy, (NE, Kcal/kg)	2,578.60	2,562.84
Dig. Protein (%)	11.58	11.85
Calcium (%)	0.52	0.52
Total phosphorus (%)	0.41	0.41
Dig. phosphorus (%)	0.21	0.21
Dig. lysine (%)	0.55	0.82
Dig. methionine (%)	0.20	0.28
Dig. methionine + cis (%)	0.42	0.49
Dig. threonine (%)	0.43	0.53
Dig. tryptophan (%)	0.13	0.16

† These two diets were blended to achieved 0.55, 0.61, 0.68, 0.75, and 0.82% digestible lysine levels.
‡ Provided per kilogram of premix: Zn 25 g, Cu 40 g, Fe 15 g, Mn 13 g, I 350 mg.
§ Provided per kilogram of premix: Se 75 mg, Folic acid 125 mg; Niacin 825 mg, Biotin 12.5 mg, Pantothenic acid 4,000 mg; Vitamin A 2,100,000 IU, Vitamin B1 250 mg, Vitamin B12 6000 mcg, Vitamin B2 1,350 mg, Vitamin B6 250 mg, Vitamin D3 350,000 IU, Vitamin E 5,000 IU, Vitamin K3 850 mg.

Table 2 - Effect of digestible lysine levels on performance and carcass characteristics of immunocastrated finishing male pigs

	Digestible lysine levels (%)						*P*-value	
Itens[†]	0.55	0.61	0.68	0.75	0.82	SEM[‡]	Linear[§]	Quadratic
Performance								
Initial BW (kg)	98.48	99.05	97.98	98.82	98.30	0.836	0.8197	0.9290
Final BW (kg)	130.40	130.95	131.22	132.70	131.56	0.855	0.0788	0.5367
ADG (kg)	1.68	1.68	1.75	1.79	1.75	0.045	0.0848	0.5008
ADFI (kg)	4.23	4.38	4.56	4.43	4.60	0.107	0.0232	0.5037
F:G (kg/kg)	2.53	2.61	2.61	2.49	2.61	0.070	0.6296	0.9802
DLys. intake (g/kg)	23.07	26.9	31.05	33.19	37.60	0.749	<0.001	0.7638
Lys:ADG (g/kg)	12.95	15.21	17.01	17.90	20.81	0.486	<0.001	0.8257
Carcass characteristics								
Hot carcass (kg)	104.95	105.93	105.40	106.39	106.67	0.885	0.0831	0.9581
Chilled carcass (kg)	102.25	103.36	102.80	103.91	104.11	0.714	0.1276	0.8774
Carcass yield (%)	77.31	77.41	77.86	77.73	78.60	0.417	0.0313	0.5175
Loin area (cm^2)	55.62	54.60	59.97	61.46	55.56	2.079	0.1905	0.0290
Backfat thickness (mm)	13.04	14.32	11.84	12.73	12.73	0.611	0.2644	0.7306
Temperature 24h (°C)	7.38	7.46	7.27	7.51	7.49	0.198	0.6564	0.8258
pH 24h	5.53	5.51	5.57	5.55	5.56	0.026	0.3690	0.8314
Carcass composition (%)								
Ham	13.50	13.49	13.45	14.09	13.45	0.170	0.3725	0.3874
Loin	6.74	6.85	6.89	7.28	6.99	0.139	0.0463	0.4174
Shoulder	7.57	7.36	7.47	7.42	7.22	0.203	0.1509	0.9485
Boston butt	3.57	3.93	3.56	3.25	3.78	0.212	0.5205	0.6469
Belly	3.73	3.69	3.63	3.95	3.67	0.164	0.8011	0.8591
Ribs	7.49	7.23	7.05	7.09	7.23	0.237	0.2878	0.1431

[†] BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed gain ratio; DLys = digestible lysine.

[‡] SEM, standard error means.

[§] A Linear Broken-Line (LBL) adjustment was observed for ADG ($P < 0.001$), where Y = 1.7677 + 0.5245 * (0.7357 - X).

Table 3 - Meat quality characteristics and composition of *Longissimus lumborum* steaks from immunocastrated male pigs in the finishing phase

	Digestible lysine levels (%)						*P*-value	
Itens	0.55	0.61	0.68	0.75	0.82	SEM[§]	Linear	Quadratic
Warner-Bratzler shear force (N)	30.88	29.44	30.96	31.31	29.89	1.924	0.9692	0.8511
Sarcomere length (µm)	1.71	1.74	1.76	1.72	1.71	0.023	0.7592	0.0844
Drip loss (%)	6.97	8.50	8.88	10.88	8.55	0.730	0.0139	0.0113
Thawing (%)	14.47	14.46	14.34	14.55	14.49	0.550	0.7656	0.8259
Cooking loss (%)	23.38	23.00	21.35	23.51	23.81	0.865	0.6237	0.1319
Lightness (*L**)	57.3	57.1	58.5	56.8	57.4	1.069	0.9802	0.7126
Redness (*a**)	8.0	7.7	6.8	7.5	8.0	0.336	0.8194	0.0167
Yellowness (*b**)	16.5	15.8	15.6	15.5	16.6	0.346	0.8884	0.0120
Chemical composition (%)								
Dry matter	25.02	25.71	25.30	25.29	25.36	0.252	0.7384	0.4101
Mineral matter[†]	4.37	4.58	4.44	4.47	4.43	0.078	0.9837	0.1583
Ethereal extract[†]	16.15	15.19	13.42	13.64	15.17	0.628	0.0901	0.0068
Crude protein[†]	84.88	83.59	82.77	83.80	83.91	0.601	0.3581	0.0496
Mineral matter[‡]	1.11	1.22	1.12	1.14	1.12	0.021	0.1767	0.0179
Ethereal extract[‡]	3.90	3.78	3.61	3.34	3.77	0.224	0.2836	0.2364
Crude protein[‡]	21.21	22.06	21.36	21.36	21.09	0.334	0.4199	0.2571

[†] Based on dry matter.

[‡] Based on natural matter.

[§] SEM, standard error means.

Table 4 - Effect of digestible lysine levels on body composition of immunocastrated finishing male pigs

	Digestible lysine levels (%)						*P*-value	
Itens (kg)	0.55	0.61	0.68	0.75	0.82	SEM[¶]	Linear	Quadratic
Body composition[†]								
Initial lean mass	85.89	82.62	84.08	85.64	82.86	0.973	0.2795	0.7334
Initial fat mass	14.04	16.90	15.17	14.28	16.16	0.795	0.7085	0.8920
Initial BMC[‡] mass	1.76	1.87	1.73	1.76	1.78	0.037	0.5621	0.9040
Final lean mass	101.63	103.12	102.03	102.77	100.11	1.120	0.3559	0.1390
Final fat mass	28.89	31.23	31.63	29.84	33.04	1.071	0.0493	0.9053
Final BMC mass	2.57	2.59	2.59	2.49	2.57	0.068	0.6577	0.9494
Productive gain								
Lean mass gain	17.68	19.35	17.44	19.26	17.17	1.092	0.7439	0.3337
Fat mass gain	13.41	14.69	16.47	15.58	17.48	0.896	0.0018	0.6374
BMC mass gain	0.70	0.82	0.84	0.69	0.76	0.046	0.9458	0.0749
Body chemical componentes[§]								
Initial body protein	16.34	15.74	16.03	16.34	15.79	0.176	0.3526	0.8656
Initial body fat	17.54	20.17	18.43	17.39	19.58	0.923	0.6315	0.9579
Initial body ash	0.39	0.40	0.38	0.39	0.39	0.007	0.5468	0.9257
Final body protein	19.54	19.84	19.62	19.77	19.24	0.224	0.3507	0.1443
Final body fat	35.84	37.19	37.66	33.45	38.76	1.232	0.5952	0.4890
Final body ash	0.55	0.55	0.55	0.52	0.55	0.015	0.3697	0.6691
Productive gain								
Body protein gain	3.54	3.87	3.49	3.85	3.43	0.218	0.7407	0.3315
Body fat gain	14.51	17.16	19.24	17.33	20.41	0.984	< 0.001	0.3847
Body ash gain	0.15	0.17	0.18	0.15	0.17	0.011	0.4312	0.2556

[†] Body composition was obtained by DXA assessment.

[‡] BMC = bone mineral content.

[§] Body chemical components were calculated using the equations of Kasper et al. (2021).

[¶] SEM, standard error means.