

LUCAS MEDINA TEIXEIRA

**SUPPLEMENTATION OF FUNCTIONAL AMINO ACIDS AND MINERALS IN
DIETS FOR GROWING-FINISHING PIGS**

Dissertation presented to the Universidade Federal de Viçosa, as part of the requirements of the Graduate Program in Animal Science, to obtain the title of *Magister Scientiae*.

Adviser: Gabriel Cipriano Rocha

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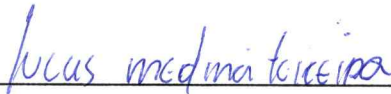
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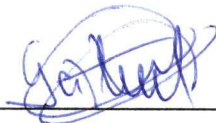
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ABSTRACT

TEIXEIRA, Lucas Medina, M.Sc., Universidade Federal de Viçosa, July, 2023. **Supplementation of functional amino acids and minerals in diets for growing-finishing pigs.** Adviser: Gabriel Cipriano Rocha.

This study aimed to evaluate the effect of functional amino acid and mineral supplementation on growth performance, carcass characteristics, meat quality, and fatty acid profile of finishing pigs. 120 castrated male pigs with an initial weight of 59.7 ± 2.7 kg were used, distributed into four diets: control diet (C); amino acids (A), C + arginine and leucine; Minerals (M), C + chromium picolinate and magnesium oxide and A + M. From 127 to 159 days, treatment A showed higher ADG and AC compared to group C. Pigs fed A + M showed better AC compared to group C. For the total period (98 to 159 days), the group fed with diet A showed higher GPD and final weight in relation to group C. Pigs fed with A + M showed greater insulin release, lower serum urea nitrogen concentration, and less subcutaneous fat deposition. The quality of the meat was affected by the treatments, pigs fed diet A showed less water loss during defrosting and total, in addition to a greater amount of oleic fatty acid. Pigs fed the M diet had greater amounts of oleic fatty acid and monounsaturated fatty acids compared to C. In summary, it has been shown that amino acid supplementation affects meat performance and quality, while mineral supplementation only affects meat quality. of the meat. The combination of amino acids and minerals improves performance and carcass characteristics.

Keywords: Arginine. Leucine. Chrome. Magnesium. Performance. Meat quality.

RESUMO

TEIXEIRA, Lucas Medina, M.Sc., Universidade Federal de Viçosa, julho de 2023. **Suplementação de aminoácidos funcionais e minerais em dietas para suínos em crescimento e terminação.** Orientador: Gabriel Cipriano Rocha.

Este estudo teve como objetivo avaliar o efeito da suplementação de aminoácidos funcionais e minerais sobre o desempenho de crescimento, características de carcaça, qualidade de carne e perfil de ácidos graxos de suínos em terminação. Foram utilizados 120 suínos machos castrados com peso inicial de $59,7 \pm 2,7$ kg, distribuídos em quatro dietas: dieta controle (C); aminoácidos (A), C + arginina e leucina; Minerais (M), C + picolinato de cromo e óxido de magnésio e A + M. Dos 127 aos 159 dias, o tratamento A apresentou maior GPD e CA em relação ao grupo C. Os suínos alimentados com A + M apresentaram melhor CA em relação ao grupo C. Para o período total (98 a 159 dias), o grupo alimentado com a dieta A apresentou maior GPD e peso final em relação ao grupo C. Suínos alimentados com A + M apresentaram maior liberação de insulina, menor concentração sérica de nitrogênio ureico e menor deposição de gordura subcutânea. A qualidade da carne foi afetada pelos tratamentos, suínos alimentado com a dieta A apresentaram menor perda de água no descongelamento e total, além de maior quantidade de ácido graxo oleico. Já suínos alimentados com a dieta M tiveram maior quantidade de ácido graxo oleico e ácidos graxos monoinsaturados em comparação ao C. Em resumo, foi demonstrado que a suplementação de aminoácidos afeta o desempenho e a qualidade da carne, enquanto a de minerais afetam apenas a qualidade da carne. Já a combinação de aminoácidos e minerais melhora o desempenho e a característica de carcaça.

Palavras-chave: Arginina. Leucina. Cromo. Magnésio. Desempenho. Qualidade de carne.

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1. INTRODUCTION

The slaughter weight of pigs has increased significantly over the years, the approximate average slaughter weight went from 110 kg in 2003 to 123 kg in 2023 (IBGE Sidra, 2023), in addition, weight is an important factor, affecting the profitability of the pork production chain and the quality of the meat produced (Kim et al., 2005; Soares et al., 2022). However, increased slaughter weight may result in reduced feed efficiency, greater deposition of subcutaneous fat, and worsens in carcass characteristics (Čandek-Potokar et al., 1998; Park et al., 2011; Choi et al., 2016).

On the other hand, heavier slaughtered pigs, with a weight close to 130 kg, may increase the lean tissue yield compared to lighter animals (Choi et al., 2016). In addition, there is a high correlation between subcutaneous fat deposition and intramuscular fat, which is associated with improving marbling, providing succulence, tenderness, and flavor to pork. One of the potential methods to improve performance and reduce subcutaneous fat deposition in pigs slaughtered at heavier weight is including feed additives in the diets, such as functional amino acids and minerals.

Leucine (Leu) and arginine (Arg) in isolation supplementation affect performance (Tan et al., 2009; Yin et al., 2010) and improve carcass traits as well as the meat quality of growing-finishing pigs. Similarly, 1% supplementation of both Arg and Leu reduced body fat in finishing pigs (Hu et al., 2019).

Leucine acts by regulating protein synthesis and degradation in skeletal muscle and also in regulating energy balance (Dardevet et al., 2000; Li et al., 2011). In addition, Leu can increase the secretion of hormones such as insulin and act by modulating adipose tissue (Madeira et al., 2014a; Hu et al., 2019). Hyun et al. (2003) verified that the supplementation of high levels of Leu in the diets increases the intramuscular fat content of growing-finishing pigs. Likewise, Arg acts by stimulating the synthesis and deposition of proteins in the muscle, in addition to promoting greater release of insulin and growth hormone (Cochard et al., 1998; Yao et al., 2008). Additionally, Arg can act by modulating the adipose tissue and the fatty acid profile (Tan et al., 2011), culminating in increased intramuscular fat content (Ma et al., 2015).

Regarding minerals, chromium (Cr) supplementation can potentiate insulin signaling in cells, in addition to increasing the uptake of amino acids and glucose in tissues (Evans & Bowman, 1992), consequently improving pig performance. Additionally, Cr supplementation in diets for growing-finishing pigs improved weight gain and feed conversion (Lindemann et al., 2008; Valente Júnior et al., 2021). Furthermore, it was shown that Cr increased ribeye area

and improved nutrient absorption (Kornegay et al., 1997; Marcolla et al., 2017). Magnesium (Mg) supplementation, on the other hand, can reduce the negative effects of pre-slaughter management, due to the reduction in the release of catecholamines, resulting in better meat quality (D'Souza et al., 1998). Also, Mg may act by modulating the fatty acid profile, as demonstrated in mice and pigs (Mahfouz & Kummerow, 1989; Albuquerque et al., 2019).

However, there is a scarcity of studies that evaluate the effect of functional amino acids (Arg + Leu) and mineral (Cr + Mg) supplementation, alone or in combination, especially in diets free of additives that improve feed efficiency and act by modifying the carcass of finishing pigs, such as ractopamine.

Thus, the hypothesis is that the supplementation of functional amino acids (Arg + Leu) and mineral (Cr + Mg), alone or in association, improves performance, carcass traits, meat quality and acts by modulating the fatty acid profile of finishing pigs. Therefore, the objective was to evaluate the effect of functional amino acids and minerals supplementation on growth performance, carcass trait, meat quality and fatty acid profile of finishing pigs.

2. MATERIAL AND METHODS

All methods involving the handling of pigs followed the ethical principles of animal research (CONCEA) and were approved by the Commission of Ethics in the Use of Production Animals (CEUAP) of the Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG (protocol 16/2021).

2.1. Animals, experimental design, and diets

A total of 120 castrated males (AGPIC 415 × Camborough) aged 98 days and with initial body weight (BW) of 59.7 ± 2.7 kg were assigned to a randomized block design according to the initial weight of the animals, in four diets with ten replicates and three pigs per experimental unit, represented by the pen.

Pigs were fed with corn-soybean meal-based diets (Table 1), formulated to meet the nutritional requirements of pigs from 98 to 127 and 128 to 159 days of age, according to Rostagno et al. (2017).

Diets consisted of a Control diet (C), Amino acids (A), C + arginine (L-arginine, purity > 99%) and leucine (L-leucine, purity > 99.5%), Minerals (M), C + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO) and A + M.

Pigs were housed in 2.50 × 1.50 m concrete-floored pens with a dry feeder and a nipple drinker each. Pigs had free access to feed and water throughout the 61-d feeding trial. The temperature and humidity inside the shed were recorded daily throughout the experimental period at every hour of the day with the aid of a data logger. During the experimental period, the maximum average temperature was $31.4 \pm 2.25^{\circ}\text{C}$ and the minimum was $22.7 \pm 1.21^{\circ}\text{C}$ and the average humidity was $81.7 \pm 10.9\%$.

During the trial, feed was weighed each day before feeding and feed wastage and leftovers were manually collected and daily weighed to determine average daily feed intake (ADFI). At 98 (beginning of the trial), 127, and 159 days of age (end of the trial), pigs were individually weighed to determine final body weight (BW), average daily gain (ADG), and feed conversion ratio (FC).

2.2. Blood sampling and serological analysis

At the end of the trial, pigs were submitted to 12-hour fasting with free access to water. After fasting, blood was collected in 10 mL tubes without anticoagulants by puncturing the orbital sinus using hypodermic needles (40 x 1.6 mm) to determine the concentration of serum urea nitrogen (SUN), triglycerides, total cholesterol, IGF-1 (Somatomedin C) and insulin. SUN concentration was evaluated using the automated enzymatic method, whereas the concentration of triglycerides and total cholesterol were determined using the enzymatic colorimetric method (Ureal Cobas C311, Linklab, software PNCQ, Roche Diagnostics, Indianapolis, IN). To evaluate the concentration of IGF-1 and insulin, the method used was chemiluminescence (IMMULITE, Siemens and Attelica - IM Analyser, Siemens, respectively).

2.3. Slaughter procedures and tissue sampling

After blood sampling, pigs were transported (4 km) in an appropriate vehicle to a commercial slaughterhouse.

One pig per pen, with BW closest to the average BW of the three pigs in the respective pen was slaughtered. The animals were stunned by the electronarcosis technique, bled, and subsequently eviscerated.

After slaughter, the carcasses were divided longitudinally and refrigerated at 5°C for 24 h. All measurements (pH, temperature, backfat thickness, and loin muscle area) were performed on the left half of each carcass. The temperature and pH of *Longissimus dorsi* (LD) were measured at 45 min and 24h after slaughter, using a pH meter with penetration probe and

thermometer coupled (Testo SE & Co., Lenzkirch, FR, Germany) inserted into the LD at the level of the last lumbar vertebra.

For meat quality, a 20 cm sample was collected after a postmortem chill of 24 h. Samples were obtained from the LD of each left half of the carcasses, between the tenth rib and the first lumbar vertebra. Upon collection, LD samples received a craniocaudal identification and then vacuum packed, stored at -20°C for 24 h, and then sectioned into five 2.54 cm thick chops. The 2.54 cm thick chops were individually vacuum packed, identified according to the pig and the position in the muscle from which they originated, and stored at -20°C for further analysis (Bridi, 2006).

2.4. Carcass traits

After 24h cooling, half carcasses were divided at the height of the 10th rib, and the backfat thickness over the LD (6 cm away from the midline) was measured using a digital caliper. To determine loin muscle area (LMA), the muscular surface of the LD between the 10th and 11th rib was covered with a polyethylene sheet and contoured using a permanent fine-tipped marker. The sheets were digitally scanned and colored. Colored areas within the contour were measured using image analysis software (ImageJ version 1.49 t, National Institutes of Health, Bethesda, MD).

2.5. Pork quality

For the determination of water losses, the analyses were performed as described by Soares et al. (2022). Frozen samples were removed from the plastic packaging, weighed, and placed to thaw at 4°C for 16 h. After thawing, the samples were gently wiped dry using a paper towel and weighed. Thaw water loss was considered to be the gravimetric difference between the steaks before and after thawing. For cooking water loss, the thawed chops were vacuum packed and cooked in a digital water bath with a stirrer (WEALAB) at 71°C for 40 minutes. After this time, the samples were removed from the water bath and placed in an ice bath for 10 minutes to stop cooking. At the end of cooking, the samples were weighed again and the cooking water loss was expressed as a percentage of the weight of the samples before and after cooking. The sum of water losses was estimated by the difference in weight between the frozen and cooked samples.

After weighing, cooked chops were used for Warner-Bratzler shear force (WBSF) determination as proposed by American Meat Science Association (2016) with minor

modifications. From each sample, 6 round cores measuring 1.27 cm in diameter were removed parallel to the longitudinal orientation of the muscle fibers, using a sharp stainless steel coring device. Care was taken to avoid sampling at areas containing visible fat and connective tissue. These round cores were sheared once through the center, perpendicularly to the longitudinal orientation of the muscle fibers, using a V notch blade with 1.016 mm thickness and 60° angle at a fixed speed of 20 m/min, coupled to a Warner-Bratzler Shear machine (G-R Electrical Manufacturing Company, Manhattan, KS). The WBSF was determined by the average of 6 measures and expressed in Newtons (N).

For color evaluation, samples of chops were thawed at a temperature of 4°C for a period of 12 hours, and removed from the packages, leaving them exposed to oxygen for 30 min. After blooming period, meat color was determined using a handheld spectrophotometer (Hunter MiniScan EZ, 4500L; Hunter Associates Laboratory, Inc., Reston, VA), calibrated against a white and a black tile. The mean L* (lightness), a* (redness), and b* (yellowness) values of each chop were determined as the average from 3 readings on 3 different points of chop surface, using illuminant D65, a 31.8 mm port size and a 10° standard observer.

To determine intramuscular fat (IMF) chops (2.54 cm thick) were thawed at 4°C for 16 hours and hand trimmed for visible fat and connective tissues, and then ground in a TURRAX CT-132 tissue homogenizer. A 100 g-sample of each chop was evaluated for IMF using near infrared spectrophotometry (FoodScan, FOSS NIR systems Inc., Laurel, MD; AOAC, Official method 2007.04; AOAC, 2007).

Fatty acid extraction and determination from the samples were followed as previously described by Ribeiro et al. (2021). The composition of total fatty was determined based on Ichichara and Fukubayashi (2010) and Guihéneuf et al. (2015). Fat acids methyl esters (FAMES) were prepared by derivatization of lipids extracted from LD. Briefly, 25 mg of samples were transferred into tubes. Then, 2 mL of 3% sulfuric acid in methanol were added. Tubes were capped and heated in a dry bath under stirring (Labnet, D1200-230V, USA) at 90°C and 90 min. After cooling down at room temperature, 2 mL of hexane (HPLC grade, Sigma Aldrich, San Luis, Missouri, USA) and 1 mL of deionized water were added to the system that was vortexed (Phoenix Luferto, AP-56, Brazil), to promote the extraction of FAMES. After phase separation, the upper phase (1 mL) was transferred to a flask (Eppendorf, Germany) containing 0.05 g of anhydrous sodium sulfate (VETEC Quimica Fina, Rio de Janeiro, Brazil). The mixture was centrifuged at $5,000 \times g$ for 5 min at 25°C. A volume of 200 µL of supernatant was removed and added to the appropriate vial with 800 µL of hexane. Finally, FAMES were

quantified by gas chromatography analysis (GC-FID Shimadzu, 2010, Japan), using a 100 m x 0.25 mm capillary column (SP-2560, Sigma Aldrich, USA). The fatty acids were identified through the retention times of the sample FAMES compared to the retention times of the FAME standard calibration mix (Supelco® 37 Component FAME Mix, Sigma Aldrich, USA).

2.6. Statistical analysis

For performance data analysis the pen was considered the experimental unit. One pig per pen was considered the experimental unit for the other analysis. The normality of experimental errors was evaluated using Shapiro-Wilk. Statistical analysis was performed using the SAS 9.4 GLM procedure (SAS Inst., Inc., Cary, NC, USA). Data were subjected to analysis of variance (ANOVA). In the case of significant differences, treatments were compared to control group diets, by the Dunnett test. Differences were considered significant at $\alpha = 0.05$.

3. RESULTS

3.1. Performance and serological analysis

From 98 to 127 d of age, the diets did not affect ($P > 0.05$) pigs performance (Table 2). From 127 to 159 d of age, pigs fed A diet had improved ADG ($P < 0.01$) and FC ($P = 0.04$) compared to diet C. Moreover, pigs fed A + M diet had improved FC ($P < 0.01$), compared to C. In the total experimental period, from 98 to 159 d of age, pigs fed with A diet had higher ADG ($P = 0.02$) and final BW ($P = 0.02$) compared to pigs in the C group.

Pigs fed A diet had higher ($P < 0.01$) concentration of SUN (Table 3). However, there was no effect ($P > 0.05$) of the diets on total cholesterol and triglycerides.

Supplementation with the A + M resulted in higher insulin concentration ($P < 0.01$) compared to C diet (Table 3). There was no effect ($P > 0.05$) of the diets on IGF-1.

3.2. Carcass traits and pork quality

Pigs fed A + M diet had lower ($P < 0.01$) BF compared to the C group (Table 4). There was no effect ($P > 0.05$) of the diets on carcasses pH, temperature and LMA.

Pigs fed diet A had lower ($P < 0.01$) thaw water loss and lower ($P = 0.04$) sum of water losses compared to group C. There was no effect ($P > 0.05$) of diets on color, shear force, and IMF (Table 5).

Pigs fed A diet had higher ($P = 0.04$) percentage of oleic fatty acid in LD muscle (C18:1-n9) compared to the C group (Table 6). In addition, pigs fed M diet had higher ($P < 0.01$) percentage of oleic fatty acid and monounsaturated fatty acids (MUFA) compared to C group.

4. DISCUSSION

The slaughter of heavy pigs can result in low feed efficiency and compromised carcass quality (Tan et al., 2009; Park et al., 2011; Zhang et al., 2011). In order to improve growth performance and maintaining or improving carcass traits nutritional strategy such as the supplementation functional amino acids and minerals can be used.

In the present work, functional amino acids and minerals did not affect pig performance in the initial experimental period (98 to 127 d). On the other hand, in the final experimental phase (127 to 159 d), Arg + Leu supplementation improved the performance of finishing pigs. The improved FC of pigs fed Arg + Leu was directly related to the higher ADG, since the ADFI was not influenced by the treatment. Therefore, the higher ADG may be the result of higher protein deposition provided by Arg + Leu supplementation, both amino acids responsible for activating key enzymes in protein synthesis and deposition (Dardevet et al., 2000; Yao et al., 2008).

Dardevet et al. (2002) reported that the physiological effects of amino acids are more effective at higher supplementation levels, especially in older animals. In the present study, despite amino acid supplementation with levels above those recommended by Rostagno et al. (2017) we did not find improvement in the performance of pigs in the initial period (98 to 127 d). However, in the final phase (127 to 159 d) and in the whole experimental period (98 to 159 d), we found better performance of the animals supplemented with Arg + Leu. This would indicate that the response to amino acid supplementation might be time-dependent.

On the other hand, supplementing the combination of Arg and Leu, Tous et al. (2016) did not find improvement in the growth performance of finishing pigs. Compared to the present study, the difference in results may be due to the higher level of amino acid supplementation (0.6% of L-arginine and 1.4% of L-leucine) used by those authors. Excess Leu can result in antagonism with other branched-chain amino acids, due to the increase in catabolic enzymes, which can reduce the plasma concentration of amino acids such as isoleucine and valine, reducing animal performance (Smith & Austic, 1978). In fact, as shown by Hyun et al. (2003), 2.0% Leu supplementation decreased ADG of finishing pigs.

Supplementation of Arg + Leu in combination with Cr + Mg had an additive effect improving the FC from 127 to 159 d. As there was no effect on ADFI and ADG, the observed improvement in FC allows us to infer that the A + M diet changed the composition of the animal's weight gain, providing greater deposition of muscle tissue in relation to fat. This inference is consistent with the lower BF observed in A + M diet. This is because Cr and Mg

act by influencing lipid deposition. Chromium increases the sensitivity of tissues to insulin, as a result of which there is a greater uptake of glucose by cells, which will be transformed into energy later. This greater contribution energy favors the gain of lean mass and reduces fat content (Clarkson, 1997; Matthews et al., 2001; Jackson et al., 2009; Zhang et al., 2011). Additionally, Apple et al. (2000) suggested that a greater supplementation of Mg in diets for growing and finishing pigs acts by improving the energy efficiency of the animals, and may contribute to the reduction of lipid deposition in the carcass.

The efficiency rate of nitrogen utilization for protein synthesis can be evaluated through the concentration of SUN (Bush et al., 2002). On the other hand, we observed that pigs fed Arg + Leu had a higher concentration of SUN even though they had a higher ADG. This probably occurred due to the excess of amino acids provided by the diet, this excess is not stored and is metabolized in the urea cycle and nitrogen is then excreted (Rezaei et al., 2013).

It was expected that pigs supplemented with Arg + Leu would have a higher concentration of blood insulin based on the reported effects of these amino acids on increasing insulin secretion (Yao et al., 2008; Madeira et al., 2014a). However, in the present study, a higher insulin concentration was observed only with the combination of Arg + Leu and Cr + Mg. Chrome can potentiate insulin signaling and increase membrane fluidity (Evans & Bowman, 1992), but does not necessarily act by stimulating insulin release (Lien et al., 1998). Additionally, Evans & Bowman (1992) report that supplementation with Cr picolinate increased Leu and glucose uptake by rat muscle cells. Thus, Cr can act by enhancing the action of amino acids, mainly Leu, resulting in greater insulin release. In addition, BF was significant lower in pigs supplemented with A + M, indicating that pigs may had less glucose being stored as fat.

The decline in pH and temperature of the post-mortem carcass is important and has been used as an indicator of pork quality, directly influencing color and water holding capacity (WHC), being decisive for tenderness and juiciness, as well as acceptance of the product consumer (Castellini et al., 2002; Scheffler & De Gerrard, 2007; Marcolla et al., 2017). In the present study, although supplementation with treatments A and A + M improved the performance of finishing pigs, the additives did not influence the pH and temperature of the carcass, in addition to not having influenced the shear force (Warner-Bratzler).

Tenderness and juiciness are the most important characteristics for pork meat palatability (De Vol et al., 1988), tenderness can be measured by the content of IMF and WBSF, with a negative correlation between both analyses (Van Laack et al., 2001; Alonso et al., 2010).

In the present study, no treatment effects were observed regarding WBSF, indicating that both functional amino acids and minerals do not have a deleterious effect on the tenderness of pork.

Furthermore, we did not observe differences between treatments in IMF content. Our hypothesis was that supplementation of functional amino acids would increase the IMF content of finishing pigs. This is because Leu is capable of donating its carbon skeleton for the formation of Acetyl-CoA, a precursor for the synthesis of fatty acids in muscle tissue (Hyun et al., 2007). Like Leu, Arg can act by increasing the content of IMF, as it contributes to lipogenesis in muscle tissue by regulating the expression of metabolic genes (Tan et al., 2011). Previous studies by Hyun et al. (2003) supplementing 2% Leu and Ma et al. (2015) supplementing 1% Arg, unlike the present study, reported increased IMF content. This difference in results may be associated with the level of inclusion of amino acids, as discussed previously, high levels of Leu cause catabolism of other amino acids, such as isoleucine and valine, which may worsen the animals' performance. In fact, in the work mentioned above, supplementation with 2% Leu increased the IMF content and, on the other hand, worsened the performance of the animals.

On the other hand, Arg + Leu supplementation reduced thawing water loss and total water loss (thawing loss plus cooking loss). Interestingly, in our study, we observed this higher WHC without any effect on the pH and color of the meat. It is known that WHC is directly influenced by pH, which directly affects the structures of myofibrils, changing WHC, and also the color of the meat (Castellini et al., 2002).

Based on these results, we can infer that Arg + Leu supplementation increased WHC in meat. This greater retention capacity may indicate that meat from pigs fed Arg + Leu is juicier. In addition, the greater water retention capacity has aroused great interest in the industry, as it can affect meat processing and storage (Rosenvold et al., 2003).

In the present study, supplementation with functional amino acids and minerals was able to modulate the fatty acid profile in the LD of finishing pigs.

Initially, fatty acid research focused mainly on porcine adipose tissue due to its diversity of fatty acids. Currently, there is an increased interest in the composition of the fatty acid profile in muscle tissue, which is of great importance, as it can affect human health (Martin et al., 2006; Cai et al., 2010). In addition, the fatty acid profile directly affects the quality of the meat. Unsaturated fatty acids have greater oxidizing capacity and can negatively affect the shelf life of pork (Wood et al., 2004).

In the present study, pigs fed the diet supplemented with Arg + Leu had higher percentages of oleic fatty acid. In agreement, Tan et al. (2011) supplementing 1% of Arg in the diet of growing and finishing pigs, found higher percentages of oleic fatty acid, and suggested that Arg activates important enzymes in the formation of oleic fatty acid, such as stearoyl CoA desaturase (SCD-1) (Smith et al., 1999). However, Madeira et al. (2014b) reported no effect of Arg and Leu supplementation on the fatty acid profile of growing pigs.

The supplementation of minerals also affected fatty acid composition, pigs fed diets with Cr + Mg had higher percentages of oleic fatty acid and MUFA. Mg modifies the fatty acid profile of finishing pigs (Albuquerque et al., 2019) and can act as a cofactor for several fatty acid desaturases, such as delta 6-desaturase and delta 9-desaturase. Using rats, Mahfouz & Kummerow (1989) demonstrated that Mg deficiency can affect the fatty acid profile through the reduction of delta 6-desaturase, responsible for the desaturation of fatty acids. Furthermore, in the same study, magnesium-deficient rats had reduced levels of oleic fatty acid. This may suggest that Mg deficiency also leads to a decrease in the activity of the enzyme delta 9-desaturase, responsible for the formation of MUFA, such as oleic fatty acid, from saturated fatty acids (Ntambi & Miyazaki, 2003; Nakamura & Nara, 2004).

The modulation of the lipid profile of pigs through Cr supplementation still poorly understood. As in the present study, other studies in the literature supplementing Cr also found higher concentrations of oleic fatty acid and MUFA. However, they did not observe any effect of Cr supplementation in relation to the activity of the enzyme delta-9-desaturase in LD (Bučko et al., 2013; Alencar et al., 2021). A possible explanation for the modulation of the lipid profile by Cr would be the increased expression of genes related to the synthesis of unsaturated fatty acids, such as the SCD-1 gene, which was increased as the dose of Cr increased (Sadeghi et al., 2015). Differing from the present study, Tian et al. (2015) and Untea et al. (2017) supplementing Cr, did not observe an increase in oleic fatty acid and MUFA levels, although they observed a reduction in saturated fatty acids. Differences in results can be attributed to differences in Cr bioavailability, which can be influenced by the source used (Caramori et al., 2017), as well as time and level of inclusion.

5. CONCLUSION

Arg + Leu improves growth rate of finishing pigs. Arg + Leu or Cr + Mg alters fatty acid profile in LD and when combined results in lower backfat thickness.

6. REFERENCES

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Table 1 - Composition of control diets

Ingredient, %	98-127 d	127-159 d
Corn, 7,8%	80.3	83.3
Soybean meal, 46,0%	15.7	13.5
Soybean oil	0.91	0.73
Dicalcium phosphate	0.76	0.57
Limestone	0.58	0.50
Salt	0.39	0.37
L-lysine HCl, 98.5%	0.47	0.38
DL – methionine, 99.0%	0.13	0.08
L – threonine, 98.5%	0.17	0.11
L – tryptophan, 98.0%	0.05	0.04
L – valine, 96.5%	0.07	0.02
Mineral premix	0.15	0.15
Vitamin premix	0.20	0.20
Antibiotic ¹	0.05	0.05
Calculated nutritional composition of experimental diets ²		
EM (kcal/kg)	3350.0	3350.0
Crude protein (%)	14.50	13.50
SID ³ Lys (%)	0.927	0.805
SID Met+Cys (%)	0.547	0.483
SID Thr (%)	0.603	0.523
SID Trp (%)	0.185	0.161
SID Val (%)	0.640	0.555
SID Leu (%)	1.194	1,148
SID Arg (%)	0,777	0,715
Sodium (%)	0.176	0.165
Calcium (%)	0.575	0.497
Available phosphorus (%)	0.341	0.301
Chrome (mg/kg) ⁴	0.360	0.360
Magnesium (dag/kg) ⁴	0.139	0.137

¹Antibiotic = Provided per ton of diet: 0.2% Florfenicol Amphenor (Sanphar).

²Values calculated according to Rostagno et al. (2017). All diets contained phytase (0.005 % of Natuphos® Basf enzyme).

³SID = standardized ileal digestible.

⁴Mineral analysis values of control diets.

Table 2 - Performance of pigs fed different combination of functional amino acids and minerals

Item	Treatments				SEM	<i>P</i> -value*		
	C	A	M	A+M		C x A	C x M	C x A+M
IBW, Kg	59.2	59.2	59.2	59.2	0.12	0.33	0.47	0.80
127 d BW, kg	94.1	95.4	95.5	93.1	0.69	0.39	0.34	0.62
159 d BW, kg	129.1	133.8	131.5	130.0	0.05	0.02	0.36	0.92
98 to 127 d of age								
ADFI, g	2.97	3.01	3.09	2.83	0.06	0.92	0.35	0.23
ADG, g	1.20	1.25	1.25	1.17	0.02	0.38	0.31	0.61
FC	2.47	2.41	2.48	2.43	0.03	0.31	0.99	0.60
127 to 159 d of age								
ADFI, g	3.36	3.52	3.47	3.26	0.06	0.08	0.27	0.39
ADG, g	1.09	1.20	1.13	1.16	0.02	<0.01	0.59	0.16
FC	3.07	2.94	3.09	2.83	0.04	0.04	0.93	<0.01
98 to 159 d of age								
ADFI, g	3.18	3.28	3.29	3.05	0.05	0.34	0.25	0.22
ADG, g	1.14	1.22	1.19	1.15	0.02	0.02	0.34	0.99
FC	2.77	2.68	2.79	2.67	0.03	0.14	0.98	0.07

* Dunnett's test.

C = control diet; A = control diet + L-Arginine (L-arginine; purity > 99%) and L-Leucine (L-leucine; purity > 99.5%); M = control diet + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation.

IBW = initial body weight; BW= body weight; ADG = average daily gain; ADFI = average daily feed intake; FC= feed conversion ratio.

SEM = Standard error of the mean.

Table 3 - Concentration of serum metabolites of pigs fed different combination of functional amino acids and minerals

Item	Treatments				SEM	<i>P</i> -value*		
	C	A	M	A+M		C x A	C x M	C x A+M
CHO, mg/dL	86.7	91.4	85.5	85.0	3.37	0.36	0.95	0.90
TG, mg/dL	32.1	32.0	32.0	34.9	1.76	0.96	0.99	0.43
SUN, mg/dL	24.8	29.9	24.9	24.1	1.15	<0.01	1.00	0.92
IGF-1, ng/mL	168.0	147.5	161.0	169.7	9.98	0.34	0.93	0.99
Insulin, μU/mL	4.4	5.5	5.5	8.0	0.72	0.55	0.50	<0.01

*Dunnett's test ($P \leq 0.05$).

C = control diet; A = control diet + L-Arginine (L-arginine; purity > 99%) and L-Leucine (L-leucine; purity > 99.5%); M = control diet + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation.

CHO = total cholesterol; TG = triglycerides; SUN = serum urea nitrogen.

SEM = Standard error of the mean.

Table 4 - Carcass traits of pigs fed different combination of functional amino acids and minerals

Item	Treatments				SEM	P-value*		
	C	A	M	A+M		C x A	C x M	C x A+M
LMA, cm ²	57.71	58.35	60.42	60.21	1.39	0.97	0.36	0.42
BF, mm	18.32	16.58	17.74	15.68	0.63	0.10	0.82	<0.01
Temperature (°C)								
45 min	38.88	39.60	38.91	39.43	0.30	0.22	0.99	0.42
24 hours	2.69	2.65	2.66	2.29	0.44	0.99	0.99	0.20
pH								
45 min	6.43	6.30	6.36	6.45	0.06	0.09	0.53	0.93
24 hours	5.82	5.89	5.84	5.88	0.05	0.45	0.95	0.54

*Dunnett's test.

C = control diet; A = control diet + L-Arginine (L-arginine; purity > 99%) and L-Leucine (L-leucine; purity > 99.5%); M = control diet + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation.

LMA = loin muscle area; BF = backfat thickness.

SEM = Standard error of the mean.

Table 5 - Pork quality parameters evaluated in *Longissimus dorsi* of pigs fed different combination of functional amino acids and minerals

Item	Treatments					P-value*		
	C	A	M	A+M	SEM	C x A	C x M	C x A+M
TL, %	16.22	13.17	15.49	16.65	0.67	<0.01	0.71	0.91
CL, %	29.73	28.62	27.99	28.56	10.42	0.79	0.50	0.76
SL, %	45.96	41.79	43.48	45.22	1.16	0.04	0.31	0.94
WBSF, kgf	2.27	2.25	2.45	2.27	0.12	0.82	0.99	0.99
<i>L</i> *	53.01	52.61	53.13	51.23	0.59	0.93	0.99	0.09
<i>a</i> *	6.67	6.85	6.36	7.40	0.36	0.97	0.87	0.35
<i>b</i> *	14.65	14.83	14.76	14.70	0.28	0.94	0.98	0.99
IMF, %	2.64	2.97	2.79	2.38	0.22	0.58	0.94	0.72

*Dunnett's test ($P \leq 0.05$).

C = control diet; A = control diet + L-Arginine (L-arginine; purity > 99%) and L-Leucine (L-leucine; purity > 99.5%); M = control diet + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation.

TL = thaw water losses; CL = cooking water losses; SL = sum of water losses; WBSF = Warner Bratzler shear force; IMF = intramuscular fat content.

SEM = Standard error of the mean.

Table 6 - Fatty acid profile in *Longissimus dorsi* of pigs fed different combination of functional amino acids and minerals

Item, %	Treatments					P-value*		
	C	A	M	A+M	SEM	C x A	C x M	C x A+M
C14:0	1.53	1.45	1.43	1.50	0.05	0.28	0.17	0.69
C16:0	25.73	24.67	25.03	25.31	0.48	0.12	0.31	0.53
C16:1	2.34	2.25	2.52	2.55	0.13	0.62	0.32	0.25
C17:0	0.20	0.22	0.24	0.25	0.02	0.48	0.18	0.13
C17:1	0.22	0.22	0.28	0.28	0.02	0.90	0.11	0.10
C18:0	12.73	12.71	11.98	11.90	0.40	0.96	0.18	0.14
C18:1 n9	43.78	45.20	45.94	44.70	0.48	0.04	<0.01	0.17
C18:2 n6	11.01	11.05	10.44	11.37	0.42	0.94	0.30	0.52
C18:3 n6	1.14	1.18	1.09	1.13	0.05	0.55	0.46	0.85
C20:2	0.38	0.37	0.39	0.38	0.02	0.71	0.85	0.90
C20:3 n6	0.36	0.38	0.46	0.44	0.06	0.77	0.13	0.23
SFA	40.35	39.29	38.83	39.10	0.65	0.25	0.10	0.18
MUFA	46.38	47.70	48.77	47.54	0.57	0.11	<0.01	0.15
PUFA	12.98	13.04	12.41	13.35	0.46	0.92	0.36	0.55

*Dunnett's test ($P \leq 0.05$).

C = control diet; A = control diet + L-Arginine (L-arginine; purity > 99%) and L-Leucine (L-leucine; purity > 99.5%); M = control diet + chromium (402 mg/kg of Cr from chromium picolinate) and magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation.

C14:0 = miristic; C16:0 = palmitic; C16:1 = palmitoleic; C17:0 = heptadecanoic; C17:1 = heptadecenoic; C18:0 = stearic; C18:1 n9 = oleic; C18:2 n6 = linoleic; C18:3 n6 = linoic;

C20:2 = eicosadienoic; C20:3 n6 = eicosatrienoic; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

SEM = Standard error of the mean.