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Supplementation of functional amino acids and minerals in diets for growing-finishing pigs

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ABSTRACT - 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. One hundred and twenty castrated male pigs with an initial weight of 59.7 ± 2.7 kg were used, distributed into four dietary treatments: control; amino acids, control + 0.5% Arg and 0.5% Leu; minerals, control + 0.1% Cr and 0.05% MgO, and amino acids + minerals. From 127 to 159 days of age, pigs fed the amino acid diet had greater average daily gain (ADG; P = 0.015), while those fed the amino acid + mineral diet showed improved feed conversion ratio (FCR; P<0.001) compared with the control diet. Across the entire experimental period (98 to 159 days), amino acid supplementation increased ADG (P = 0.043), whereas amino acid and amino acid + mineral diets improved FCR (P = 0.029). However, pigs fed the amino acid + mineral diet had lower ADG compared with pigs fed the amino acid diet. Carcass traits were largely unaffected, but pigs fed the amino acid + mineral diet had reduced backfat thickness (P = 0.015). Meat quality was influenced by amino acid supplementation, with reduced thawing loss (P = 0.001) and total water loss (P = 0.041). Regarding the fatty acid profile, amino acid and mineral diets increased oleic acid concentrations (P = 0.021), and mineral supplementation increased monounsaturated fatty acids (MUFA; P = 0.047). In summary, amino acid supplementation improved growth performance, water-holding capacity, and oleic acid concentration, whereas mineral supplementation increased the concentration of oleic acid and MUFA. The combination of amino acids and minerals improved FCR and reduced backfat thickness, but this was accompanied by lower ADG, indicating a lack of consistent synergistic benefits.

Keywords: arginine, chromium, leucine, magnesium, meat quality, swine

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1. Introduction

Pig farming has faced increasing challenges related to production efficiency, meat quality, and sustainability (Gunnarsson et al., 2020; Correia et al., 2022; Andretta et al., 2022). Increasing slaughter weight has become a common strategy to enhance profitability and meat yield (Kim et al., 2005; Soares et al., 2022), with the average slaughter weight in Brazil rising from 110 kg in 2003 to 123 kg in 2023 (IBGE, 2023). However, higher slaughter weights may reduce feed efficiency and increase

subcutaneous fat deposition, which can negatively affect carcass quality (Čandek-Potokar et al., 1998; Park and Lee, 2011). Despite this, heavier pigs (~130 kg) may yield more lean tissue and improved marbling, contributing to meat juiciness, tenderness, and flavor (Fortin et al., 2005; Zhang et al., 2022). Thus, nutritional strategies are needed to improve growth performance without increasing undesirable fat deposition.

Among the nutritional approaches explored to enhance productive efficiency, the strategic use of bioactive feed additives has gained increasing attention. Functional amino acids, particularly leucine (Leu) and arginine (Arg), together with trace minerals such as chromium (Cr) and magnesium (Mg), have been investigated for their capacity to modulate metabolic pathways related to protein accretion and lipid metabolism (Wang et al., 2009; Wu, 2010; Tarsitano et al., 2013; Albuquerque et al., 2019). Leucine and Arg have been shown to improve growth and carcass traits in pigs (Tan et al., 2009; Yin et al., 2010), largely through activation of the mTOR (mechanistic target of rapamycin) pathway, which promotes protein synthesis and inhibits muscle degradation (Cota et al., 2006; Li et al., 2011). Leucine also stimulates insulin release and modulates lipid deposition via gene expression regulation (Madeira et al., 2014a; Hu et al., 2019; Hyun et al., 2003). Similarly, Arg stimulates insulin and growth hormone release, enhancing glucose uptake for muscle anabolism and influencing intramuscular fat content and fatty acid profile (Yao et al., 2008; Ma et al., 2015; Tan et al., 2011).

Chromium supplementation improves insulin signaling and nutrient uptake, contributing to improved growth performance and carcass characteristics (Evans and Bowman, 1992; Lindemann et al., 2008; Valente Júnior et al., 2021). Magnesium, in turn, may reduce carcass fat deposition and improve meat quality by modulating catecholamine release and lipid metabolism (Tarsitano et al., 2013; Albuquerque et al., 2019).

Although the individual effects of these additives are well documented, the potential synergistic interaction between amino acids and minerals remains poorly understood in growing–finishing pigs. Therefore, this study aimed to evaluate the effect of Arg + Leu and Cr + Mg supplementation, administered alone or in combination on growth performance, carcass characteristics, meat quality, and the fatty acid profile of growing and finishing pigs.

2. Material and methods

All methods involving the handling of pigs were conducted in accordance with the ethical principles for animal research established by CONCEA and were approved by the Committee 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 surgically castrated males (AGPIC 415 × Camborough) aged 98 d with an initial BW of 59.7 ± 2.7 kg were assigned to a randomized block design. Blocking was based on initial BW, with pigs classified into two blocks (heavy and light). Within each block, animals were randomly allotted to one of the four dietary treatments. Each treatment had 10 replicates, with three pigs per pen, and the pen was considered the experimental unit. All pigs were individually identified with ear tags at the beginning of the trial.

Pigs were fed corn-soybean meal-based diets 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 (1) control diet (Table 1), (2) amino acids diet, control + 0.5% Arg (L-arginine, purity > 99%) and 0.5% Leu (L-leucine, purity > 99.5%), (3) minerals diet, control + 0.1% Cr (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO) and (4) amino acids + minerals diet, control supplemented with the same levels of amino acids and minerals as in the individual amino acid and mineral diets. The amino acids and minerals evaluated in this study were supplemented on top of the basal diet. The supplemental amino acids were based on previous studies for Leu (Yin et al., 2010;

Tous, 2016; Wang et al., 2018; Yin et al., 2022) and for Arg (Yao et al., 2008; Chen et al., 2013; Tous et al., 2016), with adaptations for the present diets and experimental phase. Chromium picolinate was supplemented based on the manufacturer's recommendations and Mg was based on previous studies (Tang et al., 2008; Albuquerque et al., 2019; Rey et al., 2021).

Pigs were housed in 2.50 × 1.50 m concrete-floored pens, each equipped with a dry feeder and a nipple drinker each. Pigs had free access to feed and water throughout the 61-d feeding trial. The pigs were housed in facilities with natural ventilation and side curtains, which allowed airflow adjustment but did not provide active temperature or humidity control. Ambient conditions were continuously

Table 1 - Composition of control diets

Item	98-127 days of age	127-159 days of age
Ingredient (%)		
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[1]	0.15	0.15
Vitamin premix[2]	0.20	0.20
Antibiotic[3]	0.05	0.05
Calculated nutritional composition of experimental diets[4,5]		
Metabolizable energy (kcal/kg)	3350.0	3350.0
Crude protein (%)	14.5 (14.8)	13.5 (13.4)
SID Lysine (%)	0.927 (0.956)	0.805 (0.849)
SID Methionine+Cysteine (%)	0.547 (0.499)	0.483 (0.508)
SID Threonine (%)	0.603 (0.600)	0.523 (0.543)
SID Trytophan (%)	0.185 (0.201)	0.161 (0.150)
SID Valine (%)	0.640 (0.835)	0.555 (0.706)
SID Leucine (%)	1.194 (1.327)	1.148 (1.192)
SID Arginine (%)	0.777 (0.641)	0.715 (0.663)
Sodium (%)	0.176	0.165
Calcium (%)	0.575	0.497
Available phosphorus (%)	0.341	0.301
Chromium (mg/kg)[6]	0.360	0.360
Magnesium (dag/kg)[6]	0.139	0.137

SID - standardized ileal digestible.
[1] Content per kilogram of premix: Fe as iron sulfate (15.0 g), Cu as copper sulfate (40.0 g), I as calcium iodate (350.0 mg), Zn as zinc oxide (25.0 g), Mn as manganese sulfate (13.0 g).
[2] Content per kg of premix: folic acid (125.00 mg), pantothenic acid (4000 mg), bioin (12.50 mg), niacin (825.00 mg), selenium (75.00 mg), vitamin B6 (250.00 mg), vitamin B2 (1350.00 mg), vitamin B1 (250.00 mg), vitamin A (2,100,000 IU), vitamin B 12 (6000.00 µg), vitamin D3 (350,000 IU), vitamin E (5,000 IU), vitamin K3 (850.00 mg).
[3] Antibiotic = Provided per ton of diet: 0.2% Florfenicol Amphenor (Sanphar), supplied only until 144 days of age, according to veterinary prescription.
[4] Values calculated according to Rostagno et al. (2017). All diets contained 50 mg/kg phytase (0.005% of Natuphos® Basf enzyme).
[5] Crude protein and total amino acids analyzed are included in the parenthesis.
[6] Mineral analysis values of control diets.

monitored throughout the experimental period using a data logger. No pigs were removed from the study, and no animals required medication during the experimental period.

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 (FCR).

2.2. Blood sampling and serological analysis

At the end of the trial, pigs were submitted to 12-hour fast with free access to water. One pig per pen, with BW closest to the pen average BW, was selected for blood sampling. After fasting, blood was collected in 10 mL tubes without anticoagulants by orbital sinus puncture using hypodermic needles (40 × 1.6 mm). The blood samples were centrifuged immediately after collection at 4000 rpm for 12 min to separate the serum. Serum was then stored at −20 °C until analysis. The serum samples were sent to the Viçosa Clinical Laboratory (Viçosa, Brazil) for determination of serum urea nitrogen (SUN), triglycerides, total cholesterol, IGF-1 (Somatomedin C), and insulin concentrations. SUN concentration was evaluated using the automated enzymatic method (Ureal Cobas C311, Linklab, software PNCQ, Roche Diagnostics, Indianapolis, IN). The concentration of triglycerides (n° 80115310039 Kovalent do Brasil Ltda, Rio de Janeiro, Brazil) and total cholesterol (n° 80115310195 Kovalent do Brasil Ltda, Rio de Janeiro, Brazil) were determined using the enzymatic colorimetric method (Ureal Cobas C311, Linklab, software PNCQ, Roche Diagnostics, Indianapolis, IN). Levels of IGF-1 (IMMULITE®, Siemens, Malvern, PA, USA) and insulin (Atellica IM, Attelica® IM Analyzer, Siemens, Malvern, PA, USA) were determined by chemiluminescent immunoassay.

2.3. Slaughter procedures and tissue sampling

After blood sampling, pigs were transported (4 km) to a commercial slaughterhouse. The pig selected for blood collection was the same animal evaluated for carcass and meat quality traits. Selected pigs were identified using a livestock pencil. The animals were stunned by the electronarcosis, exsanguinated, 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 the *longissimus thoracis* (LT) were measured at 45 min and 24 h after slaughter, using a pH meter with a penetration probe and a coupled thermometer (Testo SE & Co., Lenzkirch, FR, Germany) inserted into the LT at the level of the last lumbar vertebra.

For meat quality, a 20-cm sample was collected after a 24-h postmortem chilling period. Samples were obtained from the LT of each left half of the carcasses, between the tenth rib and the first lumbar vertebra. Upon collection, LT samples received craniocaudal identification, were 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 and Silva, 2006).

2.4. Carcass traits

After 24 h of cooling, half carcasses were divided at the height of the 10th rib, and the backfat thickness over the LT (6 cm away from the midline) was measured using a digital caliper. To determine loin muscle area, the muscular surface of the LT 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 blotted dry using a paper towel and weighed. Thawing water loss was defined as 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 min. After this time, the samples were removed from the water bath and placed in an ice bath for 10 min to stop cooking process. 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 total 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 the American Meat Science Association (AMSA, 2016) with minor modifications. From each sample, six 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 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 a thickness of 1.016 mm and 60° angle, at a fixed speed of 20 mm/min, coupled to a Warner-Bratzler shear machine (G-R Electrical Manufacturing Company, Manhattan, KS). The WBSF was determined by the average of six measurements and expressed in Newtons (N).

For color evaluation, samples of chops were thawed at a temperature of 4 °C for a period of 12 h, and removed from the packages, leaving them exposed to oxygen for 30 min. After the 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 three readings on three different points on the 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 h and hand-trimmed to remove visible fat and connective tissue, 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 were performed as previously described by Ribeiro et al. (2021). The composition of total fatty acids was determined based on Ichihara and Fukubayashi (2010) and Guihéneuf et al. (2015). Fat acids methyl esters (FAMEs) were prepared by derivatization of lipids extracted from LT. Briefly, 25 mg of sample were transferred into tubes, and 2 mL of 3% sulfuric acid in methanol was added. Tubes were capped and heated in a dry bath under stirring (Labnet, D1200-230V, USA) at 90 °C for 90 min. After cooling to 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, which was vortexed (Phoenix Luferco, 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 × 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 × 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, with BW closest to the pen average BW of the respective pen, was considered the experimental unit for the other analyses. The normality of experimental errors was evaluated using Shapiro-Wilk test. Statistical analysis was performed using the GLM procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA). Data were subjected to analysis of variance (one-way ANOVA). In the case of significant differences, treatments were compared to control group diets, by the Tukey test. Differences were considered significant at α = 0.05.

The data were analyzed according to the following model:

$$Y_{ij} = \mu + \alpha_i + b_j + e_{ij},$$

in which Y_{ij} = response variable, μ = mean, α_i = fixed effect of treatment, b_j = fixed effect of block and e_{ij} = random error.

3. Results

During the experimental period, the maximum average temperature was 31.4 ± 2.25 °C and the minimum was 22.7 ± 1.21 °C and the average humidity was 81.7 ± 10.9%.

3.1. Growth performance and serological analysis

At 127 d of age, BW was not affected by dietary treatments (Table 2). At 159 d of age, pigs fed the amino acid diet had greater BW compared with pigs fed the control diet and the amino acid + mineral diet, whereas pigs fed the mineral diet were intermediate (P = 0.049). From 98 to 127 d of age, ADFI, ADG, and FCR were not affected by dietary treatments. From 127 to 159 d of age, ADFI was not affected

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

Item	Treatment[1]				SEM	P-value
	C	A	M	A+M		
IBW (kg)	59.2	59.2	59.2	59.2	0.12	0.582
127 d BW (kg)	94.1	95.4	95.5	93.1	0.69	0.059
159 d BW (kg)	129.1b	133.8a	131.5ab	130.0b	0.05	0.049
98 to 127 d of age						
ADFI (kg)	2.97	3.01	3.09	2.83	0.06	0.230
ADG (kg)	1.20	1.25	1.25	1.17	0.02	0.052
FCR	2.47	2.41	2.48	2.43	0.03	0.328
127 to 159 d of age						
ADFI (kg)	3.36	3.52	3.47	3.26	0.06	0.082
ADG (kg)	1.09c	1.20a	1.13bc	1.16ab	0.02	0.015
FCR	3.07a	2.94ba	3.09a	2.83b	0.04	<0.001
98 to 159 d of age						
ADFI (kg)	3.18	3.28	3.29	3.05	0.05	0.229
ADG (kg)	1.14b	1.22a	1.19ab	1.15b	0.02	0.043
FCR	2.77a	2.68b	2.79a	2.67b	0.03	0.029

IBW - initial body weight; BW - body weight; ADG - average daily gain; ADFI - average daily feed intake; FCR - feed conversion ratio; SEM - standard error of the mean

[1] C = control diet; A = control diet + 0.5% L-arginine (L-arginine; purity > 99%) and 0.5% L-leucine (L-leucine; purity > 99.5%); M = control diet + 0.1% chromium (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation. Data are means of 10 repetitions per dietary treatment.

Means in the same row followed by different letters differ according to Tukey's test (P<0.05).

by dietary treatments. Pigs fed the amino acid diet had the highest ADG, followed by those fed the amino acid + mineral diet, while pigs fed the mineral diet showed intermediate values, and those fed the control diet had the lowest ADG (P = 0.015). Feed conversion ratio was improved in pigs fed the amino acid + mineral diet compared with those fed the control diet and the mineral diet (P<0.001).

Across the entire experimental period (98 to 159 d of age), ADFI was not affected by dietary treatments. However, pigs fed the amino acid diet had greater ADG compared with pigs fed the control diet, with the mineral diet being intermediate (P = 0.043). Feed conversion ratio was improved in pigs fed the amino acid diet and the amino acid + mineral diet compared with those fed the control diet and the mineral diet (P = 0.029).

Dietary treatments did not affect total cholesterol, triglycerides, IGF-1, or insulin concentrations. Pigs fed the amino acid diet had greater SUN compared with those fed the control, mineral, or amino acid + mineral diets (P = 0.003; Table 3).

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

Item	Treatment[1]				SEM	P-value
	C	A	M	A+M		
CHO (mg/dL)	86.7	91.4	85.5	85.0	3.37	0.209
TG (mg/dL)	32.1	32.0	32.0	34.9	1.76	0.496
SUN (mg/dL)	24.8b	29.9a	24.8b	24.1b	1.15	0.003
IGF-1 (ng/mL)	168.0	147.5	161.0	169.7	9.98	0.401
Insulin (µU/mL)	5.1	5.4	5.5	7.9	0.85	0.080

CHO - total cholesterol; TG - triglycerides; SUN - serum urea nitrogen; SEM - standard error of the mean.
[1] C = control diet; A = control diet + 0.5% L-arginine (L-arginine; purity > 99%) and 0.5% L-leucine (L-leucine; purity > 99.5%); M = control diet + 0.1% chromium (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation. Data are means of 10 repetitions per dietary treatment.
Means in the same row followed by different letters differ according to Tukey's test (P<0.05).

3.2. Carcass traits and pork quality

Loin muscle area, carcass temperature, and pH were not affected by dietary treatments (Table 4). Pigs fed the amino acid + mineral diet had lower backfat thickness than those fed the control and mineral diets, whereas pigs fed the amino acid diet showed intermediate values (P = 0.015).

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

Item	Treatment[1]				SEM	P-value
	C	A	M	A+M		
LMA (cm^2)	57.71	58.35	60.42	60.21	1.39	0.410
BFT (mm)	18.32a	16.58ab	17.74a	15.68b	0.63	0.015
Temperature (°C)						
45 min	38.88	39.60	38.91	39.43	0.30	0.318
24 hours	2.69	2.65	2.66	2.29	0.44	0.257
pH						
45 min	6.43	6.30	6.36	6.45	0.06	0.058
24 hours	5.82	5.89	5.84	5.88	0.05	0.547

LMA - loin muscle area; BFT - backfat thickness; SEM - standard error of the mean.
[1] C = control diet; A = control diet + 0.5% L-arginine (L-arginine; purity > 99%) and 0.5% L-leucine (L-leucine; purity > 99.5%); M = control diet + 0.1% chromium (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation. Data are means of 10 repetitions per dietary treatment.
Means in the same row followed by different letters differ according to Tukey's test (P<0.05).

Pigs fed the amino acid diet had lower TL than those fed the control, mineral, and amino acid + mineral diets (P = 0.001; Table 5). The amino acid diet also reduced SL compared with the control diet and the amino acid + mineral diet, with the mineral diet showing intermediate values (P = 0.041). Cooking loss, WBSF, color, and IMF were not affected by dietary treatments.

Pigs fed the amino acid diet and the mineral diet had greater C18:1 n9 (oleic acid) concentrations in the LT muscle compared with pigs fed the control diet, while pigs fed the amino acid + mineral diet were intermediate (P = 0.021; Table 6). Moreover, pigs fed the mineral diet had greater MUFA

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

Item	Treatment[1]				SEM	P-value
	C	A	M	A+M		
TL (%)	16.22a	13.17b	15.49a	16.65a	0.67	0.001
CL (%)	29.73	28.62	27.99	28.56	10.42	0.688
SL (%)	45.96a	41.79b	43.48ab	45.22a	1.16	0.041
WBSF (N)	22.73	22.49	24.51	22.62	0.18	0.840
*L**	53.01	52.61	53.13	51.23	0.59	0.105
*a**	6.67	6.85	6.36	7.40	0.36	0.237
*b**	14.65	14.83	14.76	14.70	0.28	0.972
IMF (%)	2.64	2.97	2.79	2.38	0.22	0.280

TL - thaw water losses; CL - cooking water losses; SL - sum of water losses; WSBF - Warner Bratzler shear force; IMF - intramuscular fat; SEM - standard error of the mean.
[1] C = control diet; A = control diet + 0.5% L-arginine (L-arginine; purity > 99%) and 0.5% L-leucine (L-leucine; purity > 99.5%); M = control diet + 0.1% chromium (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation. Data are means of 10 repetitions per dietary treatment.
Means in the same row followed by different letters differ according to Tukey's test (P<0.05).

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

Item (%)	Treatment[1]				SEM	P-value
	C	A	M	A+M		
C14:0	1.53	1.45	1.43	1.50	0.05	0.508
C16:0	25.73	24.67	25.03	25.31	0.48	0.463
C16:1	2.34	2.25	2.52	2.55	0.13	0.299
C17:0	0.20	0.22	0.24	0.25	0.02	0.416
C17:1	0.22	0.22	0.28	0.28	0.02	0.140
C18:0	12.73	12.71	11.98	11.90	0.40	0.279
C18:1 n9	43.78b	45.20a	45.94a	44.70ab	0.48	0.021
C18:2 n6	11.01	11.05	10.44	11.37	0.42	0.409
C18:3 n6	1.14	1.18	1.09	1.13	0.05	0.612
C20:2	0.38	0.37	0.39	0.38	0.02	0.955
C20:3 n6	0.36	0.38	0.46	0.44	0.06	0.377
SFA	40.35	39.29	38.83	39.10	0.65	0.381
MUFA	46.38b	47.71ab	48.77a	47.54ab	0.57	0.047
PUFA	12.98	13.04	12.41	13.35	0.46	0.500

C14:0 - miristic; C16:0 - palmitic; C16:1 - palmitoleic; C17:0 - heptadecanoic; C17:1 - heptadecenoic; C18:0 - estearic; C18:1 n9 - oleic; C18:2 n6 - linoleic; C18:3 n6 - loinolenic; 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.
[1] C = control diet; A = control diet + 0.5% L-arginine (L-arginine; purity > 99%) and 0.5% L-leucine (L-leucine; purity > 99.5%); M = control diet + 0.1% chromium (402 mg/kg of Cr from chromium picolinate) and 0.05% magnesium oxide (MgO); A+M = control diet + combination of amino acids and minerals supplementation. Data are means of 10 repetitions per dietary treatment.
Means in the same row followed by different letters differ according to Tukey's test (P<0.05).

compared with pigs fed the control diet, while pigs fed the amino acid and amino acid + mineral diets were intermediate (P = 0.047). All other individual fatty acids, as well as total SFA and PUFA, were not affected by dietary treatments.

4. Discussion

The slaughter of heavy pigs can result in low feed efficiency and compromised carcass quality (Tan et al., 2009; Park and Lee, 2011; Zhang et al., 2011). To improve growth performance and maintain or improve carcass traits, nutritional strategies, such as supplementing functional amino acids and minerals, can be used (Tan et al., 2009; Yin et al., 2010; Marcolla et al., 2017; Valente Júnior et al., 2021). Although Arg and Leu were supplied by the basal diet at levels exceeding minimum requirements (Rostagno et al., 2017), they can still be considered functional nutrients due to their roles in protein synthesis and metabolic regulation. Similarly, it was hypothesized that chromium and magnesium may exert functional effects by improving energy metabolism, thereby supporting growth performance and carcass quality in finishing pigs.

In the present work, functional amino acids and minerals did not affect pig performance in the initial experimental period (98 to 127 d). However, in the final phase (127 to 159 d) and across the entire experimental period (98 to 159 d), pigs supplemented with Arg + Leu showed improved performance. These findings suggest that the response to amino acid supplementation is time-dependent, with greater effects expressed during the later finishing stage. Dardevet et al. (2002) reported that the physiological effects of amino acids are more pronounced at higher supplementation levels and in older animals, likely due to reduced sensitivity of muscle protein synthesis in younger stages. In the present study, despite providing amino acid supplementation above the recommendations of Rostagno et al. (2017) and using older pigs, improvements in performance were only evident over the longer term and were not observed during the initial period (98 to 127 d).

The improved FCR of pigs fed Arg + Leu was directly related to the higher ADG because the treatment did not influence ADFI. Therefore, the higher ADG may have resulted from greater protein deposition promoted by Arg and Leu supplementation, as Leu activates the mTOR pathway and its downstream enzymes (e.g., S6 kinase, 4E-BP1), while Arg contributes through nitric oxide and polyamine synthesis, both of which play essential roles in protein metabolism and muscle growth (Dardevet et al., 2000; Yao et al., 2008; Correia et al., 2023). Despite this improvement in ADG, carcass traits such as loin muscle area and backfat thickness were not affected by amino acid supplementation. One possible explanation is that Arg and Leu supplementation enhanced protein deposition without substantially altering muscle cross-sectional area or fat thickness within the slaughter weight range evaluated in this study.

In contrast, 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 antagonize other branched-chain amino acids by increasing catabolic enzyme activity, which can reduce the plasma concentration of amino acids such as isoleucine and valine, reducing animal performance (Selle et al., 2020). Hyun et al. (2003) have shown that 2.0% Leu supplementation decreased the ADG of finishing pigs. Furthermore, depending on the balance relative to Lys, excess Arg or Leu can adversely affect the growth performance of pigs (Cervantes-Ramírez et al., 2013; Gomes et al., 2022; Teixeira et al., 2026) thus, less than 1% of each amino acid might be more effective (Madeira et al., 2014a,b; Tous et al., 2016).

Supplementation of Arg + Leu in combination with Cr + Mg improved FCR from 127 to 159 d. However, this result seems to be more closely related to the numerically lower ADFI observed in this group, since ADG and 159 d BW were not the highest among treatments. Interestingly, pigs fed the amino acid + mineral diet also had reduced backfat thickness compared with pigs fed the control and mineral diets, which may suggest changes in nutrient partitioning and carcass composition rather than enhanced overall growth. This combination of responses indicates that the improvement in feed efficiency

may be partially associated with lower feed intake together with leaner carcasses. Similarly, carcass and pork quality traits were influenced by amino acids or minerals separately, but the combination did not yield additive benefits. This pattern, together with the absence of an additive effect on the lipid profile, suggests that the potential interactions between amino acids and minerals may be more complex than initially expected and may depend on the specific response variable evaluated.

These performance results prompted the evaluation of biochemical parameters to better understand the underlying metabolic effects. Serum urea nitrogen concentration is commonly used to assess the efficiency of nitrogen utilization for protein synthesis (Bush et al., 2002), with lower SUN values generally indicating more efficient amino acid utilization and greater lean tissue deposition. However, in the present study, pigs supplemented with Arg + Leu exhibited higher SUN concentrations despite showing greater ADG. This result is due to amino acid oversupply in the diet, particularly of Leu and Arg. As excess amino acids cannot be stored, they are deaminated and processed via the urea cycle, leading to increased nitrogen excretion (Torres et al., 2023; Teixeira et al., 2026).

It was expected that supplementation with functional amino acids and minerals could affect insulin concentration, given the reported roles of Arg and Leu in stimulating insulin secretion (Yao et al., 2008; Madeira et al., 2014a) and the ability of Cr to potentiate insulin signaling (Evans and Bowman, 1992; Wang et al., 2009; Hung et al., 2014). However, in the present study, dietary treatments did not influence serum insulin levels. This absence of effect suggests that, under our experimental conditions, the mechanisms by which Arg, Leu, and Cr may modulate insulin secretion or sensitivity were not sufficient to alter circulating insulin concentrations.

The decline in pH and temperature of the post-mortem carcass has been used as an indicator of pork quality because it directly influences color and water-holding capacity (WHC), is decisive for tenderness and juiciness, and determines consumer acceptance of the product (Castellini et al., 2002; Scheffler and Gerrard, 2007; Marcolla et al., 2017). In the present study, although A and A + M diets improved the performance of the finishing pigs, the additives did not influence carcass pH or temperature, nor did they affect Warner-Bratzler shear-force.

Tenderness and juiciness are the most important characteristics of pork meat palatability (Listrat et al., 2016; Yi et al., 2023). Tenderness can be measured by the content of IMF and WBSF, with a negative correlation (Van Laack et al., 2001; Alonso et al., 2010). In the present study, no treatment effects were observed regarding WBSF, indicating that neither functional amino acids nor minerals had a deleterious effect on the tenderness of pork. Although our initial expectation was that supplementation might improve quality traits, the absence of differences in tenderness confirms that these nutritional strategies can be applied without deleterious effects on pork palatability.

Furthermore, no differences in IMF content were observed among treatments. It was hypothesized that supplementation with functional amino acids would increase the IMF content of finishing pigs because Leu can donate its carbon skeleton to form acetyl-CoA, a precursor for fatty acid synthesis in muscle tissue (Hyun et al., 2007). Similarly to 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).

Unlike the present study, Hyun et al. (2003) and Ma et al. (2015) supplemented 2% Leu and 1% Arg, respectively, and 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 the catabolism of other amino acids, such as isoleucine and valine, which may worsen performance. In the aforementioned studies, supplementation with 2% Leu increased the IMF content but worsened the performance of the animals.

However, Arg + Leu supplementation reduced thawing water loss and total water loss (thawing loss plus cooking loss). Based on these results, Arg + Leu supplementation increased the WHC in meat. This greater retention capacity suggests that meat from pigs fed Arg + Leu may be juicier. In addition, greater water retention capacity has triggered great interest in the industry, as it can affect meat processing and storage (Rosenvold and Andersen, 2003).

In the present study, supplementation with functional amino acids or minerals modulated the fatty acid profile in the LT of finishing pigs. While early research on lipid composition in pigs primarily focused on adipose tissue due to its broader diversity of fatty acids, there is growing interest in the intramuscular fatty acid profile, which is increasingly recognized for its relevance to both human health and meat quality (Martin et al., 2006; Cai et al., 2010). The composition of fatty acids in muscle tissue not only contributes to the nutritional value of pork but also influences technological properties such as shelf life. Specifically, higher proportions of unsaturated fatty acids, although beneficial nutritionally, can increase lipid oxidation and reduce meat stability during storage (Wood et al., 2004).

In the present study, pigs fed the diet supplemented with Arg + Leu had higher percentages of oleic acid in the LT. In agreement, Tan et al. (2011), who supplemented 1% Arg, and Zhang et al. (2024), who supplemented 1% Leu in the diet of growing and finishing pigs, found higher percentages of oleic acid. Arginine modulates key enzymes involved in oleic acid biosynthesis, such as stearoyl CoA desaturase (SCD1) (Smith et al., 1999; Choi et al., 2014). Although no direct influence of Leu supplementation on oleic acid content was observed, Madeira et al. (2014b) demonstrated that Leu supplementation enhanced the expression of SCD1 in muscle tissue. Additionally, Arg and Leu have been shown to directly modulate the expression of key genes involved in fatty acid oxidation within lipid metabolism, such as PPAR coactivator-1α (PGC1α), AMP-activated protein kinase (AMPK), and nitric oxide synthase-1 (NOS-1) (Fu et al., 2005).

The supplementation of minerals also affected the fatty acid composition. Pigs fed diets with Cr + Mg contained higher percentages of oleic 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. In pigs, stearoyl-CoA desaturase (SCD), a delta 9-desaturase, has been identified as a key enzyme regulating lipid metabolism. Differential expression analyses have shown SCD to be central to lipid profile variation (Puig-Oliveras et al., 2014), and polymorphisms in the SCD gene were associated with MUFA content and the 18:1/18:0 ratio in pork (Ros-Freixedes et al., 2016). Supporting this, Mahfouz and Kummerow (1989) demonstrated in rats that Mg deficiency can affect the fatty acid profile by reducing delta 6-desaturase activity, leading to lower oleic acid levels. This may suggest that Mg deficiency also decreases the activity of delta 9-desaturase, responsible for the formation of MUFAs such as oleic acid from saturated fatty acids (Ntambi and Miyazaki, 2003; Nakamura and Nara, 2004).

The modulation of the lipid profile of pigs through Cr supplementation is still poorly understood. As in the present study, other studies that supplemented Cr reported higher concentrations of oleic 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 LT (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 *SCD1* gene, which increased as the Cr dose increased (Sadeghi et al., 2015; Lalhriatpuii et al., 2024). As previously discussed, Cr can enhance insulin signaling and promote greater availability of amino acids and glucose to tissues. In response to increased insulin and nutrient availability, some proteins, such as sterol regulatory element binding protein-1c (SREBP-1c), may have increased activation (Kim et al., 1998; Azzout-Marniche et al., 2000). This protein is a transcription factor that positively regulates the expression of genes involved in lipid synthesis, including SCD1 (Zhu et al., 2019). Unlike the present study, Tian et al. (2015) and Untea et al. (2017) did not observe an increase in oleic acid and MUFA levels when supplementing Cr, although they observed a reduction in saturated fatty acids. The differences in these results can be attributed to the differences in Cr bioavailability, which can be influenced by the source of the Cr (Caramori Júnior et al., 2017), as well as the time and level of inclusion.

These results suggest that using amino acids or minerals separately positively influences the fatty acid profile. However, when combined, no additive effect was observed. The reasons for the absence of an additive or synergistic interaction remain unclear.

Overall, this study showed that functional amino acids (Arg + Leu) improved growth performance and water-holding capacity, whereas mineral supplementation (Cr + Mg) modified the intramuscular fatty acid profile, particularly by increasing oleic acid and MUFA concentrations. However, the expected synergistic effects of combining amino acids and minerals were not consistently observed. These findings highlight the complexity of nutrient interactions in finishing pigs. Future studies should investigate different supplementation levels and ratios of Arg, Leu, Cr, and Mg, as well as their underlying metabolic and molecular mechanisms.

5. Conclusions

Supplementation with functional amino acids (Arg + Leu) improved ADG and FCR in finishing pigs, while also reducing total water losses and the proportion of oleic acid in the *longissimus thoracis* muscle. Mineral supplementation (Cr + Mg) increased the proportion of oleic acid and total MUFA in the *longissimus thoracis* muscle. The combination of amino acids and minerals improved FCR and reduced backfat thickness; however, these responses were accompanied by lower final BW, indicating that no consistent or beneficial additive effects were achieved across the evaluated traits.

Data availability

Data will be made available on request.

Author contributions

Conceptualization: Rocha, G. C. **Data curation:** Gomes, M. S. and Duarte, M. E. **Formal analysis:** Duarte, M. E. and Rocha, G. C. **Investigation:** Teixeira, L. M.; Silva, F. C. O.; Saraiva, A. and Gomes, M. S. **Methodology:** Teixeira, L. M.; Silva, F. C. O.; Duarte, M. S.; Saraiva, A.; Gomes, M. S. and Duarte, M. E. **Project administration:** Rocha, G. C. **Supervision:** Silva, F. C. O. **Writing – original draft:** Teixeira, L. M.; Silva, F. C. O.; Duarte, M. S.; Saraiva, A.; Gomes, M. S.; Duarte, M. E. and Rocha, G. C. **Writing – review & editing:** Teixeira, L. M.; Duarte, M. S.; Saraiva, A. and Rocha, G. C.

Conflict of interest

The authors declare no conflict of interest.

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