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Alkaline protease isolate supplemented to reduced crude protein diets improves apparent digestibility but does not support performance in grower-finisher pigs

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ABSTRACT - This study aimed to assess an alkaline protease supplemented in diets with and without crude protein (CP) reduction on performance, apparent total tract digestibility (ATTD), blood parameters, and carcass and meat traits in growing-finishing pigs. Forty male pigs (26.2±1.2 kg) were randomly allocated into one of five treatments: negative control (NC, 2% and 1% reduction of CP in grower and finisher phases, respectively, no protease); NC150: NC + 150 mg protease kg^{-1} diet; NC300: NC + 300 mg protease kg^{-1} diet; PC: positive control (no CP reduction and protease); and PC300: PC + 300 mg protease kg^{-1} diet, with eight replicates of one pig/pen. Pigs fed NC showed greater average daily feed intake (ADFI) than pigs fed NC300 or PC and lower ADFI compared to pigs fed NC150. Pigs fed PC had lower ADFI than those fed PC300. Greater average daily gain and gain to feed ratio (G:F) were observed in pigs on NC compared with those on NC300 or NC150 and NC300, respectively. Pigs fed PC showed better G:F than pigs fed PC300. Lower coefficients of ATTD (CTTAD) of dry and organic matter (OM), digestible dry matter (DDM), digestible organic matter (DOM), and digestible protein were observed in growing II pigs fed NC compared with pigs fed NC150 or NC300. Pigs fed NC showed a lower DP compared with PC or NC150. Positive control group showed increased digestible protein compared with NC. Finishing II pigs fed NC showed lower DDM, DOM, CTTAD of OM, and gross energy than pigs fed NC150 or NC300. Pigs fed PC showed greater albumin concentration compared with pigs fed PC300 in finishing II. Pigs fed NC and PC300 showed greater luminosity in the *l. thoracis* muscle than pigs fed PC. A greater color score was evidenced in the *l. thoracis* in pigs fed PC compared with pigs fed PC300. The dietary supplementation of isolated alkaline protease and CP-reduced diets improves ATTD without supporting pig performance.

Keywords: blood parameters, carcass-meat attributes, digestibility, enzyme, growing pigs, growth performance

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1. Introduction

The pig industry has continuously searched for nutritional strategies to improve growth performance of animals. However, as the diets are based on corn and soybean meal, both commodities, they may

influence the nutritional expenses in pig production (Upadhaya et al., 2016). Moreover, plant protein sources contain digestively resistant antinutritional factors or allergenic proteins (Park et al., 2020). These compounds reduce the activity of endogenous proteolytic enzymes and protein digestibility in pigs fed diets with no acid or alkaline protease supplementation (Zaworska-Zakrzewska et al., 2022).

Alkaline exogenous proteases have an optimum activity when pH is close to the alkaline range (Cowieson and Ross, 2016; Ma et al., 2020). Thus, they have greater hydrolysis activity in the small intestine (Cowieson and Ross, 2016). So far, there has been little documented on the beneficial effects of alkaline proteases in pig diets at the level of valorization of the nutritional matrix. In addition, data in the literature is conflicting regarding the effectiveness of the isolated alkaline protease in reducing the effects of dietary antinutritional factors and/or crude protein (CP) content in diets (Cowieson and Ross, 2016).

Previous studies have not reported changes in carcass traits (Choe et al., 2017; Min et al., 2019; Lee et al., 2020; Perez-Palencia et al., 2021) and blood parameters in pigs (Tactacan et al., 2016; Min et al., 2019; Zaworska-Zakrzewska et al., 2022). However, the optimal growth performance (Tactacan et al., 2016; Choe et al., 2017; Min et al., 2019; Ma et al., 2020) and greater digestibility of dry matter, nitrogen (Nguyen et al., 2018), and CP (Chen et al., 2017; Ma et al., 2020) were observed when pigs fed diets containing proteases.

Here, we hypothesized that adding an isolated alkaline protease in corn- and soybean meal-based diets would improve ATTD, growth performance, and carcass and meat traits in pigs without affecting blood parameters if the reduction in CP content was no greater than that 2% and 1% in grower and finisher phases, respectively. Therefore, the objective of this study was to assess an alkaline protease supplemented in diets with and without CP reduction on growth performance, apparent total tract digestibility (ATTD), blood parameters, and carcass and meat traits in growing-finishing pigs.

2. Material and Methods

The study was conducted in an experimental unit located in Marechal Cândido Rondon, Paraná, Brazil (24°31'52" S and 54°01'03" W). Research on animals was conducted according to the institutional committee on animal use (protocol no. 09/2020).

2.1. Animals, experimental design, and diets

Forty entire male crossbreed pigs (26.2±1.2 kg body weight [BW]) from a commercial line hybrid (Landrace × Large White) were used. Pigs were allotted randomly to one of five dietary treatments in a randomized complete block design based on the initial BW of pigs, with eight replicate pens and one animal per pen as experimental unit.

At the beginning of the experiment, the animals were weighed and housed in a masonry facility with a ceramic roof equipped with side curtains and a skylight system. Pens (2.7 m^2) had masonry and metal fence and were equipped with feeders and suspended nipple drinkers. All the pens had metal chains and plastic bottles hanging from the pen wall.

All diets were corn and soybean meal-based with industrial aminoacids (AA), minerals, and vitamins. Diets were formulated to meet nutritional requirements according to Rostagno et al. (2017), except for CP content in negative control (Table 1), and offered as mash. Dietary treatments were as follows: NC - negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150 - NC + 150 mg protease kg^{-1} diet; NC300 - NC + 300 mg protease kg^{-1} diet; PC - positive control (no CP reduction and protease supplementation); and PC300 - PC + 300 mg protease kg^{-1} diet. The doses of the enzyme tested were based on recommendations of the company.

The experiment was divided into four experimental phases: grower I: entire male pigs, from 25 to 50 kg (d 0 to 26); grower II: entire male pigs, from 50 to 70 kg (d 26 to 44); finisher I: entire male pigs, from 70 to 100 kg (d 44 to 69); and finisher II: immunocastrated male pigs, from 100 to 120 kg (d 69 to 87). Animals were administered two doses of anti-GnRF (Vivax®): grower II (d 40) and finisher I (d 65).

Table 1 - Composition of diets offered to growing-finishing pigs (g kg^{-1}, as-fed basis)

Item	Grower I		Grower II		Finisher I		Finisher II	
	NC	PC	NC	PC	NC	PC	NC	PC
Ingredient								
Ground corn, 7.59% CP	729.85	668.06	781.81	724.02	817.78	788.89	939.90	911.01
Soybean meal, 46.07% CP	201.30	266.19	158.84	219.83	131.30	161.80	17.71	48.20
Soybean oil	21.73	24.95	18.06	21.13	15.65	17.19	8.62	10.16
Dicalcium phosphate	17.11	16.65	13.46	13.02	11.30	11.08	8.45	8.23
Calcitic limestone	7.07	6.82	6.92	6.68	6.24	6.12	5.63	5.51
Salt	4.50	4.48	4.15	4.14	3.88	3.87	3.73	3.72
Lys sulphate, 54.6%	9.57	6.80	8.48	5.71	7.22	5.83	8.63	7.25
DL-met, 99%	2.42	1.88	2.07	1.52	1.59	1.31	1.35	1.08
L-thr, 98%	2.87	2.00	2.59	1.71	2.08	1.63	2.35	1.90
L-trp, 98%	0.71	0.39	0.78	0.45	0.65	0.48	0.84	0.68
L-val, 98%	1.59	0.50	1.59	0.53	1.06	0.53	1.52	1.00
Mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Doximax 75®, doxycycline 75%	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Maximulin 80®, tiamulin 80%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated composition[3]								
Crude protein	164.10	184.10	144.40	164.40	132.00	142.00	89.60	99.60
Metabolizable energy (kcal kg^{-1})	3350	3350	3350	3350	3350	3350	3350	3350
Total Ca	7.69	7.69	6.60	6.60	5.73	5.73	4.44	4.40
STTD P	3.80	3.80	3.26	3.26	2.83	2.83	2.16	2.20
SID Lys	11.57	11.57	10.33	10.33	8.98	8.98	6.97	7.00
SID Met + cys	6.83	6.83	6.09	6.09	5.39	5.39	4.18	4.20
SID Thr	7.52	7.52	6.71	6.71	5.84	5.84	4.53	4.50
SID Trp	2.31	2.31	2.07	2.07	1.80	1.80	1.39	1.40
SID Val	7.98	7.98	7.13	7.13	6.20	6.20	4.81	4.80
Analyzed composition								
Crude protein	166.43	181.10	142. 30	160.10	132.00	141.82	89.00	99.23
Gross energy (kcal kg^{-1})	3384	3398	3384	3398	3353	3367	3353	3367
Neutral detergent fiber	127.36	140.35	117.15	145.11	105.31	113.54	101.93	111.14
Acid detergent fiber	55.76	54.20	49.42	63.68	60.43	55.72	55.67	34.87
Ether extract	46.83	46.97	49.19	48.14	47.14	46.71	41.98	41.16
Dry matter	878.63	880.52	880.93	882.76	881.64	879.79	876.17	878.90
Ash	47.18	47.81	40.76	44.85	39.11	39.73	26.70	28.64
Total Ca	8.88	8. 80	8. 00	8.00	7.00	7.00	6.60	6.70
Total P	5.40	5.50	4.50	4.60	4.30	4.40	3.30	3.40

STTD - standardized total tract digestible; SID - standardized ileal digestible.
NC: negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150: NC + 150 mg protease/kg diet; NC300: NC + 300 mg protease/kg diet; PC: positive control (no CP reduction and protease supplementation); PC300: PC + 300 mg protease/kg diet.
[1] Contained per kg of diet: Mn sulphate, 18 mg; Zn oxide, 45 mg; Fe sulphate, 35 mg; Cu sulphate, 7 mg; I, 0.5 mg; Se, 0.3 mg.
[2] Contained per kg of diet: vitamin A, 3000 IU; vitamin D_3, 600 IU; vitamin E, 10 IU; vitamin K_3, 0.9 mg; vitamin B_1, 0.4 mg; vitamin B_2, 1.9 mg; vitamin B_6, 0.4 mg; vitamin B_{12}, 7 mcg; vitamin B_3, 10 mg; vitamin B_5, 6.5 mg; vitamin B_9, 0.25 mg; BHT, 0.06 mg.
[3] Calculated using published values (Rostagno et al., 2017).

2.2. Protease traits

Exogenous protease supplemented to diets (alkaline protease EC Code: 3.4.21.14) was obtained from *Bacillus licheniformis* and had a catalytic activity of 200,000 units (U) g^{-1}, in which U is the amount of protease that releases 1 µg of tyrosine min^{-1} from casein at 40 °C and pH 10.5, with pH and temperature from 6.0 to 12.0 (optimum 11.0) and from 30 to 65 °C (ideal 55 °C), respectively.

2.3. Growth performance

Animals had free access to diet and water throughout the experiment. Offered diets and leftovers were recorded daily (UL-50 digital scale, DIGI-TRON, Curitiba, Brazil) throughout the experiment to determine the average daily feed intake (ADFI, g d^{-1}). Pigs were weighed at the beginning and at the end of each phase using a two-bar digital scale (ULB-3000, IWM bivolt, Curitiba, Brazil). Body weight and feed intake were monitored considering four different phases. Initial BW (IBW, kg), final BW (FBW, kg), ADG (g d^{-1}), and gain to feed ratio (G:F, g:g) were determined.

2.4. Apparent total tract digestibility

Feces were sampled to determine the ATTD of nutrients and the apparent digestible energy at the end of the grower and finisher II phases. Before feces sampling, the marker acid insoluble ash (AIA, celite™) was added to diets (10 g kg^{-1} diet) and then mixed for 10 min in a y-type mixer, as previously reported by Sakomura and Rostagno (2016). Pigs were fed diets containing the marker for 3 d and then subjected to partial feces sampling for 1 d, as previously reported by Kavanagh et al. (2001).

Feed intake was recorded daily. Feces were sampled for 12 h right after defecation to avoid contamination. Feces samples were pooled, placed in plastic bags, and stored at −20 °C. Then, the samples (140 g) were oven-dried (SF-325 NM, Tecnalbrand, Piracicaba, SP, Brazil) in duplicates at 55 °C for 72 h (Silva and Queiroz, 2009). After drying, the samples were ground in a ball-type mill (Solab, model SL-38; São Paulo, Brazil).

Dry matter (DM), ash, and CP were determined in samples as previously described by Silva and Queiroz (2009). Acid insoluble ash was analyzed as previously described by Van Keulen and Young (1977). Gross energy (GE) was determined using a bomb calorimeter (IKA®, model C200, Wilmington, NC, USA). The coefficients of total tract apparent digestibility (CTTAD) of DM, OM, CP, and GE, and digestible nutrients and energy were calculated as previously reported by Sakomura and Rostagno (2016).

2.5. Blood sampling and analysis

All animals were subjected to blood sampling (16:00 h) at the end of the grower and finisher II phases. Animals fasted for 8 h (08:00 to 16:00 h), and then blood samples (≅ 10 mL) were withdrawn from the anterior cranial vena cava using 1.20 × 40 mm needles as previously described (Moreno et al., 1997). Blood samples were transferred to three different sterile glass tubes containing heparin (for creatine kinase and calcium analyses), potassium fluoride (for glucose analysis), or no anticoagulant (for total protein, urea, and albumin analyses) and then placed on ice (4 °C). Afterward, blood samples were sent to the laboratory and centrifuged at 3,000 *g* for 10 min (analog centrifuge 80-2B, Centrilab, São Paulo, Brazil). A 3 mL aliquot of the supernatant was placed in eppendorf tubes and stored at −5 °C.

The following variables were assessed: urea (enzymatic-colorimetric method, Cat. 427), glucose (enzymatic-colorimetric method, Cat. 434), albumin (bromocresol green colorimetric method, Cat. 419), total protein (TP, colorimetric-biuret method, Cat. 418), creatine kinase (UV kinetic method, Cat. 458), and globulins (TP:albumin). All blood analyses were performed within 15 d after sapling using a spectrophotometer (Bel SPECTRO S05, Bel Engineering, Monza, Italy) and commercial kits (Gold Analisa Diagnóstica, Belo Horizonte, Brazil).

2.6. Slaughter procedures, carcass sampling, and meat traits

At the end of the trial (d 88), pigs were fasted for 11 h and transported (within 4 h) to a commercial abattoir (Medianeira, PR, Brazil), where they rested for a further 8-h period before slaughter. Slaughter was performed according to the National Council for the Control of Animal Experimentation (normative resolution no. 37 of February 15, 2018). All pigs were stunned by electronarcosis (200 volts for 5 s), followed by exsanguination, scalding, and evisceration. Then, the carcasses were divided into halves that were weighted.

All carcass and meat traits analyses were performed and calculated as described by Bridi and Silva (2006). Carcass quantitative traits were measured at the last rib (6 cm from the cutting line) using a Hennessy GP4/BP4 swine carcass typing pistol (Hennessy Garding Systems, Auckland, NZ). Backfat thickness (BFT), loin depth (LD), loin eye muscle area (LEA), carcass weight, muscularity index (kg carcass^{-1}), and lean meat were assessed.

Carcasses were stored at −2 °C for 4 h. Samples (15 cm) of each loin were cut in the last rib area (insertion of the last thoracic to the first lumbar vertebra) for further analyses. Then, the following measurements were taken from the right carcass half: carcass length (straight line from the forward edge of the atlas to the forward edge of the aitch bone) using a measuring tape and the BFT using a pachymeter (6 in, 150 mm MTX, Tools World, São Paulo, SP, Brazil).

Longissimus thoracis temperature was measured after 4 and 24 h *post mortem* using a digital skewer-type thermometer (Tp101 Xt-1234, São Paulo, Brazil), and pH was measured after 24 h *post mortem* using a portable digital pHmeter (AK103, Akso Instrumentos de Medição, São Leopoldo, RS, Brazil).

For qualitative analyses, 2.5-cm subsamples from the 15-cm samples were taken to determine intramuscular fat (marbling) and losses (dripping, thawing, and cooking). *Longissimus thoracis* color was measured 24 h after slaughter, and six luminosity measurements were taken on the muscle surface using a Minolta CR-400 colorimeter device (Konica Minolta's, São Paulo, Brazil). Results were expressed using the CIELAB color system with 8 mm aperture, area illumination (Illuminant C D65), and 0° viewing angle. Color parameters were measured as L* (luminosity), a* (red-green component), and b* (yellow-blue component) and expressed in the CIELAB color system. Then, the saturation (chroma or purity) and the tint (color or hue) in *l. thoracis* were calculated.

Color and marbling in *l. thoracis* were measured using Pork Quality Standards Score Table with a six-point color scale (1 = pale pinkish-gray to white; 6 = dark purplish-red) and a 10-point marbling scale (1 = devoid of marbling and 10 = abundant), respectively (NPPC, 1999).

Loin eye muscle area was determined in 2.5-cm samples of *l. thoracis* using a scanner printer (Officejet 4500 Desktop - G510a, HP, São Paulo, Brazil). A black box was used to block the lighting and obtain the scanned image and an object with a known area. Then, readings and calculation of LEA were performed using a software (ImageJ 1.53e - Java).

The cooked samples of the *l. thoracis* were used to determine shear force (g.cm^{-2}). Then, six cylindrically shaped subsamples (1.2-cm diameter) were taken longitudinally in the direction of the muscle fibers in each sample. Shear force was performed in the meat lab (Medianeira, PR, Brazil) using a texture meter (Stable Micro Systems TA-XT/plus, United Kingdom) equipped with a Warner-Bratzler shear force probe and software (Texture Expert Exponent – Stable Micro Systems, Vienna Court, United Kingdom).

2.7. Statistical procedures

Statistical analyzes were performed using the general linear model procedure of SAS (Statistical Analysis System, University Edition). A standardized residuals analysis was performed before variance (ANOVA) or covariance (ANCOVA) analysis. Outliers were set at residuals ≥ 3. The normality of variable errors was assessed using the Shapiro-Wilk test.

Pen was considered the experimental unit for growth performance data. The following general model was used:

$$Y_{ijk} = \mu + T_i + b_j + \beta\,(X_{ijk} - \bar{X}_{...}) + \varepsilon_{ijk},$$

in which Y_{ijk} = average observation of the dependent variable in each plot measured in the *i*-th treatment, in the *j*-th block, and in the *k*-th replicate; μ = overall average; T_i = fixed effect of treatment (*i* = 1, 2, 3, 4, and 5); b_j = random effect of block (*j* = 1 and 2); β = regression coefficient of Y over X; X_{ijk} = average observation of the covariate (initial BW) in each plot, measured in the *i*-th treatment, in

the *j*-th block, and in the *k*-th replicate; $\bar{X}_{...}$ = overall average for the covariate X; ε_{ijk} = random error of the plot associated with each Y_{ijk} observation. Other variables were analyzed using the model mentioned above without the covariate effect.

Treatment effects on the dependent variables were analyzed via ANCOVA or ANOVA. Whenever significant (P≤0.05), differences among treatments were compared using multiple contrast test (non-orthogonal contrasts): NC *vs* NC150, NC *vs* NC300, NC *vs* PC, PC *vs* PC300, and NC + NC150 + NC300 (NC group) *vs* PC + PC300 (PC group). Data were presented as averages with their pooled SEM.

3. Results

3.1. Growth performance

A treatment effect (P≤0.05) was observed only in grower II pigs (Table 2). Pigs fed NC diet showed (P<0.001) greater ADFI than those fed NC300 or PC diets and lower ADFI than pigs fed NC150 diet. In addition, pigs fed PC diet had lower ADFI (P<0.001) than those fed PC300 diet. Greater ADG (P = 0.039) and G:F (P = 0.001) were observed in pigs fed NC diet compared with pigs fed NC300 or NC150 diets and NC300 diet, respectively. Pigs fed PC diet showed better G:F (P = 0.001) than pigs fed PC300 diet. No treatment effect (P>0.05) was observed for growth performance variables in grower and finisher I phases.

Table 2 - Growth performance of growing-finishing pigs fed reduced dietary crude protein and supplemented with alkaline protease[1]

Item	Treatment[2]					SEM	P-value[3]	P-value[4]				
	NC	NC150	NC300	PC	PC300			A *vs* B	A *vs* C	A *vs* D	D *vs* E	NC *vs* PC
Grower I (d 0 to 26)												
IBW (kg)	26.28	26.28	26.27	26.28	26.28	0.20	-	-	-	-	-	-
ADFI (g)	1613	1683	1550	1599	1691	0.02	0.247	0.325	0.382	0.845	0.199	0.763
ADG (g)	941	955	907	984	999	0.01	0.176	0.722	0.400	0.282	0.704	0.045
G:F (g:g)	0.59	0.57	0.59	0.62	0.59	0.01	0.111	0.241	0.922	0.112	0.143	0.025
Grower II (d 26 to 44)												
ADFI (g)	2396	2591	2215	2246	2442	0.03	<0.001	0.015	0.019	0.050	0.013	0.075
ADG (g)	1174	1115	1020	1086	1075	0.02	0.039	0.229	0.003	0.071	0.814	0.518
G:F (g:g)	0.49	0.43	0.46	0.48	0.44	0.01	0.001	0.001	0.036	0.609	0.005	0.369
Finisher I (d 44 to 69)												
ADFI (g)	2848	2871	2696	2653	2696	0.05	0.433	0.882	0.359	0.195	0.790	0.167
ADG (g)	1335	1305	1297	1250	1251	0.02	0.655	0.648	0.583	0.200	0.984	0.169
G:F (g:g)	0.47	0.45	0.48	0.47	0.46	0.01	0.518	0.243	0.571	0.971	0.675	0.981
Finisher II (d 69 to 87)												
ADFI (g)	2756	3005	2948	3045	2953	0.06	0.579	0.206	0.282	0.130	0.637	0.382
ADG (g)	891	973	913	1056	1062	0.03	0.072	0.301	0.732	0.031	0.909	0.011
G:F (g:g)	0.33	0.33	0.31	0.35	0.36	0.01	0.108	0.973	0.455	0.270	0.513	0.024
Overall period (d 0 to 87)												
ADFI (g)	2359	2465	2291	2347	2395	0.03	0.441	0.259	0.474	0.895	0.600	0.884
ADG (g)	1085	1077	1026	1085	1091	0.01	0.428	0.839	0.131	0.999	0.865	0.343
G:F (g:g)	0.46	0.44	0.45	0.46	0.46	0.01	0.131	0.027	0.219	0.989	0.516	0.149

IBW - initial body weight; ADFI - averaged daily feed intake; ADG - averaged daily gain, G:F - gain to feed ratio; SEM - pooled standard error of the mean.
[1] Data are averages of eight pens replicates per treatment and one animal per pen as the experimental unit.
[2] NC: negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150: NC + 150 mg protease/kg diet; NC300: NC + 300 mg protease/kg diet; PC: positive control (no CP reduction and protease supplementation); PC300: PC + 300 mg protease/kg diet.
[3] Significance level of ANOVA.
[4] Significance level of contrasts: A = NC, B = NC150, C = NC300, D = PC, E = PC300, NC *vs* PC = dietary treatment groups (A + B + C *vs* D + E).

3.2. Apparent total tract digestibility

In grower II phase, lower (P≤0.05) CTTAD of DM and OM, and DDM, DOM, and digestible protein (DP) were observed in pigs fed NC diet compared with those fed NC150 or NC300 diets (Table 3). In addition, pigs fed NC diet showed a decrease of 17.6 and 3.6% in DP (P<0.001) compared with those fed PC or NC150 diets, respectively. Positive control treatment increased DP compared with the NC treatment.

In the finisher II phase, pigs fed the NC diet showed lower DDM, DOM, and CTTAD of OM and GE (P≤0.05) than pigs fed NC150 or NC300 diets (Table 3). In addition, pigs fed PC-based diet showed increase of 17.5% in DP (P<0.001) compared with those fed NC diet. Pigs from PC group had greater DP (P<0.001) than pigs from NC group.

Table 3 - Apparent total tract digestibility (as dry matter basis) in growing-finishing pigs fed reduced dietary crude protein and supplemented with alkaline protease[1]

Item	Treatment[2]					SEM	P-value[3]	P-value[4]				
	NC	NC150	NC300	PC	PC300			A *vs* B	A *vs* C	A *vs* D	D *vs* E	NC *vs* PC
Grower II (d 26 to 44)												
CTTAD DM	86.97	90.55	89.81	88.57	88.80	0.38	0.029	0.003	0.014	0.157	0.832	0.528
DDM	86.44	90.15	89.36	88.10	88.50	0.39	0.027	0.002	0.014	0.150	0.720	0.583
CTTAD OM	88.49	91.80	91.24	90.14	90.56	0.37	0.039	0.004	0.014	0.129	0.697	0.748
DOM	73.82	76.76	76.05	75.12	75.72	0.31	0.026	0.002	0.016	0.152	0.497	0.704
CTTAD CP	85.48	88.53	87.94	88.27	87.32	0.46	0.221	0.037	0.089	0.056	0.506	0.504
DP	12.31	12.76	12.72	14.48	14.31	0.16	<0.001	0.048	0.071	<0.001	0.434	<0.001
CTTAD GE	87.66	88.54	89.28	88.78	88.75	0.39	0.780	0.496	0.209	0.385	0.984	0.747
Finisher II (d 69 to 87)												
CTTAD DM	87.40	88.47	89.58	88.69	88.73	0.43	0.640	0.447	0.125	0.360	0.977	0.816
DDM	86.52	90.15	89.36	88.10	88.50	0.39	0.034	0.003	0.017	0.173	0.723	0.564
CTTAD OM	88.16	91.68	91.04	90.07	90.39	0.38	0.035	0.003	0.013	0.092	0.777	0.873
DOM	73.96	76.76	76.05	75.12	75.72	0.31	0.046	0.004	0.027	0.212	0.508	0.654
CTTAD CP	78.34	78.56	81.68	82.55	81.19	0.83	0.390	0.933	0.210	0.116	0.607	0.149
DP	7.03	7.02	7.34	8.26	8.11	0.11	<0.001	0.976	0.215	<0.001	0.554	<0.001
CTTAD GE	86.8	90.02	89.23	87.87	88.29	0.35	0.029	0.003	0.021	0.293	0.678	0.325

CTTAD DM - coefficient of total tract apparent digestibility of dry matter; DDM - digestible dry matter; CTTAD OM - coefficient of total tract apparent digestibility of organic matter; DOM - digestible organic matter; CTTAD CP - coefficient of total tract apparent digestibility of crude protein; DP - digestible protein; CTTAD GE - coefficient of total tract apparent digestibility of gross energy; SEM - pooled standard error of the mean.
[1] Data are averages of eight replicates per treatment.
[2] NC: negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150: NC + 150 mg protease/kg diet; NC300: NC + 300 mg protease/kg diet; PC: positive control (no CP reduction and protease supplementation); PC300: PC + 300 mg protease/kg diet.
[3] Significance level of ANOVA.
[4] Significance level of contrasts: A = NC, B = NC150, C = NC300, D = PC, E = PC300, NC *vs* PC = dietary treatment groups (A + B + C *vs* D + E).

3.3. Blood parameters

No effect (P>0.05) of dietary treatments on blood parameters was observed in grower II phase (Table 4). However, pigs fed PC diet showed an increase of 27.6% in albumin concentration (P = 0.05) compared with pigs fed PC300 diet in the finisher II phase. Additionally, pigs on PC treatment showed greater TP and globulin concentrations (P≤0.05) than those on NC treatment.

3.4. Carcass and meat traits

Pigs fed NC or PC300-based diets showed greater (P = 0.035) luminosity in the *l. thoracis* muscle than those pigs on PC diet (Table 5). Furthermore, a greater luminosity was observed in the *l. thoracis* of pigs from NC group compared with those from the PC group. In addition, a greater color score (P = 0.021) was observed in the *l. thoracis* muscle in pigs fed PC-based diet compared with pigs fed PC300 diet.

Table 4 - Blood parameters in growing-finishing pigs fed reduced dietary crude protein and supplemented with alkaline protease[1]

Item	Treatment[2]					SEM	P-value[3]	P-value[4]				
	NC	NC150	NC300	PC	PC300			A *vs* B	A *vs* C	A *vs* D	D *vs* E	NC *vs* PC
Grower II (at d 41)												
Glucose (mg dL^{-1})	82.39	69.33	93.66	83.20	88.44	2.86	0.080	0.134	0.194	0.925	0.542	0.582
Urea (mg dL^{-1})	15.70	13.61	11.19	16.24	16.53	0.88	0.275	0.453	0.110	0.844	0.917	0.130
Albumin (g dL^{-1})	3.17	3.29	3.33	3.22	3.21	0.06	0.954	0.591	0.487	0.816	0.938	0.759
Total protein (g dL^{-1})	4.83	5.47	5.23	5.17	5.56	0.14	0.501	0.155	0.372	0.453	0.371	0.683
Creatine kinase (U L^{-1})	755	596	1040	848	736	734	0.450	0.495	0.239	0.687	0.629	0.930
Globulin (g dL^{-1})	1.92	2.18	1.90	1.94	2.36	0.14	0.814	0.591	0.476	0.304	0.081	0.024
Finisher II (at d 84)												
Glucose (mg dL^{-1})	84.77	81.77	81.53	83.97	84.76	1.82	0.966	0.630	0.604	0.898	0.896	0.704
Urea (mg dL^{-1})	16.51	14.01	16.92	15.58	15.94	0.67	0.709	0.255	0.852	0.672	0.871	0.941
Albumin (g dL^{-1})	3.33	3.33	3.71	3.37	2.64	0.12	0.050	0.954	0.268	0.902	0.045	0.166
Total protein (g dL^{-1})	5.58	5.23	6.44	6.29	6.83	0.18	0.016	0.468	0.092	0.162	0.279	0.035
Creatine kinase (U L^{-1})	1087	851	1088	1026	964	639	0.769	0.266	0.995	0.772	0.770	0.982
Globulin (g dL^{-1})	2.26	1.89	2.73	2.97	4.19	0.24	0.018	0.590	0.476	0.303	0.080	0.023

SEM - pooled standard error of the mean.
[1] Data are averages of eight replicates per treatment.
[2] NC: negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150: NC + 150 mg protease/kg diet; NC300: NC + 300 mg protease/kg diet; PC: positive control (no CP reduction and protease supplementation); PC300: PC + 300 mg protease/kg diet.
[3] Significance level of ANOVA.
[4] Significance level of contrasts: A = NC, B = NC150, C = NC300, D = PC, E = PC300, NC *vs* PC = dietary treatment groups (A + B + C *vs* D + E).

4. Discussion

4.1. Growth performance

The animals remained healthy throughout the experimental period. Isolated exogenous protease supplementation has been previously reported to be beneficial on growth performance (Zuo et al., 2015; Tactacan et al., 2016; Upadhaya et al., 2016). However, in the present study, the supplemented protease enzyme alone in diets was not effective in improving pig performance. Animals fed NC diets did not show lower performance attributed to positive protein balance when dietary CP was reduced and, hence, N intake and usage are greater because N excretion is reduced, and more proteins are required to support performance (Maestá et al., 2008; Terzis et al., 2010; Monteiro et al., 2018).

Dietary nutritional factors and protein components can affect protease action and impair growth performance responses (Zuo et al., 2015). The lack of response could also be attributed to a fully developed gastrointestinal tract in grower-finisher pigs, which provides a greater capacity to use dietary nutrients efficiently (Nguyen et al., 2019). However, Chen et al. (2017) reported positive effects of protease supplementation on the growth performance of grower pigs due to the nutritional quality of the tested protein source. The authors mentioned above also reported greater feed intake in pigs fed NC diet, which is explained by their intent to meet nutritional requirements when a diet with poor or low-quality ingredients is provided.

Although protease enzymes can improve nutrient digestibility in swine diets, the lack of consistent improvement in growth performance can be attributed to a combination of factors related to diet composition (e.g., formulation, ingredients, protein type) and interaction with other ingredients. Thus, the aforementioned factors can influence the use of nutrients to promote animal growth, as well as the isolated form in which the enzyme is found in the diet. Consequently, we hypothesized that if protease supplementation in diets increases protein breakdown, this may exceed the ability of the digestive system to assimilate and utilize the resulting amino acids. This imbalance could lead to inefficient use of nutrients and, therefore, to no substantial improvement in growth performance.

Table 5 - Carcass and meat traits of growing-finishing pigs fed reduced dietary crude protein and supplemented with alkaline protease at d 87[1]

Item	Treatment[2]					SEM	P-value[3]	P-value[4]				
	NC	NC150	NC300	PC	PC300			A *vs* B	A *vs* C	A *vs* D	D *vs* E	NC *vs* PC
BFTp (mm)	18.00	16.83	16.50	16.21	18.60	0.62	0.748	0.557	0.451	0.351	0.272	0.940
LD (mm)	66.75	65.17	67.12	63.86	66.40	1.16	0.904	0.674	0.922	0.424	0.534	0.525
LEA (cm^2)	52.30	55.70	52.99	53.95	57.35	0.69	0.147	0.100	0.732	0.395	0.128	0.307
CW (kg)	80.65	88.07	85.53	83.50	84.18	0.95	0.121	0.012	0.084	0.285	0.820	0.588
CL (cm)	96.76	97.18	95.43	98.64	96.48	0.65	0.593	0.828	0.493	0.314	0.307	0.274
BFT (mm)	15.94	15.83	16.54	16.66	17.00	0.72	0.988	0.963	0.799	0.761	0.903	0.698
MI (kg $carcass^{-1}$)	59.13	60.67	56.00	54.40	53.50	1.10	0.197	0.612	0.334	0.150	0.811	0.063
LM (%)	57.40	57.78	57.16	56.88	56.53	0.49	0.960	0.801	0.881	0.746	0.851	0.557
LM (kg)	46.28	50.77	50.16	47.60	47.75	0.80	0.286	0.054	0.110	0.577	0.957	0.413
$Temp_{4h}$	18.20	15.65	16.73	14.46	15.74	0.49	0.098	0.078	0.301	0.009	0.403	0.048
$Temp_{24h}$	2.68	3.15	3.00	2.47	2.51	0.21	0.825	0.476	0.625	0.749	0.957	0.318
pH_{24h}	5.67	5.75	5.74	5.87	5.88	0.04	0.453	0.533	0.591	0.120	0.927	0.096
Luminosity	43.06	42.48	44.16	40.12	43.02	0.44	0.035	0.636	0.367	0.017	0.035	0.015
Chroma	9.62	8.51	9.69	8.23	8.80	0.25	0.200	0.139	0.927	0.057	0.481	0.098
Tint	0.72	0.67	0.71	0.72	0.70	0.01	0.318	0.051	0.730	0.835	0.649	0.535
WLD (g)	6.09	6.34	7.32	4.65	6.29	0.53	0.635	0.883	0.465	0.370	0.369	0.230
WLT (g)	5.40	5.63	5.55	4.47	6.12	0.37	0.735	0.842	0.899	0.407	0.197	0.527
WLC (g)	19.07	18.31	18.87	17.56	20.33	0.62	0.765	0.705	0.921	0.431	0.206	0.843
SF ($g.cm^{-2}$)	5001	5117	4516	4568	5111	141.30	0.515	0.792	0.276	0.309	0.261	0.668
Color score	3.94	3.42	4.00	4.00	3.00	0.12	0.021	0.105	0.842	0.835	0.006	0.583
Marbling score	3.25	2.83	3.17	2.71	2.20	0.14	0.158	0.316	0.840	0.182	0.255	0.063
Moisture (%)	73.33	73.70	72.62	74.01	73.52	0.16	0.079	0.437	0.134	0.134	0.334	0.054
Ash (%)	1.31	1.28	1.29	1.25	1.25	0.02	0.704	0.578	0.719	0.203	0.901	0.202
Crude protein (CP, %)	21.14	20.17	20.62	20.59	20.52	0.12	0.111	0.009	0.146	0.107	0.864	0.743
Ether extract (EE, %)	3.14	2.72	3.35	2.91	3.30	0.20	0.877	0.514	0.757	0.704	0.580	0.943
CP:EE ratio	7.84	9.00	6.46	7.30	7.59	0.51	0.664	0.471	0.392	0.710	0.853	0.722

BFTp - backfat thickness measured with a pachymeter; LD - loin depth measured with a pachymeter; LEA - scanned loin eye muscle area; CW - carcass weight; CL - carcass length; BFT - backfat thickness measured with a pig carcass typing pistol; MI - muscularity index; LM - lean meat; $Temp_{4h}$ - *l. thoracis* muscle temperature after 4 h *post mortem*; $Temp_{24h}$ - *l. thoracis* muscle temperature at 24 h *post mortem*; pH_{24h} - *l. thoracis* muscle pH after 24 h *post mortem*; WLD - water loss by dripping; WLT - water loss by thawing; WLC - water loss by cooking; SF - shear force; SEM - pooled standard error of the mean.
[1] Data are averages of eight replicates per treatment.
[2] NC: negative control (2 and 1% reduction of CP in grower-finisher phases, respectively, no protease supplementation); NC150: NC + 150 mg protease/kg diet; NC300: NC + 300 mg protease/kg diet; PC: positive control (no CP reduction and protease supplementation); PC300: PC + 300 mg protease/kg diet.
[3] Significance level of ANOVA.
[4] Significance level of contrasts: A = NC, B = NC150, C = NC300, D = PC, E = PC300, NC *vs* PC = dietary treatment groups (A + B + C *vs* D + E).

4.2. Apparent total tract digestibility

In the present study, animals supplemented with protease showed greater ATTD of nutrients, as previously reported by Upadhaya et al. (2016). This enzyme can improve the ATTD of nutrients by increasing the usage rate of nutritional compounds and support the digestive system (Nguyen et al., 2019; Park et al., 2020). Indeed, protease degrades nutrients that are resistant to endogenous digestive enzymes in growing-finishing pigs (O'Doherty and Forde, 1999; O'Shea et al., 2014) or neutralize antinutritional factors such as enzyme inhibitors to improve nutrient utilization (Zuo et al., 2015).

Nguyen et al. (2018) observed a lack of effect on ATTD compared with our findings, which could be explained by the inclusion of phytase in the diet. Indeed, diets containing other enzymes may affect the response to protease supplementation (Lee et al., 2018). This hypothesis agrees with Sultan et al. (2010), who reported that when protease is supplied alone, the ileal digestibility of protein increases compared with an enzyme blend supplementation.

Upadhaya et al. (2016) reported greater CTTAD of DM and GE in animals fed a diet containing protease, which agrees with what we observed in the present study, in which NC + protease treatments improved CTTAD of DM and GE compared with the NC diet. However, results on the benefits of protease supplementation are conflicting. A greater ATTD of nutrients is not always followed by an improved growth performance (O'Shea et al., 2014; Nguyen et al., 2019; Perez-Palencia et al., 2021), which corroborates the results we observed. This effect is supported by a previous study (Liu et al., 2013), in which greater starch and CP digestibility were observed, but not an improved growth performance.

Variations in the available substrates contribute to the response of a specific protease (Acamovic, 2001), as well as to the dietary dose (Perez-Palencia et al., 2021) and protease type (Lee et al., 2018; Torres-Pitarch et al., 2019). Hence, these variations can result in an inconsistent action of protease on the growth performance. Indeed, it may affect the growth rate in pigs (Nguyen et al., 2019; Perez-Palencia et al., 2021). The greater ATTD of nutrients we observed did not influence protein availability for growth and/or muscle deposition, which agrees with Selle et al. (2006) and Lei et al. (2017).

4.3. Blood parameters

Little information is found in the literature regarding studies assessing protease effects on pig blood parameters (Nguyen et al., 2019). As blood is widespread throughout the animal system, any disorder can alter the blood profile (Kohn et al., 2005). However, the pigs and environmental conditions were normal in our study, and therefore, glucose, urea, and creatine kinase concentrations were not altered by alkaline protease supplementation neither by CP content. Tactacan et al. (2016), and Zaworska-Zakrzewska et al. (2022) also observed no changes in blood glucose concentrations in pigs fed diets containing protease. Results indicated no kidney damage or muscle injuries due to treatments, as suggested by creatine kinase concentrations (Kaneko et al., 2008). The blood parameter concentrations we observed in growing-finishing pigs were within the reference range for pigs (Moreno et al., 1997; Klem et al., 2010).

Total protein, albumin, and globulins were analyzed to verify whether changes in CP dietary content and protease supplementation would cause metabolic disorders, liver diseases, and protein losses (Messer, 1995; Kaneko et al., 2008). We observed that animals from NC group showed lower TP concentration, which would negatively affect albumin and globulin production in the liver (Fischer et al., 2000). However, this TP reduction was reflected only in a lower globulin concentration in pigs fed NC diet. This result is related to the fact that changes in blood constituents regarding diet formulation and protease supplementation may not be easily detected under less challenging pig rearing conditions (Min et al., 2019). This case is supported by previous studies (Tactacan et al., 2016; Zaworska-Zakrzewska et al., 2022), in which no changes in TP concentrations, albumin, and globulin in pigs were observed.

Wang et al. (2020) reported greater serum albumin and a trend to greater TP concentrations in young pigs fed diets containing greater CP concentration (3% more). In the present study, when diets had less CP (1%), TP and globulins concentration were reduced in pigs from NC group, suggesting a dietary deficiency, as previously reported by Fischer et al. (2000) and Wang et al. (2020). Indeed, lower dietary CP reduces blood protein concentrations (Diaz González and Silva, 2008).

According to Rotter et al. (1994) and Chen et al. (2008), there is a negative correlation between albumin and globulin concentration, i.e., an increase in globulin concentration due to immune functions inhibits albumin synthesis in the liver as a compensatory mechanism to maintain constant TP concentration. This correlation supports the reduced albumin concentration in pigs fed PC300 diet. However, in cases of liver dysfunction, albumin concentration is lower than that of globulin concentration (Diaz González and Silva, 2008). This effect was observed in pigs from PC group, in which albumin concentrations reflected a change in globulin concentration.

Diaz González and Silva (2008) reported that reduced albumin and urea concentrations suggest a protein deficiency. Although we observed no changes in urea concentration in pigs as previously observed by Tactacan et al. (2016) and Zaworska-Zakrzewska et al. (2022), urea concentration was

analyzed as a sensitive and immediate indicator of protein intake. Urea concentration is also a marker of renal function (Kaneko et al., 2008; Upadhaya et al., 2016; Nguyen et al., 2019). Different results from our study were observed by Upadhaya et al. (2016), Nguyen et al. (2018), and Nguyen et al. (2019), who reported that dietary supplementation of protease increased the concentration of blood urea in pigs, differing from the findings evidenced by Zuo et al. (2015).

4.4. Carcass and meat traits

In the present study, the dietary alkaline protease supplementation did not alter the quantitative and composition traits of carcass and meat in pigs. This result agrees with previous studies (Choe et al., 2017; Min et al., 2019; Perez-Palencia et al., 2021) and is attributed to the lack of response in the growth performance of animals (Perez-Palencia et al., 2021). However, we observed a change in L* and color score of the *l. thoracis* muscle in pigs fed PC *vs* PC300 diets. Regarding L*, the average values previously reported for pigs ranged from 49.0 to 51.3 (Brewer et al., 2001; Bertol, 2019). Thus, pigs fed PC diet showed lower L* than pigs fed PC300 diet, resulting in darker meat. Color may vary within the same muscle in a small evaluation space because myoglobin concentration is variable (Lawrie and Ledward, 2006). It explains why color score did not differ among treatments as observed for L*.

In addition, the pH after slaughter could explain the differences among dietary treatments in L* because variation in pH_{24h} is also related to changes in meat color and water retention capacity (Lawrie and Ledward, 2006). However, no differences were observed in water loss from the *l. thoracis* muscle. With regard to L*, not only the animals fed protease, but all animals showed an effect for this variable. Hence, more studies are needed to assess the effects of protease on *l. thoracis* color in pigs.

The effectiveness of dietary proteases was also associated to how the protease is supplied (Ghazi et al., 2003) and whether the enzyme is incubated with protein source or only supplemented to the diet with a post-intake activation (Pan et al., 2016). Altogether, protease supplementation had low effects on diets for growing-finishing pigs, suggesting that greater dosages of the enzyme may be necessary (Perez-Palencia et al., 2021). The increased ATTD may not always benefit other variables. Hence, the beneficial effects of protease in diets for pigs may be directly influenced by dietary, metabolic, and physiological factors (Zuo et al., 2015; Tactacan et al., 2016; Upadhaya et al., 2016; Lee et al., 2018; Perez-Palencia et al., 2021).

5. Conclusions

The dietary supplementation of isolated alkaline protease and CP-reduced diets improves ATTD but does not result in better growth performance of growing-finishing pigs. In addition, diets with/without reduced CP, regardless of alkaline protease supplementation, negatively influence blood profile and alter the luminosity and color of the *l. thoracis* muscle in finishing pigs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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References

Acamovic, T. 2001. Commercial application of enzyme technology for poultry production. World's Poultry Science Journal 57:225-242. https://doi.org/10.1079/WPS20010016

Bertol, T. M. 2019. Estratégias nutricionais para melhoria da qualidade da carne suína. Embrapa, Brasília, DF.

Brewer, M. S.; Zhu, L. G.; Bidner, B.; Messinger, D. J. and McKeith, F. K. 2001. Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. Meat Science 57:169-176. https://doi.org/10.1016/S0309-1740(00)00089-9

Bridi, A. M. and Silva, C. A. 2006. Métodos de avaliação de carcaça e da carne suína. Midiograf, Londrina.

Chen, F.; Ma, Y.; Xue, C.; Ma, J.; Xie, Q.; Wang, G.; Bi, Y. and Cao, Y. 2008. The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs. Journal of Veterinary Science 9:39-44. https://doi.org/10.4142/jvs.2008.9.1.39

Chen, H.; Zhang, S.; Park, I. and Kim, S. W. 2017. Impacts of energy feeds and supplemental protease on growth performance, nutrient digestibility, and gut health of pigs from 18 to 45 kg body weight. Animal Nutrition 3:359-365. https://doi.org/10.1016/j.aninu.2017.09.005

Choe, J.; Kim, K. S.; Kim, H. B.; Park, S.; Kim, J.; Kim, S.; Kim, B.; Cho, S. H.; Cho, J. Y.; Park, I. H.; Cho, J. H. and Song, M. 2017. Effect of protease on growth performance and carcass characteristics of growing-finishing pigs. South African Journal of Animal Science 47:697-703. https://doi.org/10.4314/sajas.v47i5.13

Cowieson, A. J. and Roos, F. F. 2016. Toward optimal value creation through the application of exogenous mono-component protease in the diets of non-ruminants. Animal Feed Science and Technology 221:331-340. https://doi.org/10.1016/j.anifeedsci.2016.04.015

Diaz González, F. H. and Silva, S. C. 2008. Patologia clínica veterinária: texto introdutório. Universidade Federal do Rio Grande do Sul, Porto Alegre.

Fischer, R.; Miller, P. S. and Lewis, A. J. 2000. The use of plasma urea as an indicator of protein status in growing-finishing pigs. Nebraska Swine Reports. Available at: <https://digitalcommons.unl.edu/coopext_swine/112/>. Accessed on: Mar. 10, 2022.

Ghazi, S.; Rooke, J. A. and Galbraith, H. 2003. Improvement of the nutritive value of soybean meal by protease and α-galactosidase treatment in broiler cockerels and broiler chicks. British Poultry Science 44:410-418. https://doi.org/10.1080/0007166031000159828 3

Kaneko, J. J.; Harvey, J. W. and Bruss, M. L. 2008. Clinical biochemistry of domestic animals. 6th ed. Academic Press, New York, NY.

Kavanagh, S.; Lynch, P. B.; O'Mara, F. and Caffrey, P. J. 2001. A comparison of total collection and marker technique for the measurement of apparent digestibility of diets for growing pigs. Animal Feed Science and Technology 89:49-58. https://doi.org/10.1016/S0377-8401(00)00237-6

Klem, T. B.; Bleken, E.; Morberg, H.; Thoresen, S. I. and Framstad, T. 2010. Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. Veterinary Clinical Pathology 39:221-226. https://doi.org/10.1111/j.1939-165X.2009.00199.x

Kohn, R. A.; Dinneen, M. M. and Russek-Cohen, E. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. Journal of Animal Science 83:879-889. https://doi.org/10.2527/2005.834879x

Lawrie, R. A. and Ledward, D. A. 2006. Lawrie's meat science. 7th ed. CRC Press, Boca Raton; Woodhead Publishing, Cambridge.

Lee, S. A.; Bedford, M. R. and Walk, C. L. 2018. Meta-analysis: explicit value of mono-component proteases in monogastric diets. Poultry Science 97:2078-2085. https://doi.org/10.3382/ps/pey042

Lee, J. J.; Choe, J.; Kang, J.; Cho, J. H.; Park, S.; Perez-Maldonado, R.; Cho, J. Y.; Park, I. H.; Kim, H. B. and Song, M. 2020. Dietary protease improves growth rate and protein digestibility of growing-finishing pigs. Journal of Animal Science and Technology 62:313-320. https://doi.org/10.5187/jast.2020.62.3.313

Lei, X. J.; Cheong, J. Y.; Park, J. H. and Kim, I. H. 2017. Supplementation of protease, alone and in combination with fructooligosaccharide to low protein diet for finishing pigs. Animal Science Journal 88:1987-1993. https://doi.org/10.1111/asj.12849

Liu, S. Y.; Selle, P. H. and Cowieson, A. J. 2013. Strategies to enhance the performance of pigs and poultry on sorghum-based diets. Animal Feed Science and Technology 181:1-14. https://doi.org/10.1016/j.anifeedsci.2013.01.008

Ma, W.; Lv, Y.; Guo, L.; Wang, Z. and Zhao, F. 2020. Effects of three kinds of protease on growth performance, apparent digestibility of nutrients and caecal microbial counts in weanling pigs. Czech Journal of Animal Science 65:373-379. https://doi.org/10.17221/125/2020-CJAS

Maestá, N.; Cyrino, E. S.; Angeleli, A. Y. O. and Burini, R. C. 2008. Efeito da oferta dietética de proteína sobre o ganho muscular, balanço nitrogenado e cinética da 15N-glicina de atletas em treinamento de musculação. Revista Brasileira de Medicina do Esporte 14:215-220. https://doi.org/10.1590/S1517-86922008000300011

Messer, N. T. 1995. The use of laboratory tests in equine practice. Veterinary Clinics of North America: Equine Practice 11:345-350. https://doi.org/10.1016/s0749-0739(17)30305-x

Min, Y.; Choi, Y.; Kim, Y.; Jeong, Y.; Kim, D.; Kim, J.; Jung, H. and Song, M. 2019. Effects of protease supplementation on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. Journal of Animal Science and Technology 61:234-238. https://doi.org/10.5187/jast.2019.61.4.234

Monteiro, A. N. T. R.; Huepa, L. M. D.; Castilha, L. D. and Pozza, P. C. 2018. Síntese proteica em suínos: como fêmeas, machos não castrados e castrados respondem a este processo? Pubvet 12: 1-10. https://doi.org/10.22256/pubvet.v12n1a14.1-10

Moreno, A. M.; Sobestiansky, J.; Lopez, A. C. and Sobestiansky, A. A. B. 1997. Colheita e processamento de amostras de sangue em suínos para fins de diagnóstico. Embrapa Suínos e Aves, Concórdia. Available at: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/433735/colheita-e-processamento-de-amostras-de-sangue-em-suinos-para-fins-de-diagnostico>. Accessed on: Mar. 10, 2022.

Nguyen, D. H.; Lee, S. I.; Cheong, J. Y. and Kim, I. H. 2018. Influence of low-protein diets and protease and bromelain supplementation on growth performance, nutrient digestibility, blood urine nitrogen, creatinine, and faecal noxious gas in growing–finishing pigs. Canadian Journal of Animal Science 98:488-497. https://doi.org/10.1139/cjas-2016-0116

Nguyen, D. H.; Upadhaya, S. D.; Lei, X. J.; Yin, J. and Kim, I. H. 2019. Influence of dietary protease supplementation to corn–soybean meal-based high-and low-energy diets on growth performance, nutrient digestibility, blood profiles, and gas emission in growing pigs. Canadian Journal of Animal Science 99:482-488. https://doi.org/10.1139/cjas-2017-0104

NPPC - National Pork Producers Council. 1999. Pork quality standards. 3rd ed. National Pork Production Council, Des Moines, IA.

O'Doherty, J. V. and Forde, S. 1999. The effect of protease and α-galactosidase supplementation on the nutritive value of peas for growing and finishing pigs. Irish Journal of Agricultural and Food Research 38:217-226.

O'Shea, C. J.; Mc Alpine, P. O.; Solan, P.; Curran, T.; Varley, P. F.; Walsh, A. M. and Doherty, J. V. O. 2014. The effect of protease and xylanase enzymes on growth performance, nutrient digestibility, and manure odour in grower–finisher pigs. Animal Feed Science and Technology 189:88-97. https://doi.org/10.1016/j.anifeedsci.2013.11.012

Pan, L.; Zhao, P. F.; Yang, Z. Y.; Long, S. F.; Wang, H. L.; Tian, Q. Y.; Xu, Y. T.; Xu, X.; Zhang, Z. H. and Piao, X. S. 2016. Effects of coated compound proteases on apparent total tract digestibility of nutrients and apparent ileal digestibility of amino acids for pigs. Asian-Australasian Journal of Animal Sciences 29:1761-1767.

Park, S.; Lee, J. J.; Yang, B. M.; Cho, J. H.; Kim, S.; Kang, J.; Oh, S.; Park, D. J.; Perez-Maldonado, R.; Cho, J. Y.; Park, I. H.; Kim, H. B. and Song, M. 2020. Dietary protease improves growth performance, nutrient digestibility, and intestinal morphology of weaned pigs. Journal of Animal Science and Technology 62:21-30. https://doi.org/10.5187/jast.2020.62.1.21

Perez-Palencia, J. Y.; Samuel, R. S. and Levesque, C. L. 2021. Supplementation of protease to low amino acid diets containing superdose level of phytase for wean-to-finish pigs: effects on performance, postweaning intestinal health and carcass characteristics. Translational Animal Science 5:txab088. https://doi.org/10.1093/tas/txab088

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Teixeira, M. L.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Departamento de Zootecnia/UFV, Viçosa, MG.

Rotter, B. A.; Thompson, B. K.; Lessard, M.; Trenholm, H. L. and Tryphonas, H. 1994. Influence of low-level exposure to Fusarium mycotoxins on selected immunological and hematological parameters in young swine. Fundamental and Applied Toxicology 23:117-124. https://doi.org/10.1006/faat.1994.1087

Sakomura, N. K. and Rostagno, H. S. 2016. Métodos de pesquisa em nutrição de monogástricos. 2.ed. Funep, Jaboticabal.

Selle, P. H.; Ravindran, V.; Bryden, W. L. and Scott, T. 2006. Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: a review. The Journal of Poultry Science 43:89-103. https://doi.org/10.2141/jpsa.43.89

Silva, D. J. and Queiroz, A. C. 2009. Análise de alimentos: métodos químicos e biológicos. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.

Sultan, A.; Li, X.; Zhang, D.; Cadogan, D. J. and Bryden, W. L. 2010. Dietary enzymes alter sorghum protein digestibility and AME content. p.94. In: Proceedings of the 21st Annual Australian Poultry Science Symposium. University of Sydney, Sydney, Australia.

Tactacan, G. B.; Cho, S. Y.; Cho, J. H. and Kim, I. H. 2016. Performance responses, nutrient digestibility, blood characteristics, and measures of gastrointestinal health in weanling pigs fed protease enzyme. Asian-Australasian Journal of Animal Sciences 29:998-1003.

Terzis, G.; Spengos, K.; Mascher, H.; Georgiadis, G.; Manta, P. and Blomstrand, E. 2010. The degree of $p70^{S6k}$ and S6 phosphorylation in human skeletal muscle in response to resistance exercise depends on the training volume. European Journal of Applied Physiology 110:835-843. https://doi.org/10.1007/s00421-010-1527-2

Torres-Pitarch, A.; Manzanilla, E. G.; Gardiner, G. E.; O'Doherty, J. V. and Lawlor, P. G. 2019. Systematic review and meta-analysis of the effect of feed enzymes on growth and nutrient digestibility in grow-finisher pigs: effect of enzyme type and cereal source. Animal Feed Science and Technology 251:153-165. https://doi.org/10.1016/j.anifeedsci.2018.12.007

Upadhaya, S. D.; Yun, H. M. and Kim, I. H. 2016. Influence of low or high density corn and soybean meal-based diets and protease supplementation on growth performance, apparent digestibility, blood characteristics and noxious gas emission of finishing pigs. Animal Feed Science and Technology 216:281-287. https://doi.org/10.1016/j.anifeedsci.2016.04.003

Van Keulen, J. and Young, B. A. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. Journal of Animal Science 44:282-287. https://doi.org/10.2527/jas1977.442282x

Zaworska-Zakrzewska, A.; Kasprowicz-Potocka, M.; Ciolek, K.; Pruszynska-Oszmalek, E.; Stuper-Szablewska, K. and Rutkowski, A. 2022. The effects of protease supplementation and faba bean extrusion on growth, gastrointestinal tract physiology and selected blood indices of weaned pigs. Animals 12:563. https://doi.org/10.3390/ani12050563

Zuo, J.; Ling, B.; Long, L.; Li, T.; Lahaye, L.; Yang, C. and Feng, D. 2015. Effect of dietary protease supplementation on growth performance, nutrient digestibility, intestinal morphology, digestive enzymes and gene expression of weaned piglets. Animal Nutrition 1:276-282. https://doi.org/10.1016/j.aninu.2015.10.003

Wang, D.; Lindemann, M. D. and Estienne, M. J. 2020. Effect of folic acid supplementation and dietary protein level on growth performance, serum chemistry and immune response in weanling piglets fed differing concentrations of aflatoxin. Toxins 12:651. https://doi.org/10.3390/toxins12100651