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Optimal supplemental levels and effects of a 6-phytase in diets for growing and finishing pigs on their growth performance, fecal excretion and digestibility of nutrients and energy, blood and bone parameters, carcass and meat quality traits

Damares de Castro Fidelis Toledo
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VIÇOSA - MINAS GERAIS
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Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Janslller Luiz Genova

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Assent:

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Janslller Luiz Genova
Adviser

To my daughter and brothers, I dedicate it.

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To God, the author of my life, the inexhaustible source of wisdom, strength and inspiration. Without Him, nothing would be possible! “For from Him, through Him and to Him are all things. To Him be the glory forever! Amen. (Romans 11:36).
To the memory of my beloved mother, whose physical absence never diminished her living presence in my heart. I am a reflection of her persistence, her courage and her unconditional love. I am the continuation of her dreams. To my daughter Luíza, the reason for my life, I dedicate every page of this work. It was because of you and for you that I never gave up; your eyes are the window of hope that illuminates my days and renews my faith in life. To my brothers, Abraão and Ezequias, my greatest examples of integrity and humanity, I thank you for always holding my hand when I needed it most. To my sisters-in-law, Kézia and Beatriz, sisters that life has given me, I am grateful for their affection and complicity. To my nephews Enzo, Vinícius and Pietro, who share with me the most genuine and pure love I have ever received. Each one of you is forever engraved in this achievement.
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“Knowledge is power.”
(Author unknown)

ABSTRACT

TOLEDO, Damares de Castro Fidelis, M.Sc., Universidade Federal de Viçosa, February, 2025. **Optimal supplemental levels and effects of a 6-phytase in diets for growing and finishing pigs on their growth performance, fecal excretion and digestibility of nutrients and energy, blood and bone parameters, carcass and meat quality traits**. Adviser: Janslller Luiz Genova.

This study aimed to assess the effects of increasing 6-phytase levels in diets for growing and finishing pigs on performance, fecal excretion, nutrients and energy digestibility, blood metabolites, bone, carcass and meat traits. Forty-two pigs (24.93 ± 1.67 kg) were assigned for 89 days in randomized blocks to 1 of 6 treatments: positive control (PC) with nutritional requirements met and without phytase, negative control (NC) with reduced nutritional requirements and without phytase, NC + 500, 1000, 1500, or 2000 FTU/kg diet. Pigs fed NC had worse feed conversion ratio than NC500, NC1000, and NC2000 in total period, with an optimal phytase level of 1261 FTU/kg. Growing pigs fed PC had higher fecal Ca, while fecal P was higher with PC or NC than phytase-supplemented diets. Fecal Ca and P, and digestible energy decreased linearly, whereas fecal gross energy and digestible Ca increased linearly. Apparent digestibility coefficients (ADC) of nutrients showed quadratic effects. Finishing pigs fed PC had higher fecal Ca and P, while NC-fed pigs had higher values than phytase-supplemented diets. Fecal Ca, P and Zn decreased linearly, while Ca, P and Zn ADC and digestible minerals increased linearly. Blood P concentration was higher with PC or NC500 than NC or NC1500. Pigs fed NC had reduced loin depth than PC, NC1000 or NC1500, with an optimal phytase level of 1174 FTU/kg. Increasing dietary 6-phytase levels enhanced nutrient digestibility, reduced mineral excretion, and slightly improved meat traits. A 1000 FTU/kg dosage was optimal for growing-finishing pigs, despite minor performance effects.

Keywords: Blood metabolites. ; Bone parameters. ; Fecal excretion. ; Feed conversion. ; Growing-finishing pigs. ; Nutrient digestibility. ; Phytase.

RESUMO

TOLEDO, Damares de Castro Fidelis, M.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **Níveis de suplementação ideais e efeitos de uma 6-fitase suplementada em dietas para suínos em crescimento e terminação sobre seu desempenho de crescimento, excreção fecal, digestibilidade de nutrientes e energia, parâmetros sanguíneos e ósseos e características de qualidade de carcaça e carne**. Orientador: Janslller Luiz Genova.

Este estudo objetivou avaliar os efeitos do aumento dos níveis de 6-fitase em dietas para suínos em crescimento e terminação sobre o desempenho, a excreção fecal, a digestibilidade de nutrientes e energia, os metabólitos sanguíneos, as características ósseas, de carcaça e de carne. Quarenta e dois suínos (24,93 ± 1,67 kg) foram designados por 89 dias em blocos casualizados para 1 dos 6 tratamentos: controle positivo (CP) com requerimentos nutricionais atendidos e sem fitase, controle negativo (CN) com requerimentos nutricionais reduzidos e sem fitase, CN + 500, 1000, 1500 ou 2000 FTU de fitase/kg de dieta. Os suínos alimentados com CN tiveram uma taxa de conversão alimentar pior do que CN500, CN1000, e CN2000 no período total, com um nível ideal de fitase de 1261 FTU/kg. Os suínos em crescimento alimentados com CP apresentaram maior teor de Ca fecal, enquanto o P fecal foi maior com CP ou CN do que com dietas suplementadas com fitase. O Ca e o P fecais e a energia digestível diminuíram linearmente, enquanto a energia bruta fecal e o Ca digestível aumentaram linearmente. Os coeficientes de digestibilidade aparente (CDA) dos nutrientes mostraram efeitos quadráticos. Os suínos em fase de terminação alimentados com CP apresentaram maior teor de Ca e P fecais, enquanto os suínos alimentados com CN apresentaram valores maiores do que as dietas suplementadas com fitase. O Ca, P e Zn fecais diminuíram linearmente, enquanto os CDA do P, Ca e Zn e minerais digestíveis aumentaram linearmente. A concentração de P no sangue foi maior com CP ou CN500 do que com CN ou CN1500. Os suínos alimentados com CN apresentaram profundidade de lombo reduzida do que com CP, CN1000 ou CN1500, com um nível ideal de fitase de 1174 FTU/kg. O aumento dos níveis de 6-fitase na dieta melhorou a digestibilidade dos nutrientes, reduziu a excreção de minerais e melhorou ligeiramente as características da carne. Uma dosagem de 1000 FTU/kg foi ideal para suínos em crescimento e terminação, apesar dos efeitos menores no desempenho.

Palavras-chave: Metabólitos sanguíneos. ; Parâmetros ósseos. ; Excreção fecal. ; Conversão alimentar. ; Suínos em crescimentos e terminação. ; Digestibilidade de nutrientes. Fitase

SUMÁRIO

2 INTRODUCTION

Corn and soybean meal are widely used in formulating diets for pigs (Genova et al., 2023a). These ingredients contain phytic phosphorus (P) (e.g. 0.18% and 0.36%) and non-starch polysaccharides (6.8% and 16.3%), respectively (Rostagno et al., 2024), and other anti-nutritional compounds (e.g. phytic acid, trypsin inhibitors, antigenic factors, β-mannans, xylans) that are indigestible to non-ruminant animals because they are not digested by endogenous enzymes and compromise nutrient utilization (Liu et al., 2019; Baker et al., 2021; Vangroenweghe et al., 2021) and energy metabolism (Kipper et al., 2020). Phytic acid (inositol hexaphosphate or IP6) is the main storage molecule for total P (50% to 80% of P bound to phytate) present in cereal grains and oilseed meals (Rostagno et al., 2024). This molecule can bind to amino acids (AA) and enzymes (e.g. trypsin and α-amylase) (Lu et al., 2019) at pH levels above or below its isoelectric point (Dersjant-Li et al., 2015). For example, phytic acid would bind to cationic minerals at a pH above 4, resulting in the formation of phytate complexes (Dersjant-Li et al., 2020).

In this sense, the supplementation of phytase (myo-inositol hexakisphosphate phosphohydrolase) in diets reduces the negative effects of phytic acid (Valente Junior et al., 2024) by hydrolysis of the phytate molecule and efficient utilization of phytic P (Moita et al., 2021). This makes it possible to reduce dietary nutrients (e.g. to compensate for increased absorption) and energy (Valente Junior et al., 2024). This nutritional strategy represents significant savings in the final cost of formulations and less excretion of pollutants (e.g. nitrogen and minerals) into the environment (Lautrou et al., 2021) because the reduction in the formation of insoluble chelates with minerals (e.g. Ca, Zn, Mg, Na, and Fe), AA and carbohydrates promotes greater nutrients and energy release and utilization (Zeng et al., 2014; Holloway et al., 2019; Buzek et al., 2023).

In general, phytase can be classified based on its source used for production (Moita and Kim, 2023) and the site where hydrolysis of the inositol ring begins (Kumar et al., 2010). Bacterial phytase shows greater stability and improved activity under a wider range of conditions (e.g. pH, temperature, and resistance to proteolytic degradation) compared to fungal phytase (Igbasan et al., 2000). In particular, 6-phytase may be more efficient at increasing P utilization compared to 3-phytase (Moita and Kim, 2022)

due to the greater number of positions for dephosphorylation of the inositol ring (Dersjant-Li et al., 2020). However, there is no single phytase that can completely dephosphorylate it (Adeola and Cowieson, 2011). Therefore, 6-phytase reduces phytic acid to one molecule of inositol monophosphate and releases five molecules of inorganic phosphate that can be absorbed by animals (Kryukov et al., 2021).

The benefits of phytase supplementation are related not only to improvements in growth performance, but also in bone parameters (Melo et al., 2020; Czech et al., 2022; Buzek et al., 2023), carcass traits and lean meat deposition (Silva et al., 2022) due to the increase in plasma myo-inositol concentrations which directly influences pathways responsible for greater muscle protein deposition (e.g. expression of insulin genes) (Schmeisser et al., 2017) and higher release of P, which is an essential mineral for muscle development (Buzek et al., 2023). In addition, improving intestinal health (e.g. modulation of intestinal microbiota) with phytase supplementation can increase nutrient absorption (Moita and Kim, 2023), influencing the metabolism of carbohydrates, AA and lipids (e.g. Ca and P are essential minerals for biological functions) and tissue development (e.g. bone, muscle). These effects can alter blood components (e.g. urea, glucose, total cholesterol, minerals, ALP) (Cowieson et al., 2017a) linked to Na-dependent transport mechanisms for glucose (Cowieson et al., 2013) or changes in bone parameters followed by changes in serum ALP activity (Kiarie et al., 2022). However, these effects need to be better investigated.

Previous studies have reported increased P (Rutherfurd et al., 2014; Tsai et al., 2020), crude protein and ether extract digestibility (Moita and Kim, 2023), improvements in bone strength (Moita and Kim, 2023) and lean meat (Silva et al., 2019), and changes in the concentration of minerals (e.g. plasma Ca, P, Mg) (Czech et al., 2022) in pigs fed diets supplemented with phytase. No changes were observed in blood P concentrations and soft tissues (Tsai et al., 2020), feed conversion, carcass yield and loin depth (Silva et al., 2019) in pigs fed phytase. Also, the results did not always reflect better growth performance (Switkiewicz et al., 2015) because the effects of phytase in diets for finishing pigs can be variable and more evident if the contents of digestible P and other nutrients are marginal in the diet (Gonçalves et al., 2016).

Although increasing attention has been devoted to the use of exogenous enzymes in pig nutrition (Genova et al., 2023b; Rupolo et al., 2023; Arndt et al., 2024), there is an importance of understanding the mechanisms by which different levels and properties of the phytase types act on pig metabolism. Based on previous findings, a

study was conducted on the hypothesis that supplementation with increasing levels of 6-phytase improves nutrient and energy digestibility and, consequently, positively affects growth performance and bone parameters. Therefore, this study aimed to assess the effects of supplementing increasing levels of 6-phytase in diets for growing and finishing pigs on growth performance, fecal excretion and apparent total tract digestibility of nutrients and energy, blood metabolites, bone parameters, carcass and meat quality traits.

3 MATERIAL AND METHODS

The experiment was conducted at the Pig Farm Sector of the Experimental Farm belonging to the State University of Western Paraná (Unioeste), in the city of Marechal Cândido Rondon, PR, Brazil. The research protocol was approved by the Ethics Committee for the Use of Animals under certificate number P37-2022.

3.1 Animals, experimental design, housing, and diets

A total of 42 entire male 62-days-old hybrid pigs (Landrace × Large White), weighing 24.93 ± 1.67 kg were randomly assigned for 89 days in a complete randomized block design, with 6 treatments of 7 replicates, and one pig per pen as the experimental unit. The initial body weight (BW) of the animals was used as the blocking factor categorized into light (up to 25.4 kg BW) or heavy pigs (over 25.4 kg BW).

At the beginning of the experimental period (day 0, arrival of the pigs), the animals were weighed, tagged and allotted into a facility with ceramic tiles, containing pens with an entirely concrete floor (2.2 m²), arranged in two rows and divided by a central corridor. All the pens were equipped with a semi-automatic feeder (37 cm diameter and 1.15 cm high) located at the front and a pacifier-type drinking fountain. The animals received diet and water *ad libitum* throughout the experimental period.

The temperature and relative humidity were recorded daily using a datalogger with digital display (Vketech®, model UT330B digital USB UNI-T; Beijing, China) installed in the center of the facility. An average temperature of 21.4 ± 6.65°C (minimum of 11.7°C and maximum of 33.3°C) and an average relative humidity of 63.7 ± 19.30% (minimum of 26.0% and maximum of 86.0%) were recorded during the experimental period. The temperature and ventilation inside the facility were controlled using side

curtains, trees on both sides and a ridge vent system. A combination of daylight and artificial light was used, with a 12-h light:dark cycle.

The experimental period was divided into four dietary phases defined as growing I (day 0 to 27), growing II (day 27 to 42), finishing I (day 42 to 64), and finishing II (day 64 to 89). The diets were formulated following the nutritional requirements proposed by Rostagno et al. (2017) (Table1). The diets were formulated to be iso- nutritional and energetic meeting the nutritional requirements, except for the negative control diets, where animals were deficient in crude protein (CP), metabolizable energy (ME), Ca, P, and AA.

The 6 dietary treatments consisted of: 1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements [(0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine)] based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. The composition of the experimental diets is shown in Table 1.

Table 1 - Calculated chemical composition of the positive control (PC) and negative control (NC) diets fed to growing and finishing pigs (as fed basis, %)[1].

Ingredients	Growing I		Growing II[4]		Finishing I		Finishing II[4]	
	PC	NC	PC	NC	PC	NC	PC	NC
Yellow dent corn, 7.8% CP	66.84	69.97	73.13	76.26	80.02	83.15	93.20	94.96
Soybean meal, 45.4% CP	26.61	25.39	21.17	19.95	14.98	13.75	2.61	1.62
Soybean oil	2.49	1.21	2.05	0.77	1.64	0.36	0.82	0.00
Dicalcium phosphate	1.67	0.91	1.41	0.66	1.22	0.47	0.94	0.19
Calcitic limestone	0.68	0.81	0.61	0.73	0.55	0.68	0.49	0.62
Lysine sulphate, 54.6%	0.68	0.69	0.68	0.69	0.70	0.70	0.85	0.85
DL-methionine, 99.5%	0.19	0.18	0.16	0.15	0.39	0.39	0.37	0.37
L-threonine, 96.8%	0.20	0.19	0.19	0.18	0.18	0.17	0.21	0.20
L-tryptophan, 99%	0.04	0.04	0.04	0.04	0.13	0.13	0.11	0.10
L-valine, 95.5%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-isoleucine, 97.1%	-	-	-	-	-	-	0.12	0.14
Salt, NaCl	0.45	0.45	0.41	0.41	0.05	0.05	0.11	0.11
Mineral premix[2]	0.05	0.05	0.05	0.05	0.04	0.04	0.06	0.06
Vitamin premix[3]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Inert (kaolin)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.69
Halquinol®, 60%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Meeting nutritional requirements (calculated chemical composition)								
Metabolizable energy, kcal/kg	3350	3310	3350	3310	3350	3310	3350	3310
Crude protein, %	18.41	18.11	16.44	16.14	14.20	13.90	9.96	9.66
Standardized ileal digestibility lysine, %	1.157	1.137	1.033	1.013	0.898	0.878	0.697	0.677
Standardized ileal digestibility methionine + cysteine, %	0.683	0.671	0.609	0.597	0.539	0.527	0.418	0.406
Standardized ileal digestibility threonine, %	0.752	0.734	0.671	0.653	0.584	0.566	0.453	0.435
Standardized ileal digestibility tryptophan, %	0.231	0.227	0.207	0.203	0.180	0.176	0.139	0.135

Standardized ileal digestibility valine, %	0.798	0.782	0.713	0.697	0.620	0.604	0.481	0.465
Total Ca, %	0.769	0.629	0.660	0.520	0.573	0.433	0.444	0.304
Standardized total tract digestible P, %	0.380	0.240	0.326	0.186	0.283	0.143	0.216	0.076
Total Zn, %	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Total Na, %	0.190	0.190	0.176	0.176	0.165	0.165	0.158	0.158

[1]Phytase was supplemented in the NC diets at the following levels: 0.05 g/kg diet (500 FTU/kg), 0.10 g/kg diet (1000 FTU/kg), 0.15 g/kg diet (1500 FTU/kg), and 0.20 g/kg diet (2000 FTU/kg). Experimental period was divided into four dietary phases defined as growing I (day 0 to 27), growing II (day 27 to 42), finishing I (day 42 to 64), and finishing II (day 64 to 89).

[2]Contained per kg of diet: Mn sulphate, 60 mg; Zn oxide, 80 mg; Fe sulphate, 60 mg; Cu sulphate, 10 mg; I, 1 mg.

[3]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 mg; niacin, 10 mg; pantothenic acid, 6.5 mg; folic acid, 0.25 mg; Se, 0.3 mg, BHT, 0.06 mg. PC: positive control diet with nutritional requirements met and without phytase supplementation; NC: negative control diet with reduced nutritional requirements and without phytase supplementation.

[4]Acid-insoluble ash indicator was added to the experimental diets (10 g/kg) during the growing II (day 37) and finishing II (day 84) phases.

Table 2 Analyzed chemical composition of the experimental diets fed to growing and finishing pigs (based on DM).

Item	Growing I (day 0 to 27)						Growing II (day 27 to 42)					
	PC	NC	NC500	NC1000	NC1500	NC2000	PC	NC	NC500	NC1000	NC1500	NC2000
Dry matter, %	89.18	88.78	82.09	86.89	87.63	88.85	84.35	84.49	84.45	87.91	88.22	87.38
Gross energy, kcal/kg	4460	4498	5246	4466	4425	4399	4746	4579	4598	4367	4385	4445
Crude protein, %	19.05	19.03	20.1	19.45	19.65	19.64	17.68	17.57	18.07	16.78	16.91	17.56
Total Ca, %	0.76	0.54	0.58	0.67	0.62	0.61	0.73	0.53	0.51	0.57	0.56	0.57
Total P, %	0.64	0.49	0.50	0.47	0.50	0.54	0.56	0.48	0.46	0.47	0.44	0.51
Total Zn, %	0.010	0.011	0.015	0.015	0.014	0.012	0.009	0.009	0.009	0.009	0.007	0.010
Item	Finishing I (day 42 to 64)						Finishing II (day 64 to 89)					
	PC	NC	NC500	NC1000	NC1500	NC2000	PC	NC	NC500	NC1000	NC1500	NC2000
Dry matter, %	88.02	88.39	88.56	88.4	88.29	88.60	87.32	87.07	88.28	86.24	87.96	88.54
Gross energy, kcal/kg	4479	4882	4373	4404	4509	4376	4229	4343	4317	4337	4334	4276
Crude protein, %	14.75	14.97	14.49	13.22	14.13	14.3	10.64	9.64	9.92	10.43	9.97	9.91
Total Ca, %	0.66	0.5	0.53	0.51	0.56	0.52	0.48	0.38	0.36	0.33	0.39	0.39
Total P, %	0.52	0.41	0.39	0.40	0.41	0.37	0.43	0.28	0.20	0.24	0.25	0.29
Total Zn, %	0.010	0.008	0.009	0.008	0.009	0.009	0.009	0.008	0.007	0.009	0.007	0.008

11) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% crude protein, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet (NC500), 4) NC + 1000 FTU/kg diet (NC1000), 5) NC + 1500 FTU/kg diet (NC1500), and 6) NC + 2000 FTU/kg diet (NC2000).

3.2 Phytase specifications

The exogenous phytase used in the experiment was thermostable phytase (6-phytase - Bestphase HT, Bestzyme®), produced by introducing synthetic genes that simulate an *Escherichia coli* phytase gene (EC code 3.1.3.26) and express the phytase product encoded in *Aspergillus niger*, with a minimum activity of 10,000 U/g. One unit of phytase activity (U) was defined as the amount of enzyme that releases 1 µmol of organic P per min from 5.0 mmol/L of sodium phytate solution at pH 5.5 and a temperature of 37°C.

3.3 Growth performance

Pigs were weighed at the beginning and end of each phase using a digital scale (DIGI-TRON®, model ULB-3000, Curitiba, PR, Brazil) to monitor final BW (FBW) and calculate average daily BW gain (ADG, kg/day) and feed conversion ratio (FC, kg:kg). The diets, waste and leftovers in the feeder and on the floor were collected manually, dried, weighed and deducted from the total supply to calculate the average daily feed intake (ADFI, kg/day).

3.4 Fecal excretion and apparent total tract digestibility of nutrients and energy

The acid-insoluble ash indicator (AIA, Celite hyflo super cel®) was added to the experimental diets (10 g/kg) during the growing (day 37) and finishing (day 84) phases. The diets containing the fecal indicator were homogenized in a vertical mixer for 15 min (Sakomura and Rostagno, 2016).

The diets were offered for 4 days in each phase before the collection of feces began. On the fourth day of the growing (day 41) and finishing (day 88) phases, partial feces collection (indirect method) was carried out according to the methodology described by Sakomura and Rostagno (2016). The start and end of diet provision and total feed consumption per pen were recorded. Feces were collected for 12 h on the last day of feeding the diet containing the indicator in each phase. During collection, the feces were stored in identified polyethylene plastic bags and kept in thermal boxes containing ice (4ºC). After the collection period, the samples were stored at -20°C for laboratory analysis.

The feces samples (in natural matter) were then thawed at room temperature and manually homogenized using disposable gloves in a plastic container. Two technical duplicates (110 g) were then weighed on a balance (Bel engineering®, model M4102,

Monza, Italy) and dried in a forced-air oven (Tecnal®, model 394/2, Piracicaba, SP, Brazil) at 55°C for 72 h (AOAC, 2019). Afterwards, the samples were ground in a micropulverizer mill using sieves with a 1 mm hole (Tecnal®, model R-TE-350; Piracicaba, SP, Brazil).

Acid-insoluble ash analysis was carried out using hydrochloric acid (4N) digestion, following procedures described in Rupolo et al. (2023). The chemical composition of the diets and feces was carried out as described by AOAC (2019). Dry matter (DM) was determined according to method no. 930.15, while mineral matter (MM), Ca, P and Zn contents were determined following methods no. 942.05, 968.08, 931.01, 999.11, respectively. The organic matter (OM) content of the sample was estimated from the difference between the DM and MM values. Crude protein using the Kjeldahl procedure was determined according to method no. 984.13. Gross energy (GE) analysis of the diets and feces was carried out using a bomb calorimeter (IKA®, model C200, Wilmington, North Carolina, USA).

Based on the crude analysis results, the percentage of AIA in the diets and feces was used to calculate the indigestibility factor (IF) as follows (Eq. 1):

IF = (% of dietary AIA ÷ % of fecal AIA) (1)

All equations were as described by Sakomura and Rostagno (2016). The ADC of nutrients (DM, OM, CP, P, Ca, and Zn) and GE were calculated as follows (Eq. 2):

ADC = 100 - (Dietary AIA (%)) ÷ (fecal AIA (%)) × (fecal nutrient or energy (% or kcal/kg)) ÷ (dietary nutrient or energy (% or kcal/kg)) × 100 (2)

The digestible nutrient (DN) values for DM (DDM, %), OM (DOM, %), protein (DP, %), P (Pdig, %), Ca (Cadig, %), Zn (Zndig, %), and digestible energy (DE, kcal/kg) were calculated as follows (Eq. 3):

DN or DE = (Dietary nutrient or energy - (fecal nutrient or energy × IF)) (3)

3.5 Blood sampling procedures and blood metabolites analysis

Blood samples (10 mL, 08:30 am) were taken during growing II (day 40) and finishing II (day 80) phases. All the pigs were fasted for 6 h before the blood was taken. The collection procedure was carried out via jugular vein puncture, using 20 mL syringes and 1.2 × 40 mm gauge needles. The blood was then transferred to two sterile glass tubes containing potassium fluoride for analysis of glucose concentrations, and another without anticoagulant for analyses of serum urea, total protein, P, Mg, Ca, total cholesterol, and albumin concentrations, and ALP activity.

The tubes containing the collected samples were placed in a cooler with ice (4°C) and sent to the Unioeste laboratory for analysis. The blood samples were centrifuged (Centrilab®, model 80-2B analog centrifuge, Maringá, PR, Brazil) at 3,000 *g* for 10 min at room temperature. Then, 3 mL of the supernatant was transferred to Eppendorf® polyethylene microtubes (technical duplicates), duly labeled, and stored in a freezer at -20°C.

The analytical procedures for urea (enzymatic-colorimetric method, Cat. 427E), total protein (colorimetric-biuret method, Cat. 418), P (colorimetric-phosphomolybdate method, Cat. 342), Mg (colorimetric-magon sulphonate method, Cat. 450M), Ca (colorimetric-cresolphthalein method, Cat. 448M), total cholesterol (enzymatic-colorimetric method, Cat. 460), albumin (enzymatic-colorimetric method, Cat. 419), glucose (enzymatic-colorimetric method, Trinder, Cat. 434E), and ALP (kinetic-colorimetric method, Cat. 340) were carried out in technical duplicate via spectrophotometry (Bel engineering®, model Bel SPECTRO S05; Ramos, RJ, Brazil), using specific kits (Gold Analisa Diagnóstica®; Belo Horizonte, MG, Brazil).

3.6 Euthanasia procedures and carcass and meat quality traits

At day 88, all the animals were transported to a commercial packing plant (Medianeira, PR, Brazil) and euthanized after fasting for 20 h. All the pigs were stunned by CO_2 gas within 160 s (Butina® Backloader G3 Relax; Marel, Holbæk, Denmark), shackled, vertically hoisted on to a rail and bled-out within 30 s, followed by scalding and evisceration. All analysis procedures and calculations for carcass and meat quality traits were carried out in accordance with Rupolo et al. (2023) and Arndt et al. (2024).

At the packing plant, the quantitative traits of the left carcasses were measured in the region of the last rib (6 cm from the cut line), to determine the backfat thickness (BFT), percentage of muscle and lean meat in the carcass, and amount of lean meat, using a pork carcass ultrasound (SFK Technology A/S®, model UltraFom 300; Kolding, Denmark).

Hot carcass weight was determined using a scale (Sulmaq®, model 11347; São Paulo, SP, Brazil) placed on the slaughter line. The carcass yield was then calculated. The percentage of lean meat was determined by dividing the lean weight by the weight of the carcass. After cooling the carcasses in the cold room (stage 1 from -18ºC to -15ºC, stage 2 from -15ºC to -12ºC, stage 3 from -10ºC to -8ºC, totaling 180 min), a 30 cm length of the *longissimus lumborum* muscle was collected between the last thoracic

vertebra and the first lumbar vertebra in a caudal-cranial direction. The samples were immediately transferred to previously identified polyethylene plastic bags, packed in thermal boxes (4ºC), and transported to the Food Technology Laboratory (FTL) at Unioeste.

The pH value of the *l. lumborum* muscle was measured 24 h *post-mortem*, using a portable pH meter (Asko®, model AK103; São Leopoldo, RS, Brazil) in the region of the last rib. The pH meter was calibrated using the calibration solutions (2 different buffer solutions for pH 4 and pH 7), with at least a two-point calibration inside the cold room.

At the FTL, after keeping the *l. lumborum* muscle samples under refrigeration for 24 h (2ºC), the BFT and the loin depth were measured using a digital caliper (Mtx®, digital model 316119, 6 inches and 150 mm; São Paulo, SP, Brazil) by 2 trained individuals. To determine the loin eye area of the *l. lumborum* muscle, the samples were scanned on a multifunction printer (HP®, model Officejet 4500 Desktop - G510a; São Paulo, SP, Brazil) by a single trained person. The scanned samples were read using software (imageJ, version 1.53e - Java), and a black plastic box (34 cm high, 40 cm wide, 58 cm long) was used to block out light and improve image quality.

The color of the meat was determined after exposing the sample to oxygen for 15 min to oxidize the muscle. Color analyses were then carried out using a colorimeter device (Konica Minolta®, model CD400; Tokyo, Japan). The results were expressed using the CIELAB color system with an 8 mm aperture, area illumination, illuminant D65 and C, and 0° viewing angle. The color variables were measured as: 1) luminosity (L*) ranging from 0 (pure black) to 100 (pure white), 2) red-green component (a*), and 3) yellow-blue component (b*), which represent saturation (chroma or purity) and hue (color or tint).

The subjective color and marbling scores of the *l. lumborum* muscle were determined using the Pork Quality Standards Scoring Table (NPPC, 1999), with a 6-point color scale (1 = pale pinkish-gray to white, 2 = grayish pink, 3 = reddish pink, 4 = dark reddish pink, 5 = purplish red, and 6 = dark purplish-red) and a 10-point marbling scale (1 = no marbling, and 10 = abundant). Subsequently, the samples were deboned and the *l. lumborum* muscle was sequentially sectioned with 2.5 cm cross-sections to determine drip loss (DL), thawing loss (TL), cooking loss (CL), and shear force (SF). To determine DL, the samples were placed in airtight containers and hung on a nylon mesh for 48 h in a temperature-controlled chamber (Eletrolab®, model 101/3, B.O.D.;

São Paulo, SP, Brazil). The DL was calculated using the following formula: DL = 100 - [(final sample weight × 100) ÷ initial sample weight]. To assess TL, the samples were placed in plastic trays, wrapped in polyvinyl chloride plastic film and kept at 4ºC ± 1ºC for 48 h. They were then weighed again and the weight loss was expressed as a percentage of the sample's initial weight. To determine the CL, the samples were grilled on a preheated grill for 20 min at 170°C until they reached an internal temperature of 40°C, which was measured using a digital skewer thermometer (Xtrad®, model Tp101 Xt-1234; São Paulo, SP, Brazil) inserted into the center of the sample. The samples were then turned over and kept on the grill until they reached an internal temperature of 71°C. The samples were then removed from the grid, packaged, stored for 24 h at 4°C and then weighed.

After determining TL and CL sequentially, SF analysis was performed on 6 cores (1.5 cm in diameter) taken longitudinally in the direction of the muscle fibers in each sample, using a stainless steel cylinder sampler. The cores were submitted to a TA.HDplus texture meter (Stable Micro Systems®, model Texture Analyser, Stable Micro Systems; Godalming, UK), equipped with a standard Warner-Bratzler shear blade with a V-shaped cut and calibrated for force (15 g), deformation (20 mm) and speed (2.0 mm/s), and software (Stable Micro Systems®, model Texture Expert Exponent; Vienna Court, UK) for evaluation.

3.7 Bone parameters

The legs collected were deboned using tweezers and scalpels, incising the skin and sectioning the end of the forelegs at the carpometacarpal joints to expose the right and left metacarpals. The second right metacarpal of each animal was sent for analysis of bone mineral density (BMD) and bone mineral content (BMC) (Unesp Laboratory, Jaboticabal, SP, Brazil), using the Hologic Discovery Wi® small animal mode software, and bone strength (maximum applied load) carried out on a universal testing machine (EMIC®, model DL 10,000, with a load cell of 200 kgf) (Trautenmüller et al., 2021). The data collected by a computer directly attached to the machine was expressed in kgf.

The second left metacarpal was used for external morphometric measurements, in which the bones were measured using a digital caliper (Mtx®, digital model 316119; Guarulhos, SP, Brazil). Proximal and distal epiphysis width, diaphysis width, total length and wet weight (g) were measured. The Seedor index was calculated by dividing the weight of the wet bone in mg by its length in mm (Seedor et al., 1991).

Subsequently, the second left metacarpal bone was boiled for 1 h in an electric pan to facilitate the removal of the remaining soft tissue using tweezers and a scalpel (Kornegay and Thomas, 1981). The bones were then dried in a forced-air oven (Tecnal®, model SF-325 NM; Piracicaba, SP, Brazil) at 65°C for 72 h (Kornegay and Thomas, 1981). Afterwards, the bones were defatted with petroleum ether in a Soxhlet extractor (Laborchemiker®, model LCK-91793; Curitiba, PR, Brazil) and magnetic stirrer with heating plate (Quimis®, model 0261-I2; Diadema, SP, Brazil) for 6 h (Kornegay and Thomas, 1981). The samples were then ground in a ball mill (Tecnal®, model R-TE 350, micro spray mil; São Paulo, SP, Brazil) and stored in previously identified plastic flasks for later mineral analysis. After removing the fat, the metacarpals were dried in a forced-air oven (Tecnal®, model TE-393-180L; Piracicaba, SP, Brazil) at 135°C for 2 h (Lee et al., 2021). Ash analysis was then carried out using a muffle furnace (7Lab®, model BIOFM 6.7 L; Rio de Janeiro, RJ, Brazil) at 600ºC for 6 h (Traylor et al., 2005).

For mineral analysis, aliquots of 1.5 g of raw sample (wet) were weighed on an analytical balance with precision of 0.0001 g (Shimadzu®, model ATX 224; São Paulo, SP, Brazil), added to 5 mL of nitroperchloric acid solution in a ratio of 2:1 (2 parts nitric acid and 1 part perchloric acid) and digested on a digestor block at 200ºC. The final mineral solution was filtered through quantitative paper (Quanty®, model JP41 black band 12.5 cm; Londrina, PR, Brazil) using a 50 mL flask (method M-004/3) as described by Detmann et al. (2021). Subsequently, the Ca and Zn content was determined using an atomic absorption spectrophotometer (Varian®, model Spctr AA-800; Harbor, CA, USA), at the Animal Nutrition Laboratory (UFV; Viçosa, MG, Brazil). The P content in the mineral solution was estimated using an atomic absorption spectrophotometer (Bel Photonics®, model visible spectrophotometer SP1105, 680 nm; Monza, LM, Italy). The procedures for analyzing Ca, P and Zn were carried out according to the methods described by AOAC (2019).

3.8 Statistical procedures

Statistical analyses were performed using SAS Statistical Software (SAS Inst. Inc., Cary, NC, USA, version 9.4). All data were presented as averages with the pooled standard error of the mean. An analysis of Student's standardized residuals was performed before the one-way analysis of covariance (ANCOVA) or variance (ANOVA). Values greater than or equal to 3.0 standard deviations in absolute value

were considered outliers. The normality of the experimental errors was assessed using the Shapiro-Wilk test. The pen was considered the experimental unit. The statistical model included dietary treatment as a fixed effect and BW block and residual error as random factors. The initial BW of the animals was used as a covariate in the model for the growth performance data. When an effect ($P < 0.05$) of dietary treatment was detected in ANCOVA or ANOVA, the averages between treatments were compared using Tukey's post hoc test. Where possible, data were fitted to regression models when $P < 0.10$ in ANCOVA or ANOVA. The optimum phytase level was determined as a function of the quadratic regression model. Marginally different values were considered when $0.05 < P < 0.10$ according to Duncan's test.

4 RESULTS

4.1 Growth performance

Pigs in growing I phase (day 0 to 27) fed NC diet were marginally lower ($0.05 < P < 0.10$) in FBW and ADG than those fed PC, NC500 or NC1000 diets (Table 3). In the finishing I (day 42 to 64) and II (day 64 to 89) phases, pigs fed NC2000 or NC1000 diets were marginally higher ($0.05 < P < 0.10$) in FBW compared to those fed NC diet. This same trend was observed for the ADG in total period (day 0 to 89). In addition, pigs fed NC diet had ($P < 0.05$) worse FC compared to animals fed NC500, NC1000, and NC2000 diets in total period. By fitting the data to regression models ($P < 0.10$), the maximum and minimum critical points obtained from the derivative of the quadratic models were 1150 (Fig. 1A), 1216 (Fig. 1B), and 1261 FTU/kg diet (Fig. 1C) for the variables FBW, ADG in growing I phase, and FC in the total period, respectively. The other variables with $P < 0.10$ and which fitted the linear regression models showed increasing linear effect as phytase supplementation increased (Fig. 2A, B, and C).

Table 3 - Effect of supplementation with increasing levels of 6-phytase on the growth performance of growing and finishing pigs[1].

Item	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
Growing I (day 0 to 27)								
IBW, kg	24.98	24.99	25.03	24.83	24.92	24.82	0.258	-
FBW, kg	53.21^{A}	49.67^{B}	53.00^{A}	53.14^{A}	51.36^{AB}	52.14^{AB}	0.561	0.099
ADG, kg/day	1.05^{A}	0.91^{B}	1.02^{A}	1.05^{A}	0.98^{AB}	1.01^{AB}	0.016	0.087
ADFI, kg/day	1.87	1.75	1.83	1.84	1.81	1.84	0.026	0.837
FC, kg:kg	1.79	1.94	1.80	1.76	1.86	1.82	0.026	0.505
Growing II (day 27 to 42)								
FBW, kg	71.33	67.64	70.40	72.33	69.00	70.07	0.705	0.102
ADG, kg/day	1.22	1.13	1.23	1.30	1.21	1.20	0.031	0.757
ADFI, kg/day	2.62	2.34	2.29	2.37	2.49	2.44	0.047	0.427
FC, kg:kg	2.18	2.26	1.86	1.82	2.08	2.05	0.072	0.495
Finishing I (day 42 to 64)								
FBW, kg	98.43^{AB}	93.00^{B}	99.25^{AB}	101.79^{A}	98.50^{AB}	102.60^{A}	1.041	0.070
ADG, kg/day	1.23	1.15	1.28	1.33	1.28	1.36	0.024	0.158
ADFI, kg/day	3.07	2.89	2.99	3.12	3.13	3.13	0.050	0.711
FC, kg:kg	2.50	2.53	2.34	2.34	2.44	2.30	0.037	0.345
Finishing II (day 64 to 89)								
FBW, kg	129.36^{AB}	119.93^{B}	122.13^{AB}	131.67^{A}	123.50^{AB}	133.00^{A}	1.671	0.070
ADG, kg/day	1.24	1.08	1.03	1.22	1.12	1.27	0.034	0.282
ADFI, kg/day	3.38	3.26	2.78	3.11	3.15	3.50	0.077	0.206
FC, kg:kg	2.73	3.26	2.73	2.57	2.82	2.77	0.080	0.126
Total period (day 0 to 89)								
ADG, kg/day	1.18^{AB}	1.07^{B}	1.08^{AB}	1.23^{A}	1.13^{AB}	1.21^{A}	0.020	0.081
ADFI, kg/day	2.74	2.60	2.38	2.60	2.59	2.72	0.042	0.308

FC, kg:kg	2.31[ab]	2.55[a]	2.22[b]	2.11[b]	2.30[ab]	2.23[b]	0.040	0.021

[a,b]Averages followed by different lowercase letters in the row differ according to Tukey's post hoc test at a 5% probability level. [A,B]Capital letters in the same row indicate marginally higher results according to Duncan's test at a 10% probability level. [1]Data are averages from 7 pen replicates per treatment and 1 pig per pen as the experimental unit. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean. [4]IBW: initial body weight; FBW: final body weight; ADFI: average daily feed intake; ADG: average daily weight gain; FC: feed conversion ratio.

4.2 Fecal excretion and apparent total tract digestibility of nutrients and energy

Pigs in growing II phase (day 27 to 42) fed PC diet had ($P < 0.05$) higher fecal Ca contents than the others (Table 4). Additionally, higher fecal P contents were observed ($P < 0.05$) in animals fed PC or NC diets compared to those fed diets supplemented with phytase; however, pigs fed NC1500 or NC2000 diets had lower fecal P contents compared to those fed NC500 diet. Pigs fed NC2000 diet showed a marginal difference ($0.05 < P < 0.10$) in terms of higher fecal GE contents than those fed NC or NC500 diets. Fecal Ca (Fig. 3A) and P contents (Fig. 3B) of the pigs showed a decreasing linear effect, while the fecal GE contents showed an increasing linear effect as the levels of dietary phytase increased (Fig. 3C).

With regard to ADC, pigs fed NC1000 or NC1500 diets had ($P < 0.05$) higher ADCDM and ADCOM compared to animals fed NC or NC2000 diets, while pigs fed NC500 diet had intermediate results. The PC diet resulted ($P < 0.05$) in intermediate results for ADCOM, but similar compared to those fed NC1000 or NC1500 diets for ADCDM (Table 5). There was a trend ($0.05 < P < 0.10$) for ADCCP, where animals fed NC1000 diet had the highest values compared to those fed NC or NC2000 diets. The animals fed NC2000 diet had ($P < 0.05$) lower ADCGE compared to pigs fed PC diets and the diets with the other phytase levels. For ADCP, the animals fed NC2000 diet had ($P < 0.05$) higher values than those fed PC, NC or NC500 diets, while the pigs fed NC diet had the lowest values compared to all the dietary treatments. In addition, the animals fed PC diet had lower ADCP values than those fed diets supplemented with phytase levels. For ADCZn, pigs fed NC1000 diet had higher values ($P < 0.05$) than those fed NC or NC2000 diets, while pigs fed PC or NC500 diets had higher ADCZn than those fed NC2000 diet. The maximum critical points obtained from the derivative of the quadratic models were 1076 (Fig. 4A), 996 (Fig. 4B), 1083 (Fig. 4C), 855 (Fig. 4D), 1687 (Fig. 4E), and 953 FTU/kg diet (Fig. 4F) for ADCDM, ADCOM, ADCCP, ADCGE, ADCP, and ADCZn, respectively.

Regarding DN and DE, pigs fed PC diet had ($P < 0.05$) higher DDM than the others (Table 4). In addition, pigs fed NC1500 diet had higher values compared to animals fed NC, NC500 or NC2000 diets, while the animals fed NC diet were similar to those fed NC2000 diet. For DOM, animals fed NC1000 diet showed ($P < 0.05$) higher values compared to those fed PC, NC or NC2000 diets. Additionally, DOM values in pigs fed PC, NC or NC2000 diets were similar, but differed compared to those fed NC500 or NC1500 diets. Higher DP was observed ($P < 0.05$) in pigs fed NC1000 diet

than those fed NC or NC2000 diets, but they were similar to the others. Higher DE was observed ($P < 0.05$) in pigs fed PC diet compared to all other treatments, while animals fed NC500 diet had higher values than the others. In addition, lower DE values were observed in pigs fed NC1000, NC1500 or NC2000 diets than those fed NC diet. For Pdig, higher values were observed ($P < 0.05$) in pigs fed PC or NC2000 diets compared to pigs fed NC or NC500 diets. Higher Cadig values were obtained ($P < 0.05$) in pigs fed PC diet than all the others. For Zndig, pigs fed NC1000 diet had a higher value ($P < 0.05$) than those fed NC or NC2000 diets. In addition, the lowest Zndig value was observed in pigs fed NC2000 diet compared to animals fed PC, NC500 or NC1000 diets. The maximum critical points obtained from the derivative of the quadratic models were 1089 (Fig. 5A), 996 (Fig. 5B), 1082 (Fig. 5C), 1683 (Fig. 5E), and 965 FTU/kg diet (Fig. 5G) for DDM, DOM, DP, Pdig, and Zndig, respectively. Digestible energy (Fig. 5D) and Cadig (Fig. 5F) showed a decreasing and increasing linear effect as phytase supplementation increased, respectively.

Table 4 - Effect of supplementation with increasing levels of 6-phytase on fecal excretion and apparent total tract digestibility of nutrients and energy in growing pigs (on day 42)[1].

Item[4]	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
Fecal contents								
Ca, %	2.33^{a}	1.89^{b}	1.83^{b}	1.82^{b}	1.79^{b}	1.64^{b}	0.045	<0.0001
P, %	2.52^{a}	2.27^{a}	1.92^{b}	1.69^{bc}	1.50^{c}	1.49^{c}	0.074	<0.0001
Zn, %	0.0571	0.0533	0.0529	0.0514	0.0557	0.0557	0.001	0.387
CP, %	19.77	20.16	20.43	18.10	20.02	19.84	0.325	0.380
GE, kcal/kg	4430.5^{AB}	4424.34^{B}	4415.64^{B}	4506.58^{AB}	4538.21^{AB}	4577.36^{A}	20.696	0.097
Apparent digestibility coefficients of nutrients and energy								
ADCDM, %	88.04^{a}	86.97^{b}	87.74^{ab}	88.43^{a}	88.47^{a}	87.14^{b}	0.139	0.001
ADCOM, %	90.08^{ab}	89.70^{b}	90.29^{ab}	90.76^{a}	90.65^{a}	89.50^{b}	0.128	0.010
ADCCP, %	85.14^{AB}	83.97^{B}	85.83^{AB}	87.66^{A}	86.24^{AB}	84.89^{B}	0.371	0.062
ADCGE, %	88.77^{a}	88.05^{ab}	88.78^{a}	88.64^{a}	88.56^{a}	87.39^{b}	0.146	0.021
ADCP, %	45.49^{c}	30.14^{d}	48.20^{bc}	55.06^{ab}	56.14^{ab}	59.90^{a}	19.566	<0.0001
ADCCa, %	58.81	55.51	59.47	61.15	61.84	62.79	0.947	0.264
ADCZn, %	42.38^{ab}	37.22^{bc}	41.92^{ab}	46.28^{a}	40.68^{abc}	35.90^{c}	0.900	0.006
Digestible nutrients and energy								
DDM, %	77.31^{a}	72.39^{e}	73.39^{cd}	74.07^{bc}	74.30^{b}	72.68^{de}	0.270	<0.0001
DOM, %	85.12^{bc}	84.74^{c}	85.85^{ab}	86.03^{a}	85.69^{ab}	84.78^{c}	0.128	0.002
DP, %	14.00^{ab}	13.55^{c}	13.85^{abc}	14.15^{a}	13.92^{abc}	13.70^{bc}	0.060	0.049
DE, kcal/kg	4212.44^{a}	4013.27^{c}	4103.81^{b}	3870.65^{d}	3883.55^{d}	3884.37^{d}	21.038	<0.0001
Pdig, %	0.251^{a}	0.124^{c}	0.199^{b}	0.227^{ab}	0.232^{ab}	0.247^{a}	0.008	<0.0001
Cadig, %	0.389^{a}	0.289^{b}	0.309^{b}	0.318^{b}	0.322^{b}	0.326^{b}	0.007	<0.0001
Zndig, %	0.0044^{ab}	0.0039^{bc}	0.0044^{ab}	0.0049^{a}	0.0043^{abc}	0.0038^{c}	0.000	0.007

^{a,b,c,d,e}Averages followed by different lowercase letters in the row differ according to Tukey's post hoc test at a 5% probability level. ^{A,B}Capital letters in the same row indicate marginally higher results according to Duncan's test at a 10% probability level. [1]Data are averages from 7 pen replicates per treatment and 1 animal per pen as the experimental unit. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible

threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean. [4]ADCDM: apparent total tract digestibility coefficients of dry matter; ADCOM: apparent total tract digestibility coefficients of organic matter; ADCCP: apparent total tract digestibility coefficients of crude protein; ADCGE: apparent total tract digestibility coefficients of gross energy; ADCP: apparent total tract digestibility coefficients of phosphorus; ADCCa: apparent total tract digestibility coefficients of calcium; ADCZn: apparent total tract digestibility coefficients of zinc; DDM: digestible dry matter; DOM: digestible organic matter; DP: digestible protein; DE: digestible energy; Pdig: apparent digestible phosphorus; Cadig: apparent digestible calcium; Zndig: apparent digestible zinc.

Pigs in finishing II phase (day 64 to 89) fed PC diet had ($P < 0.05$) higher fecal Ca contents than the others, and pigs fed NC diet had higher values than those fed diets supplemented with phytase (Table 5). In addition, these results were the same as those observed ($P < 0.05$) for the P contents in pig feces. Pigs fed NC1500 or NC2000 diets had ($P < 0.05$) the lowest fecal Zn contents compared to pigs fed PC or NC diets. In addition, the animals fed NC2000 diet had lower values compared to those fed the other diets containing phytase.

Pigs fed PC diet had ($P < 0.05$) a higher fecal GE contents than those fed NC or NC2000 diets. In addition, the animals fed diets supplemented with phytase had the highest values compared to those fed NC diet. The contents of Ca (Fig. 6A), P (Fig. 6B) and Zn in the feces (Fig. 6C) of pigs showed a decreasing linear effect as phytase supplementation increased, while the maximum critical point obtained from the derivative of the quadratic model for fecal GE contents was 1150 FTU/kg diet (Fig. 6D).

With regard to ADC, pigs fed PC diet had ($P < 0.05$) the lowest ADCDM, ADCOM, ADCCP and ADCGE compared to others dietary treatments (Table 5). In addition, these results were the same as those observed ($P < 0.05$) for DDM and DOM. For ADCP, animals fed NC2000 diet had higher values ($P < 0.05$) than those fed PC, NC, NC500 or NC1000 diets, while pigs fed PC or NC diets had the lowest values compared to others dietary treatments. In addition, animals fed NC500 diet had lower values than those fed NC1500 diet. Higher ADCCa values were observed ($P < 0.05$) in pigs fed NC2000 diet compared to animals fed PC, NC or NC500 diets. The ADCCa values were also higher in animals fed NC1000 or NC1500 diets than in pigs fed PC or NC diets. For ADCZn, pigs fed NC2000 diet had ($P < 0.05$) higher values compared to others dietary treatments, while the pigs that consumed NC1000 or NC1500 diets had higher ADCZn than the others. In addition, pigs fed NC or NC500 diets had higher values compared to those fed PC diet. In addition, ADCP (Fig. 7A), ADCCa (Fig. 7B) and ADCZn (Fig. 7C) showed an increasing linear effect as phytase supplementation increased.

Regarding DN and DE, higher DE was observed ($P < 0.05$) in pigs fed NC1500 diet compared to pigs fed PC or NC2000 diets, and animals fed PC diet had lower values than the others (Table 5). For Pdig, higher values were observed ($P < 0.05$) in pigs fed NC2000 diet compared to pigs fed PC, NC, NC500 or NC1000 diets. In addition, pigs fed NC diet had the lowest values compared to others dietary treatments, while animals fed NC1500 diet had higher values than those fed PC, NC or NC500

diets. Higher Cadig values were obtained ($P < 0.05$) in pigs fed diets containing phytase than those fed PC or NC diets. For Zndig, pigs fed NC2000 diet had a higher value ($P < 0.05$) than the others, while the pigs fed PC diet had lower values than the others. In addition, pigs fed NC1000 or NC1500 diets showed higher values compared to animals fed NC or NC500 diets. The Pdig (Fig. 8A), Cadig (Fig. 8B) and Zndig (Fig. 8C) showed an increasing linear effect with increasing levels of phytase supplementation. The data for the other variables did not fit the linear models evaluated.

Table 5 - Effect of supplementation with increasing levels of 6-phytase on fecal excretion and apparent total tract digestibility of nutrients and energy in finishing pigs (on day 89)[1].

Item[4]	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
Fecal contents								
Ca, %	2.88^{a}	1.62^{b}	1.15^{c}	1.12^{c}	1.02^{c}	0.75^{d}	0.113	<0.0001
P, %	2.99^{a}	2.38^{b}	1.46^{c}	1.30^{c}	1.07^{c}	0.70^{d}	0.136	<0.0001
Zn, %	0.0714^{a}	0.0686^{a}	0.0629^{ab}	0.0617^{ab}	0.0557^{b}	0.0429^{c}	0.002	<0.0001
CP, %	13.30	12.14	13.27	13.47	12.93	12.79	0.219	0.537
GE, kcal/kg	4343.43^{a}	4011.77^{c}	4216.33^{ab}	4364.09^{a}	4290.11^{ab}	4164.03^{b}	27.291	0.000
Apparent digestibility coefficients of nutrients and energy								
ADCDM, %	91.32^{b}	93.66^{a}	94.17^{a}	93.90^{a}	94.27^{a}	94.23^{a}	0.209	<0.0001
ADCOM, %	93.21^{b}	95.33^{a}	95.54^{a}	95.47^{a}	95.60^{a}	95.53^{a}	0.181	<0.0001
ADCCP, %	88.88^{b}	91.91^{a}	93.11^{a}	92.19^{a}	92.48^{a}	92.50^{a}	0.294	<0.0001
ADCGE, %	90.94^{b}	94.33^{a}	94.41^{a}	94.26^{a}	94.45^{a}	94.49^{a}	0.244	<0.0001
ADCP, %	39.06^{d}	44.49^{d}	67.16^{c}	72.27^{bc}	77.69^{ab}	84.91^{a}	2.898	<0.0001
ADCCa, %	43.47^{d}	67.73^{c}	78.29^{b}	79.34^{ab}	80.15^{ab}	85.97^{a}	2.389	<0.0001
ADCZn, %	39.96^{d}	59.87^{c}	60.35^{c}	65.72^{b}	69.04^{b}	76.53^{a}	1.845	<0.0001
Digestible nutrients and energy								
DDM, %	80.90^{b}	82.84^{a}	83.28^{a}	83.06^{a}	83.39^{a}	83.38^{a}	0.179	<0.0001
DOM, %	89.21^{b}	91.81^{a}	92.03^{a}	91.72^{a}	92.35^{a}	92.52^{a}	0.212	<0.0001
DP, %	8.85	8.88	9.00	8.91	8.93	8.93	0.020	0.534
DE, kcal/kg	3845.84^{c}	4083.74^{ab}	4075.29^{ab}	4075.51^{ab}	4093.36^{a}	4040.57^{b}	14.583	<0.0001
Pdig, %	0.158^{d}	0.117^{e}	0.177^{cd}	0.190^{bc}	0.204^{ab}	0.223^{a}	0.006	<0.0001
Cadig, %	0.193^{b}	0.206^{b}	0.238^{a}	0.241^{a}	0.244^{a}	0.261^{a}	0.005	<0.0001
Zndig, %	0.0040^{d}	0.0060^{c}	0.0061^{c}	0.0067^{b}	0.0070^{b}	0.0078^{a}	0.000	<0.0001

[a,b,c,d,e]Averages followed by different lowercase letters in the row differ according to Tukey's post hoc test at a 5% probability level. [1]Data are averages from 7 pen replicates per treatment and 1 animal per pen as the experimental unit. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional

matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean. [4]ADCDM: apparent total tract digestibility coefficients of dry matter; ADCOM: apparent total tract digestibility coefficients of organic matter; ADCCP: apparent total tract digestibility coefficients of crude protein; ADCGE: apparent total tract digestibility coefficients of gross energy; ADCP: apparent total tract digestibility coefficients of phosphorus; ADCCa: apparent total tract digestibility coefficients of calcium; ADCZn: apparent total tract digestibility coefficients of zinc; DDM: digestible dry matter; DOM: digestible organic matter; DP: digestible protein; DE: digestible energy; Pdig: apparent digestible phosphorus; Cadig: apparent digestible calcium; Zndig: apparent digestible zinc

4.3 Blood metabolites

Pigs in the growing phase (on day 40) fed NC1000 diet were marginally lower ($0.05 < P < 0.10$) in total protein concentrations than those fed PC or NC500 (Table 6). In addition, blood P concentration of finishing pigs (on day 80) fed PC or NC500 diets was higher ($P < 0.05$) compared to animals fed NC or NC1500 diets. By fitting the data to regression models ($P < 0.10$), the critical points obtained from the derivative of the cubic models for the concentrations of total protein and P in the blood were 231 and 884 (Fig. 9A), and 365 and 1015 FTU/kg diet (Fig. 9B), respectively.

Table 6 - Effect of supplementation with increasing levels of 6-phytase on blood metabolites in growing and finishing pigs[1].

Item	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
Growing (day 40)								
Urea, mg/dL	33.40	35.76	28.30	33.10	31.40	31.10	1.272	0.679
Total protein, g/dL	6.33^{A}	6.23^{AB}	6.42^{A}	5.87^{B}	6.02^{AB}	6.15^{AB}	0.059	0.076
P, mg/dL	7.28	7.33	7.07	7.02	7.00	7.11	0.059	0.476
Mg, mg/dL	2.19	2.23	2.19	2.08	1.95	2.16	0.062	0.834
Ca, mg/dL	8.98	10.29	9.78	9.45	8.93	8.88	0.175	0.105
Total cholesterol, mg/dL	78.47	69.92	77.46	77.06	72.54	74.77	2.430	0.918
Albumin, mg/dL	3.27	3.25	3.34	3.02	3.22	3.32	0.039	0.170
Glucose, mg/dL	108.43	105.03	117.18	106.54	109.91	107.11	1.976	0.564
Alkaline phosphatase, U/L	225.49	297.13	249.38	255.92	241.13	240.61	9.355	0.366
Finishing (day 80)								
Urea, mg/dL	19.68	19.73	14.39	17.26	17.79	16.82	0.803	0.401
Total protein, g/dL	5.18	5.24	5.03	4.89	5.25	5.24	0.081	0.749
P, mg/dL	8.31^{a}	7.50^{b}	8.33^{a}	7.68^{ab}	7.39^{b}	7.92^{ab}	0.108	0.020
Mg, mg/dL	1.34	1.99	1.52	1.90	1.80	1.77	0.111	0.569
Ca, mg/dL	10.57	10.66	10.58	9.46	10.21	9.36	0.204	0.245
Total cholesterol, mg/dL	59.06	57.83	64.29	62.59	60.71	63.52	1.952	0.931
Albumin, mg/dL	3.09	2.98	2.72	2.89	2.94	2.93	0.049	0.426
Glucose, mg/dL	89.79	85.71	86.20	88.04	86.39	84.45	1.210	0.856
Alkaline phosphatase, U/L	186.65	217.15	238.65	211.93	213.10	200.42	7.577	0.508

^{a,b}Averages followed by different lowercase letters in the row differ according to Tukey's post hoc test at a 5% probability level. ^{A,B}Capital letters in the same row indicate marginally higher results according to Duncan's test at a 10% probability level. [1]Data are averages from 7 pigs per treatment. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean.

4.4 Carcass and meat quality traits

Pigs fed NC diet had ($P < 0.05$) a lower loin depth than those fed PC, NC1000 or NC1500 diets (Table 7). Pigs fed NC or NC500 diets showed a marginal difference ($0.05 < P < 0.10$) in terms of lower percentage of muscle in the carcass and loin eye area compared to animals fed PC or NC1000 diets. By fitting the data to regression models ($P < 0.10$), the maximum critical points obtained from the derivative of the quadratic models were 1147 (Fig. 10A), 1177 (Fig. 10B), and 1174 FTU/kg diet (Fig. 10C) for the variables of loin eye area, percentage of muscle in the carcass, and loin depth, respectively.

Table- 7 Effect of supplementation with increasing levels of 6-phytase on the carcass and meat traits of finishing pigs (at 89 days)[1].

Item[4]	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
pH_{final}	5.80	5.92	5.85	5.95	5.87	5.97	0.040	0.869
Drip loss, %	6.61	5.93	3.82	5.23	5.21	5.34	0.305	0.203
Thawing loss, %	4.09	4.60	3.72	3.24	3.04	3.98	0.227	0.444
Cooking loss, %	14.61	14.85	14.82	14.79	15.59	14.12	0.387	0.962
Subjective color score	3.17	3.43	3.83	3.29	3.57	3.50	0.089	0.380
Subjective marbling score	1.67	1.43	1.83	1.71	1.71	1.50	0.119	0.942
Loin depth, mm	67.30^{a}	58.15^{b}	64.23^{ab}	67.81^{a}	65.63^{a}	62.89^{ab}	1.042	0.046
Backfat thickness using a digital caliper, mm	19.45	20.26	16.60	16.11	18.38	17.55	0.591	0.330
Luminosity, L*	44.64	43.72	43.63	45.14	44.26	45.54	0.456	0.828
a*	5.32	5.29	5.14	5.51	4.90	5.01	0.140	0.841
b*	4.73	4.35	4.49	4.45	4.08	3.84	0.199	0.866
Backfat thickness using a pork carcass typification pistol, mm	15.36	18.18	17.02	16.43	17.90	17.55	0.628	0.838
Muscle in the carcass, %	61.20^{A}	53.50^{B}	55.00^{B}	58.29^{A}	56.83^{AB}	55.25^{AB}	0.791	0.077
Lean meat, %	58.48	56.00	57.14	57.90	56.98	56.95	0.391	0.573
Lean meat, kg	53.48	48.30	51.56	53.83	52.32	52.43	0.818	0.406
Carcass yield, %	71.01	70.68	71.28	71.97	71.41	71.54	0.211	0.569
Hot carcass weight, kg	91.30	86.28	90.32	94.26	91.88	91.93	1.242	0.551
Saturation (chroma or purity)	7.12	6.89	6.82	7.12	6.48	6.41	0.204	0.886
Hue (color or tint)	0.73	0.67	0.72	0.67	0.64	0.64	0.024	0.865
Loin eye area, cm^2	40.75^{A}	34.80^{B}	35.14^{B}	40.95^{A}	39.54^{AB}	35.70^{AB}	0.855	0.076

[a,b]Averages followed by different lowercase letters in the row differ according to Tukey's post hoc test at a 5% probability level. [A,B]Capital letters in the same row indicate marginally higher results according to Duncan's test at a 10% probability level. [1]Data are averages from 7 pigs per treatment. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean. [4]pH_{final}: 24 h *post-mortem*; a*: red-green component; b*: yellow-blue component

4.5 Bone parameters

No differences in the bone parameters were detected by an increasing supplementation of phytase in diets for growing and finishing pigs (Table 8).

Table 8 - Effect of supplementation with increasing levels of 6-phytase on bone parameters in finishing pigs (at 89 days)[1]

Item	Dietary treatments[2]						SEM[3]	*P*-value
	PC	NC	NC500	NC1000	NC1500	NC2000		
Maximum applied load, kgf	173.49	167.28	219.59	182.15	193.52	200.12	8.036	0.220
Wet bone weight, g	29.82	29.02	29.79	32.08	29.41	32.64	0.531	0.272
Total bone length, cm	7.88	7.71	8.00	8.00	8.00	8.15	0.055	0.426
Seedor index	378.42	376.00	372.24	400.85	367.69	400.82	5.521	0.331
Proximal epiphysis width, cm	8.28	8.00	8.31	8.58	8.15	8.81	0.092	0.142
Distal epiphysis width, cm	8.75	8.43	8.50	9.06	8.82	8.95	0.096	0.406
Diaphysis width, cm	6.67	6.60	6.88	7.03	6.51	6.80	0.082	0.486
Bone mineral density, g/cm²	0.40	0.35	0.37	0.37	0.38	0.40	0.006	0.319
Bone mineral content, g	55.57	48.60	53.88	54.29	50.66	58.38	1.477	0.466
Dry matter, %	88.32	87.1	87.48	87.58	88.17	87.12	0.183	0.349
Mineral matter, %	62.8	61.05	61.92	61.69	60.92	63.32	0.325	0.216
Total Ca in bone, %	21.99	20.75	21.03	21.99	21.07	21.63	0.247	0.691
Total P in bone, %	12.96	12.57	13.32	12.89	12.65	12.94	0.085	0.110
Total Zn in bone, %	0.016	0.016	0.016	0.015	0.014	0.015	0.000	0.187
Ca:P ratio	1.69	1.64	1.60	1.76	1.68	1.69	0.023	0.476

[1]Data are averages from 7 pigs per treatment. [2]1) positive control (PC) diet with nutritional requirements met and without phytase, 2) negative control (NC) diet with reduced nutritional requirements (0.3% CP, 0.04 kcal ME/kg, 0.14% Ca and P, 0.02% digestible lysine, 0.012% digestible methionine + cysteine, 0.018% digestible threonine, 0.004% digestible tryptophan, and 0.016% digestible valine) based on the bestzyme phytase® nutritional matrix and without phytase, 3) NC + 500 FTU/kg diet, 4) NC + 1000 FTU/kg diet, 5) NC + 1500 FTU/kg diet, and 6) NC + 2000 FTU/kg diet. [3]Pooled standard error of the mean.

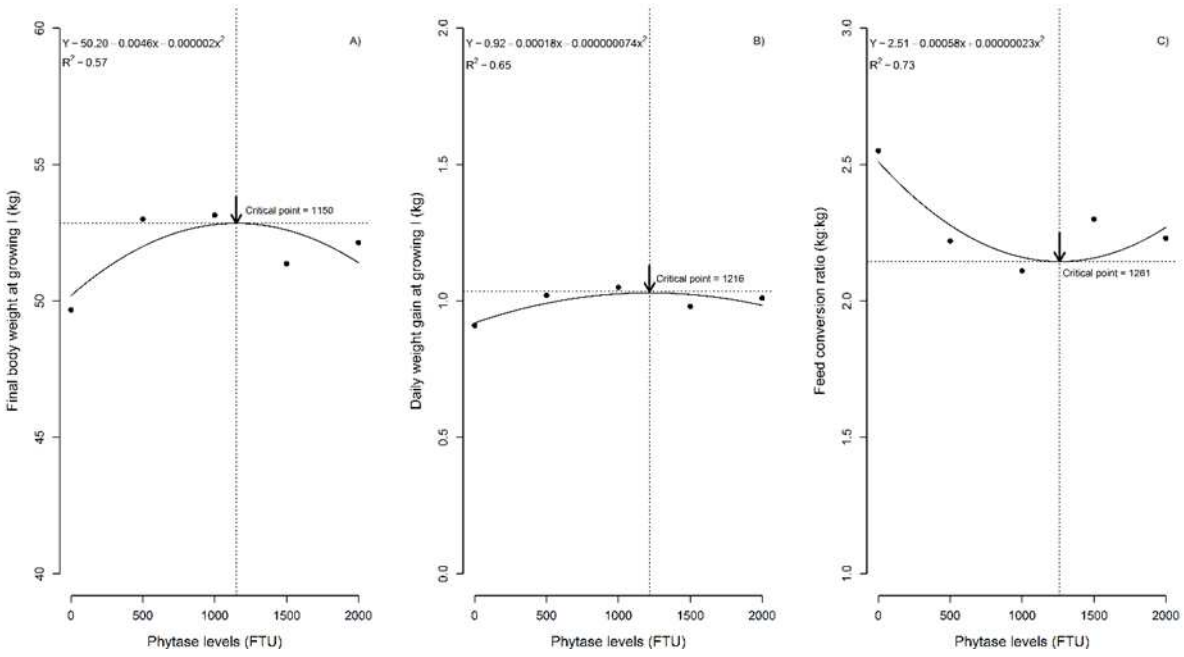

Fig. 1 - Adjusted quadratic regression models of final body weight (A), daily weight gain (B) and feed conversion ratio (C) of pigs in growing I phase (day 0 to 27) and total period (day 0 to 89) fed diets supplemented with increasing levels of 6-phytase.

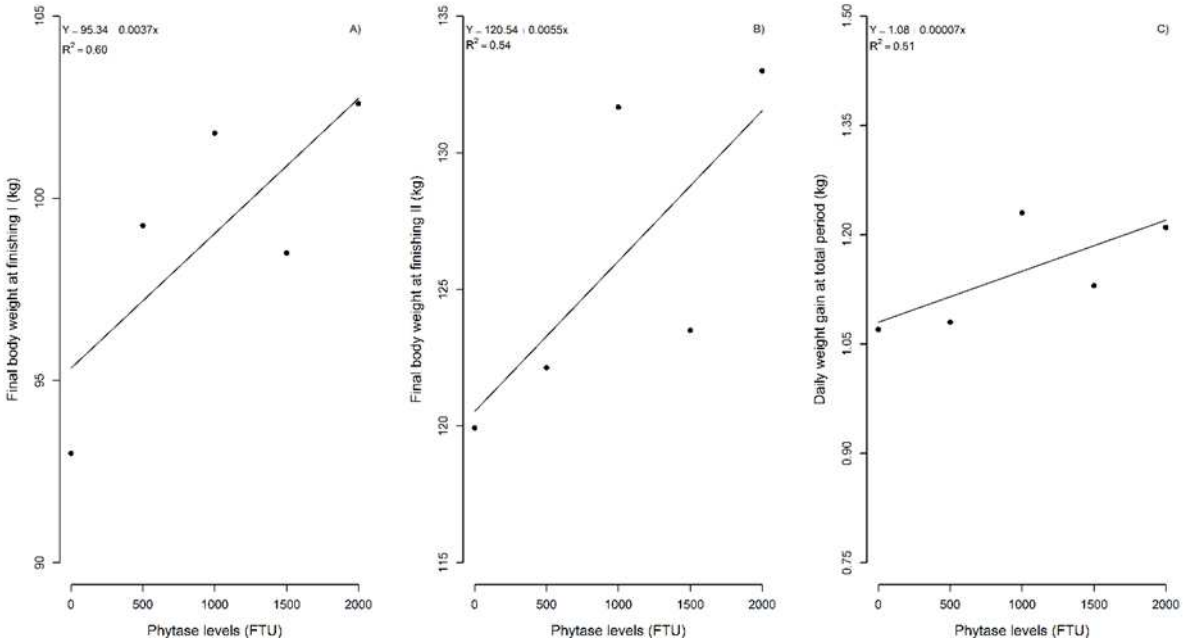

Fig. 2 - Adjusted linear regression models of final body weight in finishing I (A, day 42 to 64), final body weight in finishing II (B, day 64 to 89) and daily weight gain in total period (C, day 0 to 89) of pigs fed diets supplemented with increasing levels of 6-phytase.

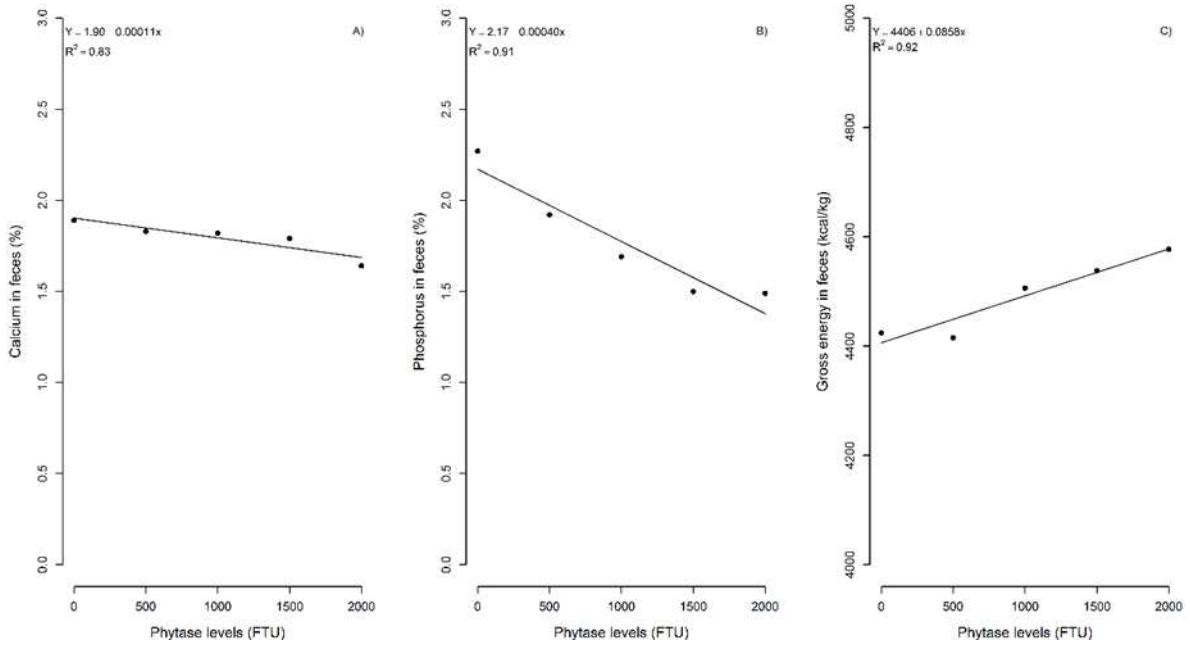

Fig. 3 - Adjusted linear regression models of calcium (A), phosphorus (B) and gross energy in feces (C) of pigs in growing II phase (day 27 to 42) fed diets supplemented with increasing levels of 6-phytase.

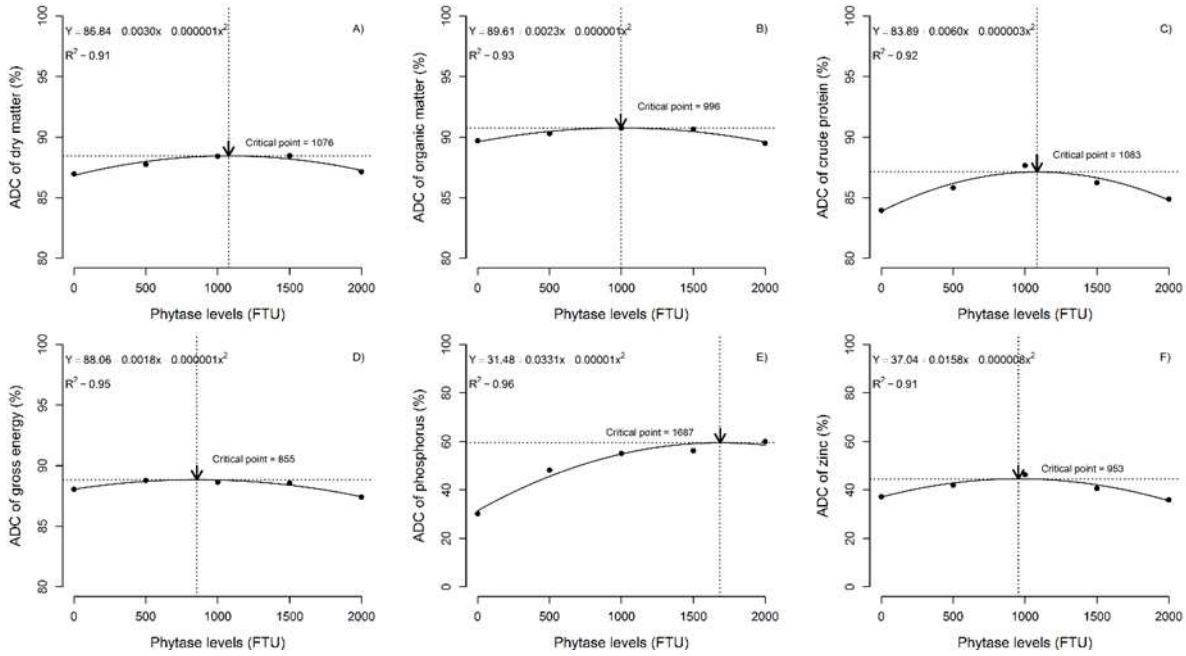

Fig. 4 - Adjusted quadratic regression models of the apparent total tract digestibility coefficients (ADC) of dry matter (A), organic matter (B), crude protein (C), gross energy (D), phosphorus (E) and zinc (F) of pigs in growing II phase (day 27 to 42) fed diets supplemented with increasing levels of 6-phytase.

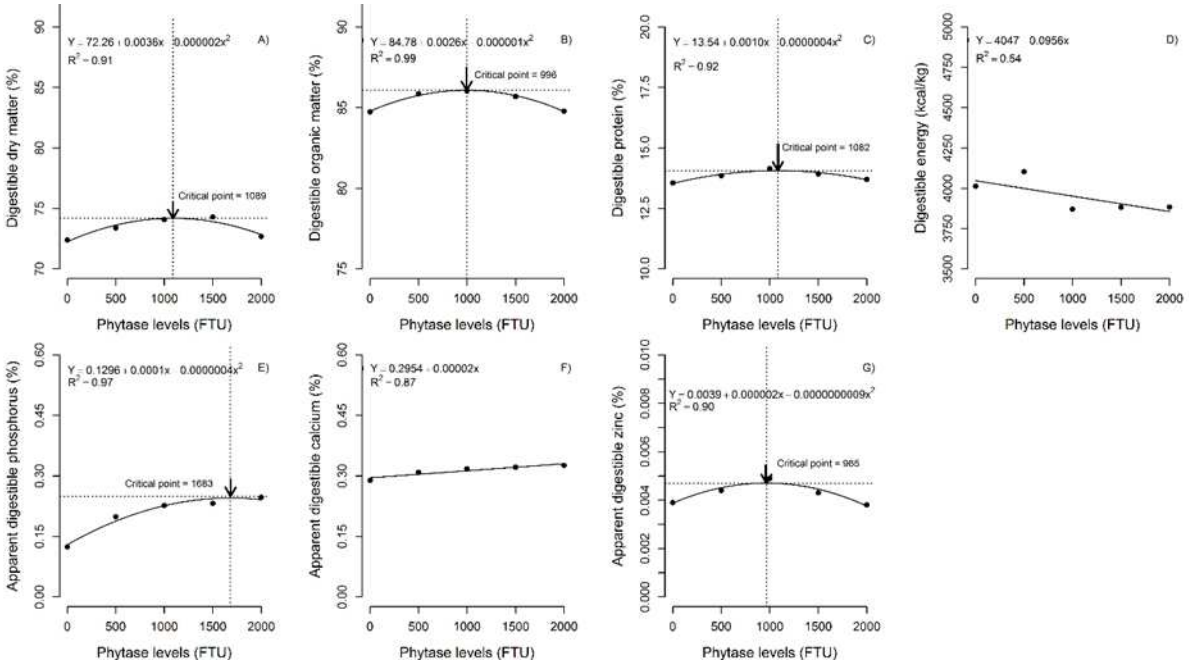

Fig. 5 - Adjusted quadratic and linear regression models of digestible dry matter (A), organic matter (B), protein (C), energy (D), phosphorus (E), calcium (F) and zinc (G) of pigs in growing II phase (day 27 to 42) fed diets supplemented with increasing levels of 6-phytase.

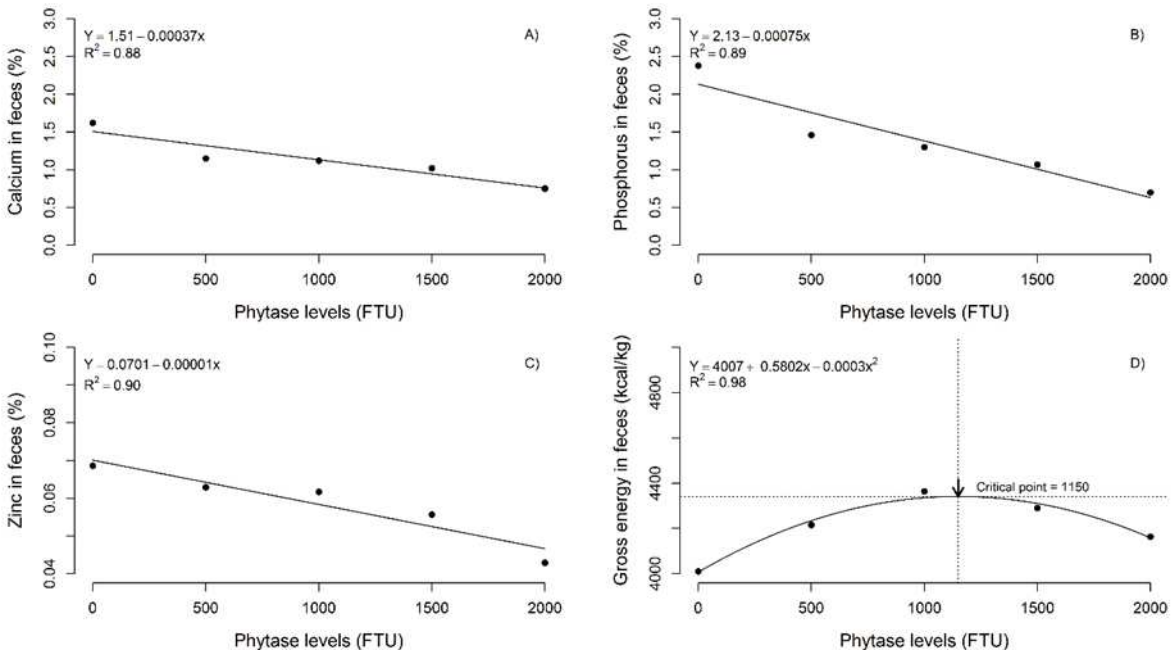

Fig. 6 - Adjusted linear and quadratic regression models of the calcium (A), phosphorus (B), zinc (C) and gross energy contents in the feces (D) of pigs in finishing II phase (day 64 to 89) fed diets supplemented with increasing levels of 6-phytase.

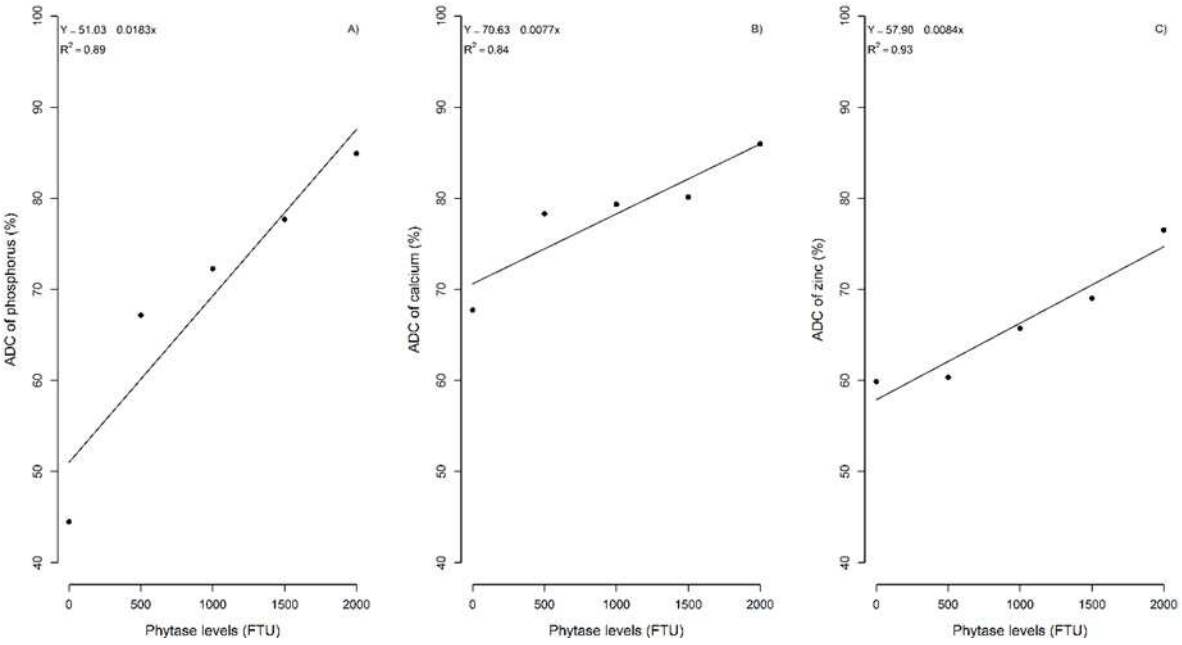

Fig. 7 - Adjusted linear regression models of the apparent total tract digestibility coefficients (ADC) of phosphorus (A), calcium (B) and zinc (C) of pigs in finishing II phase (day 64 to 89) fed diets supplemented with increasing levels of 6-phytase.

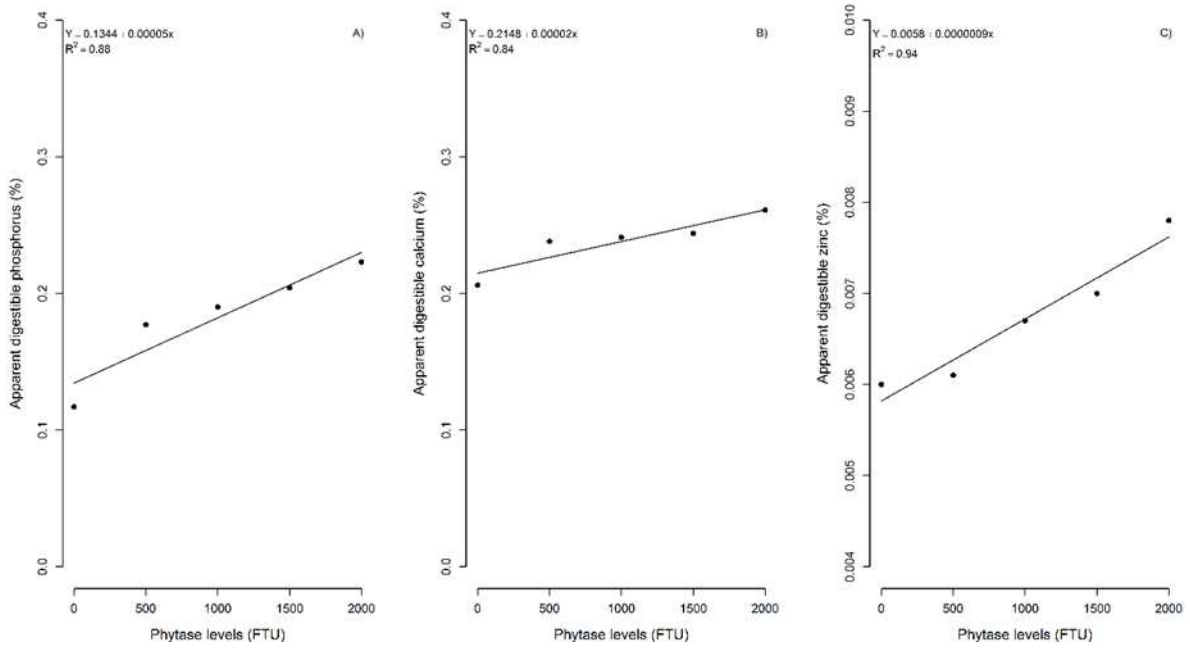

Fig. 8 - Adjusted linear regression models of apparent digestible phosphorus (A), calcium (B) and zinc (C) of pigs in finishing II phase (day 64 to 89) fed diets supplemented with increasing levels of 6-phytase.

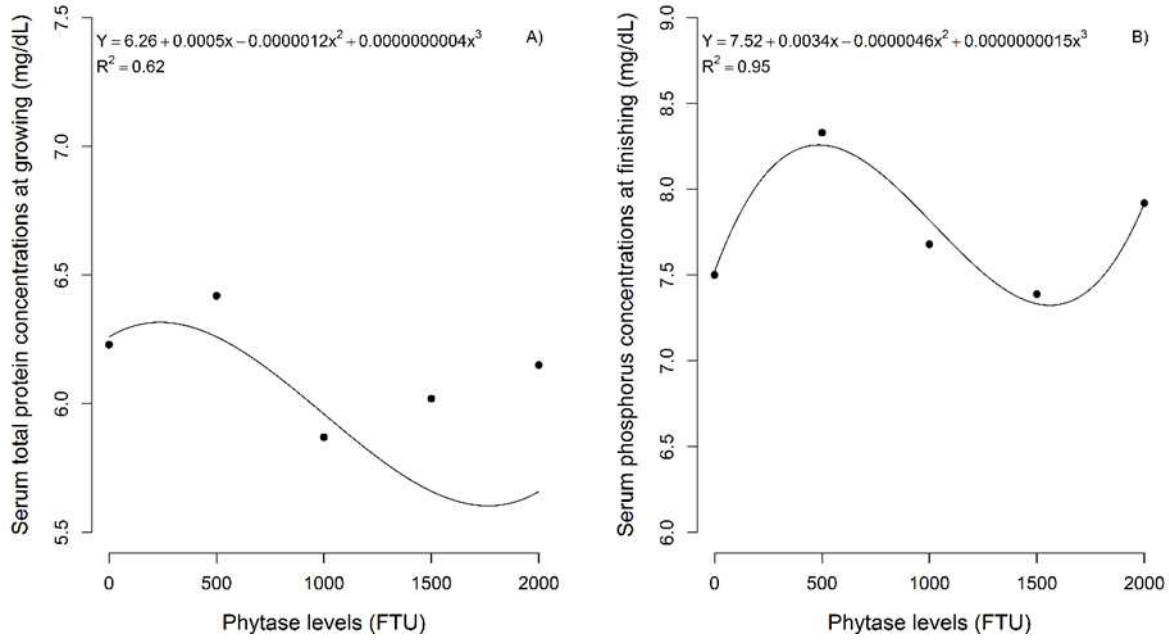

Fig. 9 - Adjusted cubic regression models of the concentrations of serum total protein (A) and phosphorus (B) in growing (day 40) and finishing (day 80) pigs fed diets supplemented with increasing levels of 6-phytase, respectively.

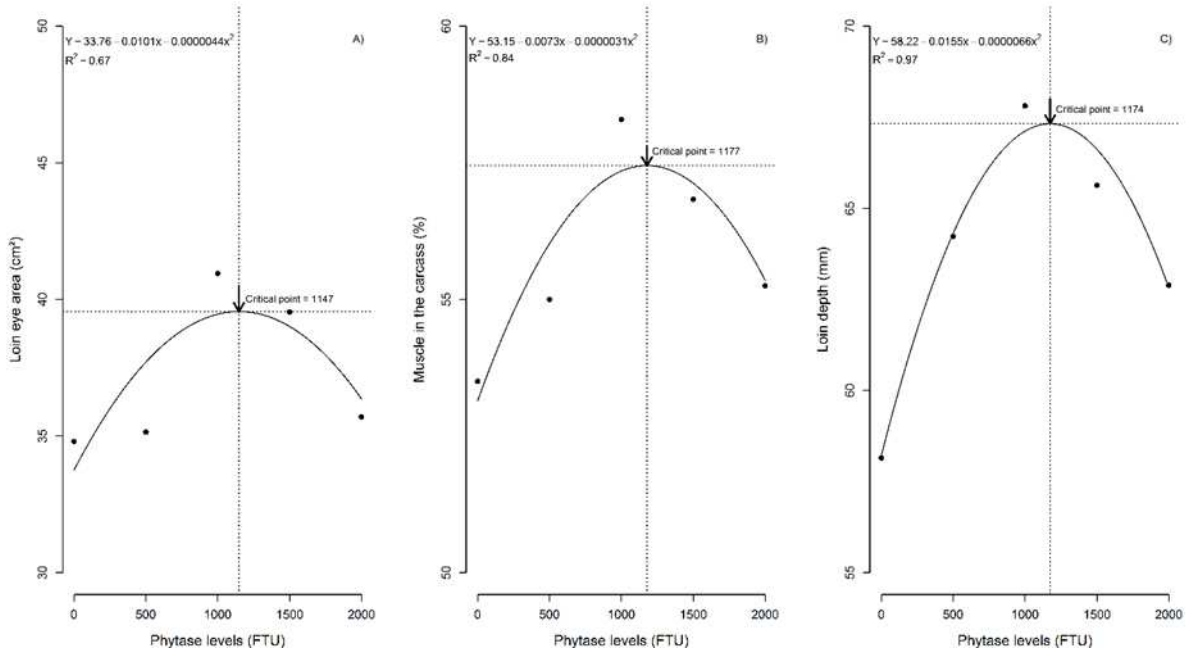

Fig. 10 - Adjusted quadratic regression models of the loin eye area (A), percentage of muscle in the carcass (B) and loin depth (C) of pigs in finishing II phase (day 89) fed diets supplemented with increasing levels of 6-phytase.

5 DISCUSSION

In the present study, we evaluated the supplementation of increasing levels of 6-phytase in nutrient- and energy-reduced diets fed to growing and finishing pigs, with the hypothesis that the enzyme would improve nutrients and energy digestibility and, consequently, positively affect growth performance and bone parameters. Phytate is an anti-nutritional factor that reduces the availability of nutrients (e.g. minerals, AA, carbohydrates) and energy (Selle et al., 2012; Zeng et al., 2014; Wensley et al., 2020), increases mucin loss and compromises Na-dependent transport of starch, glucose and AA in the intestine (Adeshina et al., 2022; Kiarie et al., 2022).

Previous studies (Faria et al., 2015; Buzek et al., 2023; Moita and Kim, 2023) have shown that phytase supplementation can release phytate-bound P by dephosphorylating phytic acid, increasing P digestibility and contributing to better mineral absorption (e.g. P, Ca, Zn, Mg, Na), as in the present study. Therefore, phytase supplementation is generally useful when the phytate content in the diet is high because phytic acid has a strong binding capacity to the AA in corn and soybean meal

(Araujo et al., 2023). In the present study, the phytic P contents calculated for the PC and NC diets in the growing I, growing II, finishing I, and finishing II phases were 0.22% and 0.22%, 0.21% and 0.21%, 0.20% and 0.20%, and 0.18% and 0.18%, respectively, based on the phytic P contents described by Rostagno et al. (2024).

In the current study, although a significant effect of supplementation with increasing levels of 6-phytase in diets on FC was observed only in the total period, the marginally higher results of ADG possibly resulted in better FC. Improved apparent digestibility has been shown to promote better nutrients absorption and utilization, and a reduction in the anti-nutritional effects of phytate in pigs fed diets supplemented with 6-phytase (500, 1000, 2000, and 5000 FTU/kg) (Moita and Kim, 2023), and phytase derived from *E. coli* (500, 1000, and 20000 FTU/kg, Zeng et al., 2014; 500, and 1000 FTU/kg, Kiarie et al., 2022). This better utilization of nutrients and energy (Holloway et al., 2019; Buzek et al., 2023) favors a better FC as observed in our study.

Previously, Silva et al. (2004) supplemented levels of a fungal phytase (400, 800, and 1200 FTU/kg) in diets for growing pigs and observed a linear improvement in FC, with no effects on ADG and ADFI. Although phytase supplementation in diets improves growth performance, the magnitude of this result varies according to the formulation of the basal diet (e.g. content of minerals and other nutrients), the phase of development of the pig, the sources and dosage of phytase, and the source of phytate (Zeng et al., 2014; Kiarie et al., 2022; Pereira et al., 2024). Other studies (Holloway et al., 2019; Grela et al., 2020) have confirmed minor improvements in the performance of finishing pigs, similar to what was observed in the present study. This is attributed to the fact that the growth performance response to phytase supplementation may involve removing the anti-nutrient effects of phytate and/or enhancing the release of other limiting compounds (e.g. inositol) from the diet (Holloway et al., 2019). In addition, the effects on performance may be marginal when the reduction in the contents of P or other nutrients in the diet are close to the animals' requirements (Araujo et al., 2023) or when the phytate content in the dietary components is lower (Buzek et al., 2023).

In a study conducted by Silva et al. (2022), the authors observed that finishing pigs fed diets supplemented with 1500 or 3000 FTU/kg of diet of a bacterial phytase had higher FBW at 112 days-old. In the present study, there was a linear trend of higher FBW in finishing pigs fed increasing levels of phytase. In discrepancy, Wensley et al. (2020) found no differences on growth performance in the growing and finishing phases in pigs fed diets containing 1500 FTU/kg. In the current study, the best

estimated levels of phytase for the pig performance in growing and total period were 1150 and 1216, and 1261 FTU/kg for FBW and ADG, and FC, respectively. This contradicts the results of other studies, for example, Holloway et al. (2019) tested three levels of phytase (1000, 1750, and 2500 FTU/kg) and observed better feed and energy efficiency in growing pigs fed diets containing the highest level of phytase, while Pereira et al. (2024) obtained a significant improvement in FC in nursery pigs fed diets containing 2000 FTU/kg diet.

In this previous conducted study, the authors believed that the reduction of the anti-nutritional effects attributed to phytate and, consequently, the release of myo-inositol may not result in better growth performance in pigs (Silva et al., 2022) due to the high levels of valorization of the enzyme matrix (Cowieson et al., 2016). In the present study, the slight improvements observed in pig performance can also be explained by the pathways stimulated by myo-inositol (e.g. protein accretion) whose action mimics insulin, with similar effects on glucose transporter-4 (not evaluated in the present study) when blood glucose concentration increases, which is involved in aspects of gluconeogenesis (Cowieson et al, 2017a; Schmeisser et al., 2017) and improved digestibility, ADG and FC (Silva et al., 2019).

Potentially, phytase supplementation has been shown to improve digestibility by reducing the formation of indigestible phytate complexes (Zeng et al., 2014; Grela et al., 2020; Kiarie et al., 2022), as well as limiting interactions between nutrients and phytate within the gastrointestinal tract (Jlali et al., 2024). Improved nutrient digestibility translates into a more efficient digestive process and reduced fecal excretion of nutrients (Adeola and Cowieson, 2011), as observed in our study in the linearly decreasing results of fecal excretion of minerals (Ca, P, Zn) as phytase levels increased. Studies conducted by Lee et al. (2017) and Holloway et al. (2019) reported a reduction in nutrient excretion in the feces of pigs fed phytase-based diets. This is because as phytase breaks down phytate, nutrients are released so that they are more easily absorbed in the gastrointestinal tract (Selle et al., 2014). In addition, the digestibility of Zn was also improved, although less than that of Ca and P because Zn is less bound to phytate (Liu et al., 2014), confirming the results found for ADC and digestible minerals.

In our study, linearly increasing ADC results for Ca, P and Zn, and digestible Ca, P and Zn were obtained as phytase levels increased in diets for finishing pigs, in agreement with the results found by Grela et al. (2020) for the ADC of Ca and P. In the

growing phase, a quadratic improvement was observed in the ADC for P (1687 FTU/kg) and Zn (953 FTU/kg), and for Pdig (1683 FTU/kg) and Zndig (965 FTU/kg). Digestible Ca showed an increasing linear improvement as dietary phytase levels increased. No effect on ADC for P and Ca was observed between two levels of microbial phytase supplementation (1107 or 2215 FTU/kg) in diets for growing pigs (Rutherfurd et al., 2014), which contradicts the findings of the present study. Previous studies have reported a reduction in fecal P contents as a result of better P utilization in growing pigs fed diets containing fungal phytase (Faria et al., 2015) and 6-phytase (250, 500, 750, and 1000 FTU/kg) (Araujo et al., 2023), confirming that phytase effectively reduces the loss of this mineral (Cowieson et al., 2011). The reduction in fecal contents of Ca and Zn can be explained by the increased bioavailability of these minerals when phytate is degraded, promoting greater retention in the animal's body (Faria et al., 2015).

In relation to fecal CP and GE contents, phytase may have a marginal influence (Cowieson et al., 2011). Although the main function of phytase is to improve mineral digestibility, there is evidence that has found a slight increase in ADC of GE and CP (Cowieson et al., 2017b; Wensley et al., 2020), due to extra phosphoric effects (Cowieson et al., 2011). However, studies of protein and amino acid digestibility in growing and finishing pigs fed phytase have had conflicting and inconclusive results (Traylor et al., 2011; Selle et al., 2012). The result of increased digestibility and energy availability due to the breakdown of phytate complexes with some nutrients (Buzek et al., 2023) may promote an increase in fecal GE and reduce DE, as observed in the present study in growing pigs. Also, the effect of phytase activity on increasing energy utilization in pigs is mainly derived from higher protein digestibility (Zeng et al., 2014). This is in line with the results found in the present study, where higher ADC for CP and GE were observed in finishing pigs fed levels of phytase in their diets.

Bones are the main storage tissue for P and, therefore, dietary P directly affects bone development. However, when dietary P contents are close to or above physiological requirements in animals, phytase supplementation in diets may not significantly affect bone parameters (Adeola and Cowieson, 2011; Zeng et al., 2011), corroborating the results observed in our study. This result suggests that the release of phytate-bound P was sufficient to maintain bone health without additional benefits. In addition, bone mineralization is a response to phytase supplementation in diets for young pigs, where P requirements for bone development are higher (Selle et al., 2012).

However, bone development is less active in finishing pigs and, therefore, improvements in bone parameters may be minimal or no significant (Cambra-López et al., 2020; Chen et al., 2023).

The results of the bone parameters contradict other studies such as those conducted by Moita and Kim (2023), who evaluated levels of bacterial 6-phytase (500, 1000, 2000, and 5000 FTU/kg) in diets for nursery pigs and observed that increasing phytase supplementation linearly increased P and ash contents in bones, while improving bone breaking strength at the estimated level of 1889 FTU/kg. Similarly, as the level of supplemented phytase increased in finishing pigs fed a P-deficient diet (75% less than PC), a linear increase and quadratic response were observed in P contents in the femur (Czech et al., 2022), while total P in the metacarpal increased significantly in finishing pigs (Buzek et al., 2023).

Improvements in the ADC for P, Ca and Zn can influence the mineral composition of bones (Grela et al., 2020), although in the present study we did not observe this or changes in the serum ALP activity, which is the enzyme responsible for bone mineralization (Kiarie et al., 2022). Grela et al. (2020) and Miller et al. (2016) reported that bone parameters are less sensitive to phytase supplementation when P contents are sufficient or when serum P concentrations are regulated, indicating a threshold beyond which additional P availability does not further improve bone growth or composition, but may influence mineral composition in other body tissues (Schmeisser et al., 2017). Changes in bone parameters (e.g. ash content, BMD, BMC) are observed due to P supply (Babatunde and Adeola, 2022), and the use of diets deficient in Ca and P even when supplemented with phytase may not reflect effects on BMC (Faria et al., 2015). This may explain the lack of effects observed on bone parameters in the present study.

Most of the blood metabolites evaluated in this study were not influenced by the dietary treatments and did not vary in the phases evaluated. In addition, the improvement in ADC, DN and DE did not promote changes in the urea, glucose and total cholesterol concentrations, because there is an interaction of phytate with dietary proteins, carbohydrates and lipids released by the hydrolysis of phytic acid caused by the action of phytase (Selle et al., 2014). Cowieson et al. (2017a) evaluated diets containing myo-inositol (2 g/kg) or phytase (1000 or 3000 FTU/kg) and found no effects on blood metabolites (e.g. ALP, urea, glucose, Ca, triacylglycerol) in pigs, in accordance with the results of this study. In contrast, Kiarie et al. (2022) observed a

linear reduction in ALP activity in pigs fed diets supplemented with phytase. Phytase can increase the availability and absorption of Ca and P, and this is not always reflected in higher blood concentrations because Ca and P homeostasis are tightly regulated (Nunes and Guggenbuhl, 1998) by parathyroid hormone and associated mechanisms (Cowieson et al., 2017a). When dietary needs are met, minerals such as Ca and P are less influenced by phytase supplementation in diets (Harper et al., 1997).

The regulatory effects of blood P concentrations have been well documented by Jendza et al. (2005) and Magnago et al. (2015), showing that increased P release with phytase supplementation does not always increase serum P concentration. Similarly, Babatunde and Adeola (2022) observed minor changes in blood P in pigs fed diets supplemented with phytase, highlighting the homeostatic mechanisms involved in regulating P concentrations. In contrast, a previous study (Madrid et al., 2013) observed that pigs fed diets deficient in P (0.13% less total P compared to PC) had a reduction in serum P concentrations, and supplementation with 500 FTU of phytase derived from *E. coli*/kg diet attenuated this effect.

On the other hand, given that blood P concentrations were similar in pigs fed PC and NC diets supplemented with 500, 1000, and 2000 FTU/kg diet, the difference in fecal P contents between both groups suggests that pigs fed diets supplemented with phytase (except 1500 FTU/kg) had greater tissue retention of P, although this was not reflected in greater P deposition in bones. When the blood P concentrations has been fitted to regression models, there is limited biological significance to cubic models even if the parameters are significant and the model has an adequate goodness of fit. Therefore, we hypothesized that the cubic response observed in the serum P concentrations of finishing pigs could be related to: 1) saturation effects, where phytase supplementation may release more P than the animal can absorb or utilize efficiently; 2) feedback regulation, where increased serum P can activate endocrine pathways that regulate P homeostasis (e.g. absorption and excretion); 3) interactions with other nutrients, where unbalanced relationships between minerals (e.g. Ca and P) may influence serum P absorption and dynamics.

Although there was a trend towards a cubic response in total protein concentration, this result may not indicate a direct response to phytase supplementation. A previous study (Kiarie et al., 2022) did not observe any change in total protein concentration in pigs fed diets containing phytase. Protein status is mainly influenced by amino acid intake and overall protein synthesis. Previously (Zeng et al., 2014; Wensley et al.,

2020, Lagos et al., 2023) an improvement in the digestibility and release of AA in the phytate molecule was observed by the action of phytase. Therefore, major changes in total protein concentrations in animals fed diets containing phytase could be supported not only by improved amino acid digestibility, but also by effects such as changes in nutritional status (e.g. metabolic effects), improved protein utilization, increased growth rate (e.g. anabolic activity), as well as improved immune function (e.g. release of minerals essential for the synthesis of immune proteins) (Adeshina et al., 2022). A possible reason could be the immunostimulant effect of dietary phytase, but this hypothesis needs to be further investigated.

The increased bioavailability of nutrients and energy due to the action of phytase may contribute to improving muscle deposition and carcass traits, consistent with previous findings (Silva et al., 2019; Silva et al., 2022). In the present study, the increase in ADC, DN and DE observed in pigs fed phytase suggests an improvement in the fractionation and utilization of dietary components, benefiting muscle growth with estimated phytase levels of 1147, 1174, and 1177 FTU/kg for loin eye area, loin depth, and percentage of muscle in the carcass, respectively. In contrast, previous studies have observed that supplementation with 1200 FTU of hybrid 6-phytase/kg (Gebert et al., 1998) and an *E. coli*-derived phytase (500, and 2000 FTU/kg) (Buzek et al., 2023) only affected meat color brightness and texture. Phosphorus is also essential in muscle development (Buzek et al., 2023) and, therefore, when the minimum need for this mineral is met (e.g. by supplementing phytase in the diet), an improvement in lean tissue deposition up to a certain limit can be observed (Silva et al., 2019).

In addition, the myo-inositol released by the action of phytase increases the expression of insulin genes and pathways related to insulin-like growth factor-1 (Schmeisser et al., 2017). A possible explanation is that these pathways (although not evaluated in this study) are responsible for increasing muscle protein deposition and negatively regulating gluconeogenesis (Silva et al., 2022). The results obtained for meat traits may suggest the role of the higher concentrations of myo-inositol (not evaluated in our study) through the action of phytase in enhancing greater lean meat deposition, an effect related to the increase in insulin sensitivity promoted by the myo-inositol molecule (Yamashita et al., 2013).

In the present study, we evaluated the supplementation of increasing levels of 6-phytase in nutrient- and energy-reduced diets fed to growing and finishing pigs, with the hypothesis that the enzyme would improve nutrients and energy digestibility and,

consequently, positively affect growth performance and bone parameters. Phytate is an anti-nutritional factor that reduces the availability of nutrients (e.g. minerals, AA, carbohydrates) and energy (Selle et al., 2012; Zeng et al., 2014; Wensley et al., 2020), increases mucin loss and compromises Na-dependent transport of starch, glucose and AA in the intestine (Adeshina et al., 2022; Kiarie et al., 2022).

Previous studies (Faria et al., 2015; Buzek et al., 2023; Moita and Kim, 2023) have shown that phytase supplementation can release phytate-bound P by dephosphorylating phytic acid, increasing P digestibility and contributing to better mineral absorption (e.g. P, Ca, Zn, Mg, Na), which agrees with the results from the present study. Therefore, phytase supplementation is generally useful when the phytate content in the diet is high because phytic acid has a strong binding capacity to the AA in corn and soybean meal (Araujo et al., 2023). In the present study, the phytic P contents calculated for the PC and NC diets in the growing I, growing II, finishing I, and finishing II phases were 0.22% and 0.22%, 0.21% and 0.21%, 0.20% and 0.20%, and 0.18% and 0.18%, respectively, based on the phytic P contents described by Rostagno et al. (2024).

In the current study, although a significant effect of supplementation with increasing levels of 6-phytase in diets on FC was observed only in the total period, the marginally higher results (e.g. FBW and ADG) possibly resulted in better FC. Improved apparent digestibility has been shown to promote better nutrients absorption and utilization, and a reduction in the anti-nutritional effects of phytate in pigs fed diets supplemented with 6-phytase (500, 1000, 2000, and 5000 FTU/kg) (Moita and Kim, 2023), and phytase derived from *E. coli* (500, 1000, and 20000 FTU/kg, Zeng et al., 2014; 500, and 1000 FTU/kg, Kiarie et al., 2022). This better utilization of nutrients and energy (Holloway et al., 2019; Buzek et al., 2023) favors a better FC as observed in our study.

Previously, Silva et al. (2004) supplemented levels of a fungal phytase (400, 800, and 1200 FTU/kg) in diets for growing pigs and observed a linear improvement in FC, with no effects on ADG and ADFI. Although phytase supplementation in diets improves growth performance, the magnitude of this result varies according to the formulation of the basal diet (e.g. content of minerals and other nutrients), the phase of development of the pig, the sources and dosage of phytase, and the source of phytate (Zeng et al., 2014; Kiarie et al., 2022; Pereira et al., 2024). Other studies (Holloway et al., 2019; Grela et al., 2020) have confirmed minor improvements in the

performance of finishing pigs, similar to what was observed in the present study. This is attributed to the fact that the growth performance response to phytase supplementation may involve removing the anti-nutrient effects of phytate and/or enhancing the release of other limiting compounds (e.g. inositol) from the diet (Holloway et al., 2019). In addition, the effects on performance may be marginal when the reduction in the contents of P or other nutrients in the diet are close to the animals' requirements (Araujo et al., 2023) or when the phytate content in the dietary components is lower (Buzek et al., 2023).

In a study conducted by Silva et al. (2022), the authors observed that finishing pigs fed diets supplemented with 1500 or 3000 FTU/kg of diet of a bacterial phytase had higher FBW at 112 days-old. In the present study, there was an increasing linear trend of higher FBW in finishing pigs fed increasing levels of phytase. In discrepancy, Wensley et al. (2020) found no differences on growth performance in the growing and finishing phases in pigs fed diets containing 1500 FTU/kg. In the current study, the best estimated levels of phytase for the pig performance in growing and total period were 1150 and 1216, and 1261 FTU/kg for FBW and ADG, and FC, respectively. This contradicts the results of other studies, for example, Holloway et al. (2019) tested three levels of phytase (1000, 1750, and 2500 FTU/kg) and observed better feed and energy efficiency in growing pigs fed diets containing the highest level of phytase, while Pereira et al. (2024) obtained a significant improvement in FC in nursery pigs fed diets containing 2000 FTU/kg diet.

In this previous conducted study, the authors believed that the reduction of the anti-nutritional effects attributed to phytate and, consequently, the release of myo-inositol may not result in better growth performance in pigs (Silva et al., 2022) due to the high levels of valorization of the enzyme matrix (Cowieson et al., 2016). In the present study, the slight improvements observed in pig performance can also be explained by the pathways stimulated by myo-inositol (e.g. protein accretion) whose action mimics insulin, with similar effects on glucose transporter-4 (not evaluated in the present study) when blood glucose concentration increases, which is involved in aspects of gluconeogenesis (Cowieson et al, 2017a; Schmeisser et al., 2017) and improved digestibility, ADG and FC (Silva et al., 2019).

Potentially, phytase supplementation has been shown to improve digestibility by reducing the formation of indigestible phytate complexes (Zeng et al., 2014; Grela et al., 2020; Kiarie et al., 2022), as well as limiting interactions between nutrients and

phytate within the gastrointestinal tract (Jlali et al., 2024). Improved nutrient digestibility translates into a more efficient digestive process and reduced fecal excretion of nutrients (Adeola and Cowieson, 2011), as observed in our study in the linearly decreasing results of fecal excretion of minerals (Ca, P, Zn) as phytase levels increased. Studies conducted by Lee et al. (2017) and Holloway et al. (2019) reported a reduction in nutrient excretion in the feces of pigs fed phytase-based diets. This is because as phytase breaks down phytate, nutrients are released so that they are more easily absorbed in the gastrointestinal tract (Selle et al., 2014). In addition, the digestibility of Zn is also improved, although less than that of Ca and P because Zn is less bound to phytate (Liu et al., 2014), confirming the results found for ADC and digestible minerals.

In our study, linearly increasing ADC results for Ca, P and Zn, and digestible Ca, P and Zn were obtained as phytase levels increased in diets for finishing pigs, in agreement with the results found by Grela et al. (2020) for the ADC of Ca and P. In the growing phase, a quadratic improvement was observed in the ADC for P (1687 FTU/kg) and Zn (953 FTU/kg), and for Pdig (1683 FTU/kg) and Zndig (965 FTU/kg). Digestible Ca showed an increasing linear improvement as dietary phytase levels increased. No effect on ADC for P and Ca was observed between two levels of microbial phytase supplementation (1107 or 2215 FTU/kg) in diets for growing pigs (Rutherfurd et al., 2014), which contradicts the findings of the present study. Previous studies have reported a reduction in fecal P contents as a result of better P utilization in growing pigs fed diets containing fungal phytase (Faria et al., 2015) and 6-phytase (250, 500, 750, and 1000 FTU/kg) (Araujo et al., 2023), confirming that phytase effectively reduces the loss of this mineral (Cowieson et al., 2011). The reduction in fecal contents of Ca and Zn can be explained by the increased bioavailability of these minerals when phytate is degraded, promoting greater retention in the animal's body (Faria et al., 2015).

In relation to fecal CP and GE contents, phytase may have a marginal influence (Cowieson et al., 2011). Although the main function of phytase is to improve mineral digestibility, there is evidence that has found a slight increase in ADC of GE and CP (Cowieson et al., 2017b; Wensley et al., 2020), due to extra phosphoric effects (Cowieson et al., 2011). However, studies of protein and amino acid digestibility in growing and finishing pigs fed phytase have had conflicting and inconclusive results (Traylor et al., 2011; Selle et al., 2012). The result of increased digestibility and energy

availability due to the breakdown of phytate complexes with some nutrients (Buzek et al., 2023) may promote an increase in fecal GE and reduce DE, as observed in the present study in growing pigs. Also, the effect of phytase activity on increasing energy utilization in pigs is mainly derived from higher protein digestibility (Zeng et al., 2014). This is in line with the results found in the present study, where higher ADC for CP and GE were observed in animals fed levels of phytase in their diets.

Bones are the main storage tissue for P and, therefore, dietary P directly affects bone development. However, when dietary P contents are close to or above physiological requirements in animals, phytase supplementation in diets may not significantly affect bone parameters (Adeola and Cowieson, 2011; Zeng et al., 2011), corroborating the results observed in our study. This result suggests that the release of phytate-bound P was sufficient to maintain bone health without additional benefits. In addition, bone mineralization is a response to phytase supplementation in diets for young pigs, where P requirements for bone development are higher (Selle et al., 2012). However, bone development is less active in finishing pigs and, therefore, improvements in bone parameters may be minimal or no significant (Cambra-López et al., 2020; Chen et al., 2023).

The results of the bone parameters contradict other studies such as those conducted by Moita and Kim (2023), who evaluated levels of bacterial 6-phytase (500, 1000, 2000, and 5000 FTU/kg) in diets for nursery pigs and observed that increasing phytase supplementation linearly increased P and ash contents in bones, while improving bone breaking strength at the estimated level of 1889 FTU/kg. Similarly, as the level of supplemented phytase increased in finishing pigs fed a P-deficient diet (75% less than PC), a linear increase and quadratic response were observed in P contents in the femur (Czech et al., 2022), while total P in the metacarpal increased significantly in finishing pigs (Buzek et al., 2023).

Improvements in the ADC for P, Ca and Zn can influence the mineral composition of bones (Grela et al., 2020), although in the present study we did not observe this or changes in the serum ALP activity, which is the enzyme responsible for bone mineralization (Kiarie et al., 2022). Grela et al. (2020) and Miller et al. (2016) reported that bone parameters are less sensitive to phytase supplementation when P contents are sufficient or when serum P concentrations are regulated, indicating a threshold beyond which additional P availability does not further improve bone growth or composition, but may influence mineral composition in other body tissues

(Schmeisser et al., 2017). Changes in bone parameters (e.g. ash content, BMD, BMC) are observed due to P supply (Babatunde and Adeola, 2022), and the use of diets deficient in Ca and P even when supplemented with phytase may not reflect effects on BMC (Faria et al., 2015). This may explain the lack of effects observed on bone parameters in the present study.

Most of the blood metabolites evaluated in this study were not influenced by the dietary treatments and did not vary in the phases evaluated. In addition, the improvement in ADC, DN and DE did not promote changes in the urea, glucose and total cholesterol concentrations, because there is an interaction of phytate with dietary proteins, carbohydrates and lipids released by the hydrolysis of phytic acid caused by the action of phytase (Selle et al., 2014). Cowieson et al. (2017a) evaluated diets containing myo-inositol (2 g/kg) or phytase (1000 or 3000 FTU/kg) and found no effects on blood metabolites (e.g. ALP, urea, glucose, Ca, triacylglycerol) in pigs, in accordance with the results of this study. In contrast, Kiarie et al. (2022) observed a linear reduction in ALP activity in pigs fed diets supplemented with phytase. Phytase can increase the availability and absorption of Ca and P, and this is not always reflected in higher blood concentrations because Ca and P homeostasis are tightly regulated (Nunes and Guggenbuhl, 1998) by parathyroid hormone and associated mechanisms (Cowieson et al., 2017a). When dietary needs are met, minerals such as Ca and P are less influenced by phytase supplementation in diets (Harper et al., 1997).

The regulatory effects of blood P concentrations have been well documented by Jendza et al. (2005) and Magnago et al. (2015), showing that increasing P with phytase supplementation does not always increase serum P concentration. Similarly, Babatunde and Adeola (2022) observed minor changes in blood P in pigs fed diets supplemented with phytase, highlighting the homeostatic mechanisms involved in regulating P concentrations. In contrast, a previous study (Madrid et al., 2013) observed that pigs fed diets deficient in P (0.13% less total P compared to PC) had a reduction in serum P concentrations, and supplementation with 500 FTU of phytase derived from *E. coli*/kg diet attenuated this effect.

On the other hand, given that blood P concentrations were similar in pigs fed PC and NC diets supplemented with 500, 1000, and 2000 FTU/kg diet, the difference in fecal P contents between both groups suggests that pigs fed diets supplemented with phytase (except 1500 FTU/kg) had greater tissue retention of P, although this was not reflected in greater P deposition in bones. When the blood P concentrations has

been fitted to regression models, there is limited biological significance to cubic models even if the parameters are significant and the model has an adequate goodness of fit. Therefore, we hypothesized that the cubic response observed in the serum P concentrations of finishing pigs could be related to: 1) saturation effects, where phytase supplementation may release more P than the animal can absorb or utilize efficiently; 2) feedback regulation, where increased serum P can activate endocrine pathways that regulate P homeostasis (e.g. absorption and excretion); 3) interactions with other nutrients, where unbalanced relationships between minerals (e.g. Ca and P) may influence serum P absorption and dynamics.

Although there was a trend towards a cubic response in total protein concentration, this result may not indicate a direct response to phytase supplementation. A previous study (Kiarie et al., 2022) did not observe any change in total protein concentration in pigs fed diets containing phytase. Protein status is mainly influenced by amino acid intake and overall protein synthesis. Previously (Zeng et al., 2014; Wensley et al., 2020, Lagos et al., 2023) an improvement in the digestibility and release of AA in the phytate molecule was observed by the action of phytase. Therefore, major changes in total protein concentrations in animals fed diets containing phytase could be supported not only by improved amino acid digestibility, but also by effects such as changes in nutritional status (e.g. metabolic effects), improved protein utilization, increased growth rate (e.g. anabolic activity), as well as improved immune function (e.g. release of minerals essential for the synthesis of immune proteins) (Adeshina et al., 2022). A possible reason could be the immunostimulant effect of dietary phytase, but this hypothesis needs to be further investigated.

The increased bioavailability of nutrients and energy due to the action of phytase may contribute to improving muscle deposition and carcass traits, consistent with previous findings (Silva et al., 2019; Silva et al., 2022). In the present study, the increase in ADC, DN and DE observed in pigs fed phytase suggests an improvement in the fractionation and utilization of dietary components, benefiting muscle growth with estimated phytase levels of 1147, 1174, and 1177 FTU/kg for loin eye area, loin depth, and percentage of muscle in the carcass, respectively. In contrast, previous studies have observed that supplementation with 1200 FTU of hybrid 6-phytase/kg (Gebert et al., 1998) and an *E. coli*-derived phytase (500, and 2000 FTU/kg) (Buzek et al., 2023) only affected meat color brightness and texture. Phosphorus is also essential in muscle development (Buzek et al., 2023) and, therefore, when the minimum need for this

mineral is met (e.g. by supplementing phytase in the diet), an improvement in lean tissue deposition up to a certain limit can be observed (Silva et al., 2019).

In addition, the myo-inositol released by the action of phytase increases the expression of insulin genes and pathways related to insulin-like growth factor-1 (Schmeisser et al., 2017). A possible explanation is that these pathways (although not evaluated in this study) are responsible for increasing muscle protein deposition and negatively regulating gluconeogenesis (Silva et al., 2022). The results obtained for meat traits may suggest the role of the higher concentrations of myo-inositol (not evaluated in our study) through the action of phytase in enhancing greater lean meat deposition, an effect related to the increase in insulin sensitivity promoted by the myo-inositol molecule (Yamashita et al., 2013).

6 CONCLUSION

Based on the criteria evaluated in this study, dietary supplementation with increasing levels of 6-phytase marginally improved the quantitative traits of meat compared to pigs fed nutritionally reduced diets without phytase, despite the minor influence on growth performance. In addition, supplementation with increasing levels of 6-phytase in diets promoted greater nutrients digestibility, while reduced mineral fecal excretion in pigs. Although the observed serum P concentration showed an oscillatory response, bone parameters were not altered, suggesting that phytase levels did not affect the pigs' bone health. Therefore, a level of 1000 FTU/kg using the bestzyme's enzyme matrix was optimal in nutritionally reduced diets for growing and finishing pigs.

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