



## Levels of digestible phosphorus for growing gilts

[ Níveis de fósforo digestível para leitoas em crescimento ]

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**Abstract:** The aim of this study was to evaluate the effects of digestible phosphorus levels in diets for growing gilts (30 to 50 kg), which have high potential for lean meat deposition, on the performance and characteristics of waste. Sixty gilts were used, with an initial weight of  $29.99 \pm 3.37$  kg, distributed in a randomized block design with five levels of digestible phosphorus (0.219, 0.299, 0.319, 0.369, and 0.419 %), six replicates, and two animals per experimental unit. Phosphorus levels did not influence ( $P>0.05$ ) final weight, total weight gain, daily weight gain, daily feed intake, total feed intake, crude protein consumption, digestible lysine consumption, metabolizable energy, and feed conversion. The daily intakes of digestible phosphorus and calcium increased linearly ( $P<0.01$ ) with increasing levels of phosphorus and calcium in the diet. The dry matter, natural matter, residue coefficient, total solids, and total nitrogen of the manure were not influenced ( $P>0.05$ ) by the phosphorus level. On the other hand, a linear increase ( $P<0.01$ ) was observed for volatile solids and total phosphorus in swine manure as the digestible phosphorus level increased. The level of 0.219 % digestible phosphorus, corresponding to an intake of 3.67 g of daily digestible phosphorus, meets the nutritional requirements of phosphorus for growing gilts (30 to 50 kg) and allows for a reduction in phosphorus excretion in waste.

**Keywords:** manure; minerals; nutrition; nutritional requirements.

**Resumo:** Realizou-se este estudo com o objetivo de avaliar níveis de fósforo digestível em dietas para leitoas em crescimento (30 aos 50 kg), com alto potencial para deposição de carne magra, sobre desempenho e características dos dejetos. Foram utilizadas 60 leitoas, com peso inicial de  $29,99 \pm 3,37$  kg, distribuídos em delineamento de blocos ao acaso, com cinco níveis de fósforo digestível (0,219; 0,269; 0,319; 0,369 e 0,419 %), seis repetições e dois animais por unidade experimental. Os níveis de fósforo não influenciaram ( $P>0,05$ ) o peso final, ganho de peso total, ganho de peso diário, consumo de ração diário, consumo de ração total, consumo de proteína bruta, consumo de lisina digestível, consumo de energia metabolizável e a conversão alimentar. Os consumos diários de fósforo digestível e de cálcio aumentaram linearmente ( $P<0,01$ ) de acordo com o aumento do nível de fósforo e cálcio na dieta. A matéria seca, matéria natural, coeficiente de resíduo, sólidos totais e nitrogênio total dos dejetos não foram influenciados ( $P>0,05$ ) pelos níveis de fósforo. Por outro lado, foi possível observar aumento linear ( $P<0,01$ ) para sólidos voláteis e fósforo total nos dejetos dos suínos de acordo com o aumento dos níveis de fósforo digestível. Conclui-se que o nível de 0,219 %



de fósforo digestível, correspondente ao consumo de 3,67 g de fósforo digestível diário, atende às exigências nutricionais de fósforo para leitoas em crescimento (30 aos 50 kg) e possibilita a redução da excreção de fósforo nos dejetos.

**Palavras-chave:** exigência nutricional; dejetos; minerais; nutrição.

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## 1. Introduction

To improve economic efficiency in the production of lean meat in modern pig farming, the nutritional requirements of certain nutrients need constant updates. Among these, phosphorus is of particular interest because of its importance for many processes within organisms, such as energy metabolism and protein synthesis <sup>(1)</sup>.

Inadequate phosphorus intake can result in reduced muscle and bone growth rates and decreased feed efficiency in pigs <sup>(2)</sup>. Therefore, formulating diets for swine requires precise and up-to-date information on phosphorus requirements during different rearing phases; otherwise, animal performance may be compromised <sup>(3)</sup>.

An adequate concentration of phosphorus in the diet provides the animal with maximum deposition of muscle tissue <sup>(4)</sup>. Therefore, pigs with a high capacity for lean meat deposition will mobilize phosphorus from bones and will not achieve maximum muscle protein deposition if they are fed deficient diets. On the other hand, a diet with excess phosphorus may compromise calcium absorption and increase production costs, as phosphorus is the third most expensive nutrient in swine diets, in addition to causing greater excretion of this nutrient, which can have a negative environmental impact <sup>(5)</sup>.

Over the years, there have been changes in the daily phosphorus recommendations for swine. In 2005, the recommended available phosphorus value in the diet for high-genetic-potential gilts was 0.332 % <sup>(6)</sup>. However, with successive updates of the Brazilian tables of nutritional requirements for poultry and swine, recommendations have also begun to be presented for digestible phosphorus, with recommended levels of 0.319 % <sup>(7)</sup>, 0.349 % <sup>(8)</sup>, and 0.360 % <sup>(9)</sup>. The updates in nutritional levels demonstrate a linear increase in requirements, and in this scenario, continuous evaluation of the nutritional phosphorus requirements for swine becomes necessary. Therefore, this study was conducted to evaluate the levels of digestible phosphorus in the diets of gilts ranging from 30 to 50 kg, with a focus on the performance and quantitative and qualitative characteristics of manure.

## 2. Material and methods

The research was conducted at the Federal University of Mato Grosso do Sul (UFMS). The research was carried out in accordance with the animal ethics committee of UFMS (protocol number 721/2015). Sixty crossbred gilts (Duroc/Pietran × Large White/Landrace) with an initial weight of  $29.99 \pm 3.37$  kg and a final weight of  $48.19 \pm 4.11$  kg were used. The animals were housed in a masonry shed containing 30 pens measuring 1.15 m in width × 2.86 m in length and equipped with a water trough, semiautomatic feeders, and nipple drinkers.

The dry bulb, wet bulb, black globe temperatures and relative humidity (%) were measured daily at 08:00 and 16:00 h at six points at the height of the animals' backs via a portable digital thermometer model ITWTG 2000. The black globe temperature and humidity index (BGHI) was calculated via the equation proposed by Buffington <sup>(10)</sup>. The average minimum and maximum

temperatures, relative humidity, and BGHI observed during the experimental period were  $22.0 \pm 2.32^{\circ}\text{C}$ ,  $31.0 \pm 2.50^{\circ}\text{C}$ ,  $81.92 \pm 10.98 \%$ , and  $76.85 \pm 3.01$ , respectively. The experimental period lasted 21 days.

The animals were distributed in a randomized block experimental design, with five levels of digestible phosphorus (0.219, 0.269, 0.319, 0.369, and 0.419 %), six replicates, and two animals per experimental unit. The experimental unit was represented by the pen, and the initial weight of the animals was considered for the formation of the blocks.

The experimental diets were formulated on the basis of corn and soybean meal supplemented with minerals and vitamins to meet the nutritional requirements of gilts <sup>(7)</sup>, except for the levels of digestible phosphorus and calcium. The calcium:phosphorus ratio was kept constant. The total phosphorus concentrations of the ingredients were evaluated through laboratory tests conducted at the Animal Nutrition Laboratory of UFMS. The digestible phosphorus concentrations used to prepare the experimental diets (Table 1) were based on the digestibility coefficients established for growing swine <sup>(7)</sup>.

**Table 1.** Total values of calcium and phosphorus, digestibility coefficient, and digestible phosphorus of the ingredients used in the experimental diets.

Ingredients	Calcium, g/kg <sup>1</sup>	Total phosphorus, g/kg <sup>1</sup>	CVDP <sup>2</sup>	Digestible phosphorus, g/kg
Corn	0.30	2.50	44.00	0.97
Soybean meal, 46 %	2.40	6.40	45.70	2.60
Dicalcium phosphate	245.00	178.50	75.00	139.70
Calcite limestone	377.00	-	-	-

<sup>1</sup>Analyzed values. <sup>2</sup>CVDP = true phosphorus digestibility coefficient for swine <sup>(7)</sup>.

On the basis of the reference diet (Table 2), the experimental diets were supplemented with dicalcium phosphate in place of kaolin to prepare diets with five digestible phosphorus concentrations. All the diets were supplemented with calcitic limestone as a calcium source to maintain a constant ratio of digestible phosphorus to calcium. The diets were supplemented with crystalline amino acids to maintain the same ideal protein pattern among the diets. The experimental diets and water were provided ad libitum to the animals.

The produced waste was quantified and subjected to physical characterization through the determination of total solids and volatile solids and chemical characterization, with the analysis of total nitrogen and total phosphorus. On the days preceding each collection, the pens were completely washed, and no water blade was used during the sampling period. Each collection was carried out over 24 hours. After this period, the waste was weighed, placed in properly identified plastic bags, and stored in a freezer at  $-12^{\circ}\text{C}$ . The samples were subsequently thawed and kept at room temperature for analysis. The waste production, expressed in kg total solids/animal/day, was calculated via the data on waste weight in kg, number of housed animals, number of days, and total solids (TS) content found in the waste, according to the following equation: Waste production = kg of waste/animal/day  $\times$  total solids <sup>(11)</sup>.

The residue coefficient (CR) was also calculated, which indicates the amount of residue generated per kilogram of weight gain, considering the total amount of waste produced (dry basis) in relation to the weight gain of the animals, according to the following equation: CR = (waste production/weight gain), described by Rosa <sup>(11)</sup>.

The total solids (TS) and volatile solids (VS) contents were determined according to the methodology proposed by APHA <sup>(12)</sup>. The total nitrogen content was determined via the Kjeldahl method, as per previously reported methods <sup>(13)</sup>. The samples were previously homogenized and subjected to acid digestion with concentrated sulfuric acid in the presence of a catalyst, which promoted the conversion of organic nitrogen into ammonium ions. After digestion, the extract was alkalized, and the released ammonia nitrogen was distilled by steam distillation and subsequently collected in a boric acid solution. Quantification was performed by titration with a standard hydrochloric acid solution, and the results were calculated as a percentage. Total phosphorus was determined after acid digestion of the samples, with the aim of converting all forms of phosphorus into orthophosphate. For this purpose, the previously homogenized samples were digested with sulfuric acid combined with an oxidizing agent under controlled heating. After digestion and cooling, phosphorus was quantified via a colorimetric method in which ascorbic acid reagent was used to form the blue molybdenum complex. The absorbance was determined via a spectrophotometer at a wavelength of approximately 880 nm, and the total phosphorus concentration was calculated via a standard curve, with the results expressed as a percentage, according to the methodology described in the literature <sup>(13)</sup>.

**Table 2.** Nutritional and percentage compositions of experimental diets for growing gilts (30--50 kg).

Ingredients	Digestible phosphorus levels, %				
	0.219	0.269	0.319	0.369	0.419
Corn	68.427	68.427	68.427	68.427	68.427
Soybean meal, 46 %	26.820	26.820	26.820	26.820	26.820
Soybean oil	0.978	0.978	0.978	0.978	0.978
Dicalcium phosphate	0.532	0.892	1.252	1.612	1.972
Calcium carbonate	0.644	0.686	0.728	0.770	0.812
Salt	0.405	0.405	0.405	0.405	0.405
L-Lysine HCl 78 %	0.223	0.223	0.223	0.223	0.223
Vitamin/mineral premix <sup>1</sup>	0.150	0.150	0.150	0.150	0.150
Growth promoter <sup>2</sup>	0.100	0.100	0.100	0.100	0.100
DL-Methionine 99 %	0.072	0.072	0.072	0.072	0.072
L-Threonine 98 %	0.042	0.042	0.042	0.042	0.042
Inert (kaolin)	1.607	1.205	0.803	0.401	0.000
Calculated composition					
Crude protein, %	18.03	18.03	18.03	18.03	18.03
Metabolizable energy, Mcal/kg	3.25	3.25	3.25	3.25	3.25
Digestible lysine, %	0.988	0.988	0.988	0.988	0.988
Digestible Met+Cyst, %	0.583	0.583	0.583	0.583	0.583
Digestible threonine, %	0.642	0.642	0.642	0.642	0.642
Digestible tryptophan, %	0.187	0.187	0.187	0.187	0.187
Sodium, %	0.180	0.180	0.180	0.180	0.180
Calcium, %	0.458	0.562	0.666	0.770	0.874
Digestible phosphorus, %	0.219	0.269	0.319	0.369	0.419
Ca: P ratio	2.09:1	2.09:1	2.09:1	2.09:1	2.09:1

<sup>1</sup>Content per kilogram of feed: pantothenic acid, 9.2 mg; niacin, 18.0 mg; folic acid, 0.5 mg; copper, 15.0 mg; iron, 0.10 g; zinc, 0.13 g; iodine, 1.0 mg; selenium, 0.3 mg; manganese, 0.05 g; vitamin A, 5,000 IU; vitamin D3, 1,000 IU; vitamin E, 25.0 IU; vitamin K3, 3.0 mg; vitamin B1, 1.5 mg; vitamin B2, 4.0 mg; vitamin B6, 1.5 mg; vitamin B12, 18.0 mg; and B.H.T. (butylated hydroxytoluene) and excipient q.s.p., 1 g. <sup>2</sup>Zinc bacitracin.

The data obtained for the performance characteristics, quantification, and characterization of the waste were subjected to analysis of variance, considering the initial weight of the animals as a covariate. Linear and quadratic regression analyses were also performed, according to the best fit obtained for each variable. The statistical analysis was performed via the statistical program SAS, version 9.1<sup>(14)</sup>. A significance level of 5 % was adopted.

### 3. Results and discussion

The levels of digestible phosphorus did not influence ( $P>0.05$ ) the final weight, daily weight gain, feed intake, crude protein intake, digestible lysine intake, metabolizable energy intake, or feed conversion ratio of the pigs (Table 3). When low (0.250 %) and high (0.300 %) levels of digestible phosphorus in the diet of growing pigs were studied, Schlegel & Gutzwiller<sup>(15)</sup> did not observe effects on weight gain, feed intake, or the feed conversion ratio.

**Table 3.** Performance of gilts aged 30--50 kg fed diets containing different levels of digestible phosphorus.

Variables	Digestible phosphorus levels, %					CV, %	P Value
	0.219	0.269	0.319	0.369	0.419		
PI, kg	29.86	29.89	29.75	29.66	30.88	-	-
PF, kg	47.33	47.96	47.83	48.49	49.56	5.30	0.372
ADG, g	832	860	861	896	890	7.39	0.095
ADFI, kg	1.68	1.82	1.67	1.72	1.80	9.96	0.710
ADPD, g*	3.67	4.91	5.33	6.35	7.53	10.70	<0.001
ADCal, g	7.68	10.25	11.13	13.26	15.70	10.69	<0.001
ADPB, g	302.17	328.86	301.14	310.44	323.90	9.96	0.709
ADLys, g	16.56	18.02	16.50	17.01	17.75	9.96	0.710
DE, Mcal/kg	5.46	5.94	5.44	5.61	5.85	9.96	0.709
CA	2.03	2.12	1.94	1.95	2.02	7.65	0.271

PI= initial weight; PF = final weight; GPT= total weight gain; GPD= daily weight gain; CRD= daily feed intake; CRT= total feed intake; CPDD = daily digestible phosphorus intake; CCaD= daily calcium intake; CPBD= daily crude protein intake; CLisD= daily lysine intake; CED= daily energy intake; CA=feed conversion ratio. \*Linear effect ( $P<0.01$ ).

On the other hand, Oster<sup>(16)</sup>, when evaluating levels of 0.56 % (low), 0.84 % (medium), and 1.02 % (high) digestible phosphorus in the diet of growing pigs, reported positive effects on weight gain, feed intake, and feed conversion. Similarly, Bünzen<sup>(17)</sup> and Araujo<sup>(18)</sup> reported that diets with 0.310 % and 0.435 % digestible phosphorus promote positive effects on the weight gain of growing pigs, respectively. Drews<sup>(19)</sup> also noted improvements in the feed conversion of pigs with increasing phosphorus levels, possibly due to changes in weight gain and, consequently, increases in the proportion of protein deposition in the carcass.

Although feed intake was not influenced by phosphorus levels in the present study, it can be inferred that the average intake of 1.74 kg obtained was higher than the 1.41 kg estimated in the literature<sup>(9)</sup> for gilts in the same weight range. Studies<sup>(20)</sup> have reported that phosphorus can influence appetite control in swine and that its nutritional deficiency may reduce feed intake due to a decline in the synthesis and release of growth and thyroid hormones, especially triiodothyronine, which impacts feed efficiency. Additionally, nutritional phosphorus deficiency can lead to inadequate mineral retention, impairing the skeletal structure and affecting various physiological processes dependent on phosphorus in swine.

Thus, we can infer that in the present study, there was no extreme nutritional deficiency of phosphorus, since the levels of digestible phosphorus did not impair feed intake, according to studies <sup>(21)</sup> that reported that phosphorus deficiency does not affect intake; however, such an effect will occur if there is an extreme deficiency of this mineral. The results obtained are also consistent with the literature <sup>(15, 22-24)</sup>, which, when evaluating phosphorus levels for growing pigs, also did not observe changes in voluntary intake.

On the other hand, the daily digestible phosphorus intake increased linearly ( $P < 0.01$ ) as a function of increasing dietary phosphorus level, according to the equation  $\hat{Y} = -0.2861 + 18.32x$ ,  $r^2 = 0.98$ . A linear effect ( $P < 0.01$ ) was also observed for calcium intake, according to the equation  $\hat{Y} = -0.5499 + 38.1x$ ,  $r^2 = 0.98$ . Since there was no effect on daily feed intake, it can be inferred that the increases in digestible phosphorus and calcium intake occurred due to the increase in their concentration in the diet, given that the calcium:phosphorus ratio remained constant at 2:1.

It can be inferred that the phosphorus requirements determined in the present study differ from those reported in the literature. Saraiva <sup>(25)</sup> established a level of 0.349 % for weight gain and 0.354 % for feed conversion for females, corresponding to daily intakes of 7.45 g and 7.36 g of available phosphorus, respectively. Saraiva <sup>(22)</sup> recommended a level of 0.372 % and an intake of 8.20 g for pigs of the same category and weight. Bünzen <sup>(17)</sup> suggested a level of 0.310 % and a daily intake of 5.87 g of digestible phosphorus for castrated males and females, and Drews <sup>(19)</sup> suggested levels of 0.331 and an intake of 5.73 g and 0.330 and an intake of 5.71 g and 0.302 % and an intake of 5.18 g of available phosphorus for daily weight gain, feed intake, and feed conversion, respectively.

In more recent studies, Nieto <sup>(24)</sup> recommended a level of 0.219 % and a daily intake of 3.58 g for castrated male pigs ranging from 30 to 50 kg. The digestible phosphorus requirements to meet the performance characteristics of the animals evaluated in the present study were met by 0.219 %, corresponding to a daily intake of 3.67 g. However, most of the aforementioned studies suggest that higher levels meet the requirements of animals in this weight range.

In addition, the recommendations established in the Brazilian tables of nutritional requirements for poultry and swine <sup>(7)</sup> followed the same trend, suggesting a level of 0.319 %, which corresponds to a daily intake of 5.73 g of digestible phosphorus for female swine, from 30-50 kg. According to the NRC <sup>(26)</sup>, the recommendation for digestible phosphorus for females from 25 to 50 kg is 0.310 %. In turn, the digestible phosphorus recommended from the FEDNA tables <sup>(27)</sup> for high-performance swine in the 20--60 kg range is 0.29 %.

The level recommended as ideal by the Brazilian tables published in 2017 <sup>(8)</sup> was 0.349 %, which corresponds to a daily intake of 5.44 g of digestible phosphorus for sows. This recommendation was updated in 2024 <sup>(9)</sup>, with the nutritional requirement for digestible phosphorus increasing to 0.360 %, representing a daily intake of 4.89 g.

According to the variations in performance data reported in the present work and in other studies published by different researchers, the differences in phosphorus nutritional requirements may be related to the genetic potential of the animals for muscle tissue deposition <sup>(28)</sup>, their health status <sup>(29)</sup>, and the thermal environment to which they were subjected <sup>(30, 31)</sup>.

There was no effect ( $P > 0.05$ ) of digestible phosphorus levels on the dry matter content, natural matter content, or residue coefficient of the waste, indicating that the evaluated phosphorus levels did not quantitatively alter waste production (Table 4). The average waste production

observed in the present study was 0.175 and 0.562 kg of dry matter and natural matter/day/animal, respectively. Notably, the phosphorus levels did not affect waste production, considering that they also did not alter the animals' daily feed intake.

**Table 4.** Production of waste and bedding residue coefficients of gilts fed diets with different levels of digestible phosphorus ranging from 30 to 50 kg.

Variables	Digestible phosphorus levels, %					CV, %	P Value
	0.219	0.269	0.319	0.369	0.419		
Waste production in MN <sup>1</sup>	0.625	0.536	0.530	0.521	0.596	30.53	0.776
Waste production in MS <sup>1</sup>	0.198	0.172	0.178	0.150	0.177	33.78	0.423
Residue coefficient <sup>2</sup>	0.237	0.197	0.199	0.195	0.198	23.60	0.207

<sup>1</sup> kg/day/animal; <sup>2</sup> kg manure/kg body weight; MN: natural matter; DM: dry matter.

The average residue coefficient observed in the present study was 0.205 kg of waste per kg of body weight, which means that during the evaluated period, the animals produced 0.205 kg of waste for each 1.00 kg of weight. Orrico Junior <sup>(32)</sup> reported a residue coefficient of 0.230 kg of waste per kg of body weight for growing pigs, whereas Carvalho <sup>(33)</sup> reported residue coefficient values of 0.284 kg of waste per kg of body weight for female pigs ranging from 30--100 kg. In the growth phase, pigs are typically more efficient in utilizing dietary nutrients to convert them into body weight, which can be observed through the residue coefficient, where values below one indicate productive efficiency of the system and the analyzed animal phase.

The levels of digestible phosphorus did not influence ( $P>0.05$ ) the total solids or total nitrogen contents of the waste (Table 5). A linear effect ( $P<0.01$ ) of digestible phosphorus levels on the volatile solids content of the animal waste was observed according to the equation  $\hat{Y}=75.75+20.5x$ ,  $r^2=0.82$ . The linear increase observed in volatile solids demonstrated that as the level of digestible phosphorus in the diet increased, the concentration of organic matter in the waste also increased. This is because any nutrient provided in excess, i.e., above the animal's requirements, will not be digested and utilized by the animal, and this nutrient is excreted via waste, thereby increasing the percentage of organic material and polluting potential.

The average value for volatile solids found in the present study was 82.29 %, a value close to 85.9 % found for growing swine manure <sup>(34)</sup>. The excretion of total phosphorus increased linearly ( $P<0.01$ ) as the levels of digestible phosphorus in the diets increased, concomitantly increasing the excretion of total phosphorus into the environment, according to the equation  $\hat{Y}=-0.9509+3.32x$ ,  $r^2=0.81$ . The increase in phosphorus excretion in manure should be considered, as this is one of the nutrients with the greatest environmental impact on swine manure, since it is the limiting factor in the growth of certain algae in ponds and rivers <sup>(35)</sup>, which can cause eutrophication, leading to low oxygen concentrations and fish mortality.

**Table 5.** Physicochemical characteristics of waste from gilts fed diets containing different levels of digestible phosphorus ranging from 30 to 50 kg.

Variables <sup>1</sup>	Digestible phosphorus levels, %					CV, %	P Value
	0.219	0.269	0.319	0.369	0.419		
TS, %	31.60	31.37	34.17	28.91	29.29	9.29	0.056
VS, %*	80.19	80.97	83.34	82.34	84.63	2.80	0.001
Total N, %	3.52	3.55	3.41	3.63	3.71	6.79	0.183
Total P, %*	1.77	1.83	1.80	2.27	2.38	11.83	<0.001

TS: total solids; VS: volatile solids; N: nitrogen; P: phosphorus. <sup>1</sup>Values expressed on a DM (dry matter) basis. \*Linear effect (P<0.05).

Importantly, the diet containing 0.219 % digestible phosphorus, corresponding to a daily intake of 3.67 g, did not impair the animals' performance, resulting in a lower percentage of total phosphorus excreted in the waste. Therefore, this level can be recommended for formulating diets for gilts ranging from 30 to 50 kg. Given the above, the need to revise the nutritional recommendations of digestible phosphorus for this category becomes evident, since the requirements for maximum performance and lower phosphorus excretion into the environment were met with levels lower than those established by the nutritional requirement tables <sup>(7-9, 26, 27)</sup>.

## 4. Conclusion

The level of 0.219 % digestible phosphorus, corresponding to a daily intake of 3.67 g of digestible phosphorus, meets the nutritional requirements of phosphorus for growing gilts from 30 to 50 kg and promotes lower phosphorus excretion in waste.

### Conflict of interest statement

The authors declare no conflicts of interest.

### Data availability statement

The data will be made available upon request to the corresponding author.

### Author contributions

Conceptualization: Carvalho, K. C. N. and Kiefer, C. Data curation: Carvalho, K. C. N. and Kiefer, C. Formal analysis: Carvalho, K. C. N. and Kiefer, C. Methodology: Kiefer, C. Investigation: Carvalho, K. C. N. Funding acquisition: Kiefer, C. Project administration: Kiefer, C. Supervision: Kiefer, C. Writing (original draft): Carvalho, K. C. N. Writing (review and editing): Kiefer, C.; Nascimento, K. M. R. S.; Santos, T. M. B.; Corassa, A.; Saches, D. S.; Rodrigues, G. P.

### Generative AI use statement

The authors did not use generative Artificial Intelligence tools or technologies in the creation or editing of any part of this manuscript.

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