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Phosphorus supplementation does not affect the intake, digestibility, and meat quality of Nellore young bulls fed with high-grain diets

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ABSTRACT. This study evaluated the effect of phosphorus (P) supplementation on the intake, digestibility, and quality of aged meat from Nellore young bulls fed on high-grain diets finished in feedlot. Forty young bulls (30 months old) with an initial body weight (IBW) of 296 ± 25 kg were used. It was distributed in a completely randomized experimental design. The treatments were: without P supplementation (CO), commercial mineral supplement (CM), and supplementation with dicalcium phosphate (DP) with 2.4, 4.2, or 5.0 g of P per kg of dry matter (DM), respectively. Diets were composed of sugarcane bagasse (200 g kg^{-1}) plus concentrate (800 g kg^{-1}) on a dry matter (DM) basis. The meat quality parameters analyzed were pH, color, cooking losses, shear force, and water-holding capacity. P supplementation did not affect the intake and digestibility of nutrients There was no interaction ($p > 0.05\%$) between diets and the aging time for the meat quality parameters. However, bulls fed with DP exhibited lower pH (5.98) compared to CO and CM (6.19 and 6.14, respectively). The longer aging time increased the cooking losses and intensity of yellow (b*). Under Brazilian conditions, feedlot Nellore cattle fed with high-grain diets do not require additional mineral supplements.

Keywords: cattle; feedlot; mineral; supplement.

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Introduction

The demand for sustainable beef cattle production has increased the number of cattle experiments with the goal of obtaining the minimum amount of inputs that are needed for animal production (Zanetti et al., 2017). Phosphorus (P) has nutritional, environmental and economic importance because of its high cost and potential for soil and water contamination (Souza, Malafaia, Vieira, Granja-Salcedo, & Berchielli, 2018).

In this context, the rational use of phosphorus sources is needed, since this mineral is expensive and exhaustible. Studies have shown that increased levels of minerals in the ruminant's diet result in increased mineral excretion, suggesting increased environmental contamination (Geisert et al., 2010; Prados et al., 2017; Zanetti et al., 2017). However, inadequate supply may result in lower nutrient intakes, microbial activity and digestibility of organic constituents (Hosnedlová, Trávníček, & Šoch, 2007; Dermauw et al., 2013; Sathler et al., 2017).

In Brazil, the diets used in feedlots are characterized by a high proportion of grains (Oliveira & Millen, 2014), which are naturally rich in phosphorus (Malafaia et al., 2014), being stored mainly as phytate (Humer & Zebeli, 2015). In ruminants, phytate is hydrolyzed by the phytase produced by ruminal microorganisms (Morse, Head, & Wilcox, 1992), and 60 to 70% of the ingested organic P is used by the animal. Thus, there is no scientific evidence to justify the use of phosphorus in high-grain diets in beef cattle feedlots, once the phosphorus present in the diet meets the animals' requirement (Erickson et al., 2002; Malafaia, Cabral, Vieira, Costa, & Carvalho, 2003; Geisert et al., 2010; Souza et al., 2018). Phosphorus is involved with growth, the formation of membrane phospholipids and nucleic acid compounds (DNA and RNA). In addition, it has significant importance muscle energy load, calcium action in muscles, and therefore, has an impact on meat quality (Ternouth, 1990). However, there is currently a lack of available information in the

literature regarding the influence of P supplementation with or without the addition of other minerals, ionophores and antibiotics on nutrient digestibility and meat quality under tropical conditions.

Tokarnia, Döbereiner, and Peixoto (2000) observed that cattle can tolerate a large variation of the calcium:phosphorus ratio, which can vary from 0.6:1 to 6:1. Thus, animals ingesting diets with excess of P can mobilize calcium reserves in greater quantity to adjust the calcium:phosphorus ratio.

Calpains are enzymes that are responsible for the meat tenderness process, through the proteolysis of myofibrillar proteins and depend on the calcium concentrations in the muscle tissue for their activation (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001). We hypothesized that the mobilization of calcium caused by excess P in the diet would lead to the starvation of calcium in the muscle, which would impair the activity of calpain during the period of *rigor mortis* and meat maturation. Thus, our hypothesis is that P supplementation with or without the addition of other minerals, ionophores, and antibiotics would not alter the intake and digestibility of nutrients. Additionally, we investigated the possible effects of extra P in the diet on meat quality. Thus, the aim of the current study was to evaluate the intake and apparent digestibility of nutrients, and quality of aged meat in Nellore cattle feedlot fed with P supplementation with or without the addition of other minerals, ionophores, and antibiotics.

Material and methods

The present study was conducted in the Food and Digestibility facilities, belonging to the Animal Science Department of FCAVUNESP, Jaboticabal Campus, SP, Brazil (Brazil, 21°15′22″S, 48°18′58″ W and 595 m above sea level). All procedures involving the use of animals followed the guidelines recommended by the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal, COBEA) and were approved by the Ethics, Bioethics, and Animal Welfare Committee of the São Paulo State University (UNESP), Jaboticabal Campus, under Protocol 7741/14.

A total of 40 Nellore bulls 30 months old, with an average initial body weight (IBW) of 296 ± 25 kg and an average final body weight (FBW) of 431,2 ± 28 kg were housed in individual 9 m^2 (3×3 m^2) pens and provided with a feed and water trough. The animals spent 21 days adapting to the diets, facilities, and management. After this period, they were confined for 95 days (totaling 116 days for the adaptation and experimental period).

The diets were adapted using three diets (one per week); the diets had decreasing amounts of fiber in order to minimize the deleterious effects of excess of grains on ruminal physiology.

The experiment was conducted using a completely randomized design with three treatments and 14 replicates per treatment, considering each animal as an experimental unit. National Research Council (NRC, 2000) was used to calculate the requirements and diets with an estimated average daily gain (ADG) of 1.25 kg. The composition of the concentrate was soybean meal, citrus pulp, and ground corn, used in the proportion of 80% of the diet. The diets were provided with 200 g kg^{-1} of sugarcane bagasse and ~800 g kg^{-1} of concentrate, on a dry matter (DM) basis (Table 1) and balanced for ~130.0 g kg^{-1} of crude protein (CP) (DM basis). The treatments consisted of three different P concentrations during feeding: basal diet (CO; 2.4 g P kg^{-1}), commercial mineral mix (CM; 4.2 g P kg^{-1}), and including dicalcium phosphate (DP; 5.0 g P kg^{-1}) (Table 1). The basal diet has the mineral recommendations of NRC (2000), and the CM and DP treatments have 1.75 and 2.1 g P kg^{-1} above the expected requirements according to NRC (2000).

The DP treatment was proposed to have a P concentration similar to that of the CM treatment, but without the other additives inserted in the mineral core (vitamins, ionophores, yeasts, chelates, etc.) in order to isolate the P effect.

Limestone was used in in the DP treatment at 6.4 g kg^{-1} of diet DM to meet the Ca requirements and maintain acceptable Ca:P ratios similar between CM and DP treatments

At 6 a.m and 4 p.m, the diet was provided, allowing ~5% refusals. Samples of refusals and diets were obtained every week, and they made up one composite sample by period of each animal, which was stored at -20°C. The feces samples were collected at 3 time points during the study (at the beginning, in the middle, and at the end of the trial) for 3 days consecutively from the floor of the stalls, immediately after defecation by each animal; at 07 a.m, 11 a.m, and 4 p.m, on the first, second, and third day of collection, respectively, (Ferreira et al., 2009).

Posteriorly, all samples were dried at 55°C for 72h and ground in a Wiley mill, 1 mm screen. After that, food samples offered, refusals, and feces were analyzed for dry matter (method 934.01; Association of

Official Analytical Chemists [AOAC], 1990), mineral matter (MM; method 942.05; AOAC, 1990), ether extract (EE; method 954.02; AOAC, 1990), and lignin (method 973.18; AOAC, 1990). Neutral detergent fiber (aNDF) was obtained using α-amylase without the addition of sodium sulfite, following the methodology of Van Soest, Robertson, and Lewis (1991), adapted for an Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY). Nitrogen concentration was determined in each sample by combustion (850°C): conversion of all N-combustion products to N_2 (Leco® model FP-528; LECO Corporation, Michigan, USA).

Table 1. Ingredients proportion and chemical composition analyzed of the experimental diets (DM basis).

	Diet		
	CO	CM	DP
Ingredient (g kg^{-1})			
Sugar cane bagasse	200.0	200.0	200.0
Ground corn	480.0	464.0	480.0
Soybean meal	88.0	88.0	88.0
Citrus pulp	224.0	208.0	218.0
Urea	8.0	8.0	8.0
Commercial mineral mix[A]	0.0	32.0	0.0
Dicalcium phosphate	0.0	0.0	8.0
Limestone	0.0	0.0	6.4
Chemical composition			
Dry matter (g kg^{-1})	888.8	895.4	890.8
Crude protein (g kg^{-1})	136.6	124.4	128.0
Organic matter (g kg^{-1})	958.8	907.6	914.8
Neutral detergent fibre (g kg^{-1})	305.3	298.1	297.5
Acid detergent fibre (g kg^{-1})	162.4	164.1	164.4
Lignin (g kg^{-1})	03.4	02.9	03.8
Metabolizable energy[B] (MJ kg^{-1})	12.2	11.7	12.0
Calcium (g kg^{-1})	5.7	12.1	11.8
Phosphorus (g kg^{-1})	2.4	4.2	5.0

CO - control ration (2.4 g phosphorus (P) kg^{-1}); CM - commercial mineral mix (4.2 g P kg^{-1}); DP - dicalcium phosphate (5.0 g P kg^{-1}). [A]Containing (per kg) 130 g calcium, 40 g P, 111 g sodium, 20 g sulfur, 94 g magnesium, 60 mg cobalt, 650 mg copper, 40 mg iodine, 1960 mg zinc, 9 mg selenium, 520 mg manganese, 1120 mg iron, 400 mg monensin and 550 mg virginiamycin. [B]Values obtained in the NRC (2000).

Indigestible neutral detergent fiber (iNDF) was used to estimate fecal production according to Cochran, Adams, Wallace, & Galyean (1986) and validated by Silva et al. (2014) for Brazilian feedlot diets. The concentrations of iNDF in the fecal samples, roughage, concentrate, and refusals were obtained with the methodology of in situ incubation (288 h) as recommended by Valente et al. (2011), with extraction of the NDF according to Van Soest et al. (1991). Digestibility and balance trials were performed to assess the differences in P absorption and retention under different levels of P supplementation. The apparent absorption (AA) coefficient of P was estimated using the following equation:

$$AA = [(Nf - N0 - Nfc) / (Nf - N0)] \times 100$$

where *Nf* = nutrient in feed (g), *N*0 = nutrient in feed refusals (g), and Nfc = nutrient in feces (g).

At the end of the feedlot, the animals were slaughtered, and the carcasses obtained were divided and chilled in cold storage at 0°C for 24 h. After this, three individual samples from each carcass at different times (1, 7, and 14 days *post-mortem*) and a sample of the *longissimus* muscle obtained between the 10th and 13th rib of the left half carcass was taken for further analysis of the meat quality. The samples were individually vacuum-packed in polyethylene bags (water vapor permeability < 10 g m^{-2} $24h^{-1}$ at 38°C and oxygen permeability < 40 mL m^{-2} $24h^{-1}$ at 25°C) and at the end of the day were frozen at -20°C for further analysis of the meat quality. Samples for days 7 and 14 were aged in chambers between 0 and 2°C and stored at -20°C until quality analysis.

The pH, color, cooking losses, shear force, and water holding capacity were analyzed. Meat color was determined as described by Houben, Van Dijk, Eikelenboom, and Hoving-Bolink (2000), using a colorimeter (CR 300, Minolta Camera Co. Ltd., Osaka, Japan). Before starting the reading of the samples, calibration of the instrument was made, with a standard white and black in different points of each steak or portion of subcutaneous fat. The pH was measured (final pH) after 24 h of chilling and at 7 and 14 days after exposure to air, using a portable pH meter with a penetration electrode introduced into a cut of 2 to 4 cm depth, in the *longissimus* muscle of the left half carcass.

Thirty minutes before the evaluation at different points of the meat sample, a cross-section of the muscle was made to expose the myoglobin to oxygen. After 30 min of exposure to air, five readings were

taken at different points for L*(lightness, 0 = black; 100 = white), a* (intensity of the red color), and b* (intensity of the yellow color) determined according to the CIELAB system.

For the Warner–Bratzler shear force (WBSF) and cook loss analysis, the steaks used were thawed at 4°C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated at 150°C. When the internal temperature controlled by the thermocouples reached 35°C, the steak was turned over and allowed to reach an internal temperature of 70°C before removal from the oven. After this procedure, the steaks were cooled for 24h at 4°C (American Meat Science Association [AMSA], 1995). Eight round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA, 1995) and sheared once through the center, perpendicular to the fiber direction using a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS, USA).

Total cooking loss was calculated as the difference between the weight of the steaks before and after oven-broiling. Water-holding capacity was calculated as the difference in weight of a meat sample (approximately 2 g), before and after being subjected to a pressure of 10 kg for 5 min.

The experimental design was completely randomized. The results were evaluated for homoscedasticity of variances and normality of the data. Statistical analysis was conducted using the MIXED procedure of SAS (Statistical Analysis System, version 9.1).

The mathematical model is represented by

$$Y_{ij} = \mu + t_i + e_{ij}$$

where Y_{ij} is the observation of animal j subject to treatment i, μ=the overall mean, t_i =effect of treatment i, i=1 to 3, and e_{ij} is the residual experimental error. Treatment means were compared using the Tukey test and adopting $p < 0.05$. The standard errors of the mean derived from the model are reported in the tables.

Results

There were no effects ($p > 0.05$) of different levels of dietary P on the intake and digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, and ether extract (Table 2).

Table 2. Effect of different amounts of dietary P with or without other minerals on dry matter and nutrient intake, apparent dry matter (DM) and nutrient digestibility in Nellore bulls finished in feedlot.

	Diet			S.E.M.	P-value
	CO	CM	DP		
Intake (kg day^{-1})					
Dry matter	8.37	8.27	8.23	0.357	0.958
Organic matter	8.10	7.76	7.92	0.339	0.787
Crude protein	1.17	1.12	1.14	0.050	0.783
Ether extract	0.22	0.22	0.21	0.011	0.880
NDF	2.70	2.71	2.63	0.116	0.852
Digestibility (g kg^{-1})					
Dry matter	746.1	737.8	725.1	0.680	0.096
Organic matter	765.7	758.7	750.7	0.510	0.128
Crude protein	745.6	712.6	714.6	1.820	0.369
Ether extract	917.2	924.3	905.8	2.520	0.808
aNDF	569.1	557.0	550.8	0.900	0.180

CO – Control diet (2.4 g phosphorus (P) kg^{-1}); CM – commercial mineral mix (4.2 g P kg^{-1}); DP – dicalcium phosphate (5.0 g P kg^{-1}); aNDF, neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; s.e.m., standard error of the mean; Means with a different letter in rows are significantly differ by Tukey test (at p = 0.05)

The pH, color (L*, a*, and b*), cooking losses (CKL), shear force (SF), and water holding capacity (WHC) did not show an interaction between diet and aging time ($p > 0.05$), and the results were analyzed separately (Table 3).

Phosphorus supplementation did not influence ($p > 0.05$) the variables related to shear force (SF), water holding capacity (WHC), cooking losses (CKL), or color (L*, a*, and b*). However, the pH values were directly affected by the levels of phosphorus (Table 3). Animals fed with dicalcium phosphate (DP-5.0 g kg^{-1} of P) exhibited lower pH than the control ration (CO-2.4 g P kg^{-1}) and commercial mineral mix (CM-4.2 g P kg^{-1}).

The cooking loss (CKL) and b* variables presented differences in the longer aging time, with higher values for time 14 ($p < 0.05$, Table 3).

Table 3. Effect of different amounts of dietary P with or without other minerals on shear force (SF), pH, water holding capacity (WHC), cooking losses (CKL), Color (L*, a* and b*) in the longissimus muscle of Nellore bulls finished in feedlot.

	Diet (D)			S.E.M	*P*-value	Aging Time (AT)		S.E.M	P-value	D x AT
	CO	CM	DP			7	14			
pH	6.19a	6.14a	5.98b	0.052	0.034	6.07	6.14	0.288	0.328	0.933
Color										
L*	35.4	36.0	35.7	0.144	0.848	36.2	35.3	3.722	0.298	0.795
a*	14.7	14.6	14.2	0.138	0.615	14.1	14.87	2.076	0.123	0.778
b*	1.55	1.09	1.73	0.156	0.426	0.91b	2.01[a]	1.781	0.009	0.847
CKL, %	29.9	30.9	31.3	0.343	0.799	25.4b	35.9[a]	7.43	<0.0001	0.089
SF, kgf	3.38	3.57	3.74	0.085	0.219	3.44	3.69	0.711	0.127	0.835
WHC, %	39.3	40.5	40.6	0.329	0.639	39.7	40.6	4.639	0.459	0.408

CO – Control diet (2.4 g phosphorus (P) kg^{-1}); CM – commercial mineral mix (4.2 g P $^{k-1g}$); DP – dicalcium phosphate (5.0 g P kg^{-1}); s.e.m., standard error of the mean; Means with a different letter in rows are significantly differ by Tukey test (at p = 0.05).

Discussion

The results of this study demonstrated that the increase in supplementary phosphorus in high-grain diets of beef cattle feedlots did not affect their intake and digestibility. However, meat pH decreased in animals who were fed on diets with supplementary P. Our hypothesis that the mobilization of calcium caused by excess P in the diet, would lead to the starvation of calcium in the muscle, which would impair the activity of calpain during the period of rigor mortis and meat maturation was not confirmed in this study, since animals from the different treatments showed similar values for shear force.

The intake and digestibility were not affected by phosphorus supplementation in this study. The results corroborate with Silva et al. (2015), who found no differences in the intake and digestibility of Nellore bulls when they were fed with a phosphorus restricted diet, containing 80% of the animal's requirements.

Although phosphorus metabolism can be dependent on Ca, Prados et al. (2015) obtained results similar to that of this study, when evaluating the effects of diets containing three different concentrations of Ca (0.18, 0.30, and 0.48% in DM) and three levels of P (0.22%, 0.24 and 0, and 26% in DM) on the intake, nutrient digestibility, and performance of crossbred cattle during the finishing period in feedlot.

Lemos, Costa, Neto, and Malafaia (2013) and NRC (2000) have shown that the recommended P requirements may overestimate the dietary requirements of beef. A study conducted by Lemos et al. (2013) did not show differences in the performance of Nellore cattle confined and supplemented or not with P for 134 days. Costa et al. (2016), using different levels of P supplementation, also did not find an apparent relationship between the levels of P supplement and fertility rate of cows.

Strategies that minimize the loss of nutrient, such as P in feedlots should be employed (Hünerberg et al., 2014). One of these strategies involves the reduction of fecal excretion of P to the environment, by reducing the amount supplied in the diet via mineral supplements. Zhang et al. (2016) demonstrated that reducing dietary P from 0.42% to 0.26% did not negatively affect heifers' growth performance, although it significantly reduced their fecal P excretion into the environment. Geisert et al. (2010) and Erickson et al. (2002) suggested that dietary P concentrations of 0.17% are sufficient to maintain DM intake and ADG.

Thus, Mineral P supplementation is unnecessary in feedlots because of the high concentration of P in the diet. In addition, due to the high-concentrate diet, dietary P greatly exceeds the normal requirements (<0.17%), and all the excess phosphorus is not used, but is excreted and does not favor the intake, digestibility of nutrients, and meat quality, as shown here.

The animals without P supplementation probably had sufficient phosphorus levels not to affect the meat traits. However, according to the pH results, the control diet presented the highest pH, which could be explained by a lower enzymatic activity during glycolysis in the *post-mortem* period, possibly due to an increase in phosphorus absorption. Meat with pH of 5.3 to 5.8 is brightly red, whereas the meat with pH of 6.0 or higher is dark colored because of the increased enzymatic activity, improved water holding capacity, and lower penetration of oxygen.

There was no interaction between diet and aging ($p < 0.05$) for L* and a* variables, in agreement with previous findings (Rivaroli et al., 2016). Although the intensity of yellow (b *) increased with an increase in the maturation period, at the time of maturation, seven days (0.91) showed a lower intensity of yellow in relation to the meat that passed to the fourteen (2.01) days of maturation. In a review, Abularach, Rocha, and Felício (1998) describe the intensity of yellow, b* <3.40 as low, and b* > 8.28 as high. Thus, it was

observed that the values of yellow intensity at 7 and 14 d of maturation were lower than 3.40. During the maturation period, the meat tends to become dark, that is, the content of b* tends to be higher, as affirmed by Mancini and Hunt (2005). This increase in the intensity of the Yellow can be attributed to the fact that heme pigments are sensitive to oxidation. Cooking losses were affected by aging, increasing with aging time. Lipid and protein oxidation decreased the meat quality, leading to an increase in water loss (Pearce, Rosenvold, Andersen, & Hopkins, 2011).

Conclusion

Under Brazilian conditions, feedlot Nellore cattle fed with high-grain diets do not require any additional mineral supplements. A P concentration of 2.4 g kg^{-1} in DM was sufficient to ensure adequate intake, digestibility, and meat quality.

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