



Impacts of reduction of phosphorus in finishing diets for Holstein×Zebu steers



D. Zanetti^{a,b,*}, S.C. Valadares Filho^a, L.F. Prados^a, E. Detmann^a, M.V.C. Pacheco^a, L.A. Godoi^a, L.N. Rennó^a, T.E. Engle^b

^a Department of Animal Science, Universidade Federal de Viçosa, PH Rolfs St, 36570-900 Minas Gerais, Brazil

^b Department of Animal Science, Colorado State University, 350 W Pitkin St, 80523 CO, United States

ARTICLE INFO

Keywords:

Absorption
Calcium
Mineral
Phosphorus
Requirement
Retention

ABSTRACT

In Brazil, commonly males of dairy herd are destined to beef production. However, little is known about the mineral requirements for dairy males. Therefore, the objective of this experiment was to evaluate the calcium (Ca) requirements of Holstein×Zebu steers by determining Ca body tissue concentrations, true absorption and retention coefficients, as well as the requirements for maintenance and weight gain of steers fed diets containing or not containing dicalcium phosphate. Twenty-eight Holstein×Zebu steers with an average initial body weight of 377.5 ± 49.4 kg were utilized. The experiment was conducted as a completely randomized design with a 2×2 factorial arrangement of treatments. Factors included 1) two concentrate levels (30% or 60%); and 2) two levels of dicalcium phosphate (DP), 0 or the amount necessary to attend the estimated dietary requirements. Absorption and retention coefficients, maintenance and gain requirements were estimated. The feedstuffs, refusals, feces, urine, blood and bone were sampled for Ca and phosphorus (P) analysis. Phosphorus intake was improved by the addition of concentrate and presence of DP. There were no variations in the daily fecal and urinary phosphorus excretion as a function of DP. The serum level of inorganic phosphorus was lower in non-supplemented animals, although it was within adequate concentrations for all treatments. Dry matter and organic matter intake, organic matter digestibility, performance, and efficiency were not affected by treatment. Absorption and retention coefficients, and the net requirements for maintenance are similar across treatments. Therefore, the lack of inorganic supplementary Ca and P in diets of feedlot finishing cattle does not change the concentrations of these minerals in bone. The dietary requirements of calcium and phosphorus obtained for cattle were lower than those described by the nutritional requirement systems from Brazil, USA, and UK.

1. Introduction

The demand for sustainable beef cattle production (i.e. the use of resources for feed production) have increased the number of crossbred cattle experiments with the goal of obtaining the minimum amount of inputs that are needed for animal production. Calcium and phosphorus are common minerals supplemented to beef cattle for growth, production, and reproduction. However, studies have shown that for beef cattle in feedlot systems (Erickson et al., 1999; Costa e Silva et al., 2015b; Prados et al., 2015) Ca and P supplementation is not necessary. The desire to obtain the correct nutritional requirements of minerals, mainly P, is based on the possibility of financial savings and reducing the environmental impact of excess P being excreted by the animal (Khorasani et al., 1997; Wu et al., 2000; Bogestrand et al., 2005; Schoumans et al., 2014; Murphy et al., 2015).

In regards to the search for more efficient systems that are

financially and environmentally beneficial the nutritional aspect is the most important factor. Thus, the constant updating of nutrient databases that estimate the nutritional requirements of beef cattle is really important for the maintenance of the beef production systems. When comparing the systems of diet formulation (AFRC, 1991; Valadares Filho et al., 2010; NRC, 2016), Costa e Silva et al. (2015a) verified that the levels of recommended phosphorus for Nelore cattle are greater than the animal requirement, leading to an increase in production costs, excretion of phosphorus into the environment, and depletion of natural resources. In Brazil, the dairy herd is comprised of Holstein×Zebu (*Bos indicus*) due to their heat tolerance and disease resistance (Santana et al., 2014; Rotta et al., 2015). Commonly males of dairy herd are destined for beef production, in a system called “dairy cow, beef calf” (Menezes et al., 2015). However, little information is available describing the mineral requirements for these crossbred animals. Thus, our hypothesis is that not supplementing calcium and

* Corresponding author at: Department of Animal Science, Colorado State University, 350 W Pitkin St, 80523 CO, United States.
E-mail addresses: diego.zanetti@ufv.br, diego.zanetti@colostate.edu (D. Zanetti).

phosphorus to Holstein×Zebu feedlot cattle does not affect the concentration of these minerals in several body tissues, and that predicted nutritional requirements of Ca and P are overestimated. Our objective was to evaluate the concentration of calcium and phosphorus in body tissues, true absorption and retention coefficients of Ca and P, and the requirements for maintenance and weight gain of Holstein×Zebu steers that are fed diet either supplemented or not supplemented dicalcium phosphate.

2. Materials and methods

This experiment was conducted in Universidade Federal de Viçosa, Viçosa, MG, Brazil in the Experimental Feedlot of Animal Sciences Department, following approval by the Ethics Committee for Animal Use (CEUA/DZO/UFV process number 77/2013).

2.1. Animals, treatments and experimental design

Twenty-eight Holstein×Zebu steers with an average initial body weight of 377.5 ± 49.4 kg were utilized. After a 21-d period of acclimation, the animals were randomly divided into two groups: maintenance (n=4) and performance (n=24).

The animals from the maintenance group were fed daily at 1.1% of body weight on a dry matter basis, while the animals from the performance group had *ad libitum* access to feed throughout the day. All steers were housed during 84-d in individual pens equipped with concrete feeders and waterer. Water was available *ad libitum*. The experiment was conducted as a completely randomized design with a 2×2 factorial arrangement of treatments with factors being 1) concentrate level (30% or 60% concentrate on a DM basis), and 2) with or without supplemental dicalcium phosphate. Once different nutrient intake levels are necessary to support linear and nonlinear regressions, and the regressions are the main tool for determination of nutritional requirements, maintenance group and two concentrate levels for performance group were adopted in our experiment.

Diets were formulated according to the BR-Corte (Valadares Filho et al., 2010) for a gain of 1.0 kg per day and were isonitrogenous (12% CP). Fresh sugarcane was utilized as roughage source and a mixture of urea/ammonium sulfate in the proportion of 9:1 was used to adjust the N content of all diets. The concentrate proportion of the diet consisted of ground corn, soybean hulls, soybean meal, salt, and mineral mix. The proportions of the ingredients of the concentrates and diets as well as their chemical composition are shown in Table 1.

2.2. Intake, excretion, and serum levels of the minerals

Animals were fed twice a day at 0800 and 1600 h. Feed delivery was adjusted daily to maintain approximately 5% refusals. The appropriate amount for each animal was based in refusal collection and weight every morning, before feed delivery. According to amount of refusals, TMR offered was reduced or increased to reach *ad libitum* intake with 5% refusals (as fed basis). Refusals and feedstuffs were sampled daily. All feed and refusal samples were proportionally grouped each week, dried in a forced-air drying oven at 55 °C for 72 h, ground through a 1 mm sieve using a knife mill and were packed in plastic containers for further laboratory analyses.

For the determination of mineral excretion, fecal and urine collection was conducted during weeks 4 and 9 of the experiment. Twenty-four hour fecal and urine output was determined for all steers over three days. Feces were collected immediately after spontaneous defecation, with the aid of a shovel, and stored in a bucket over a 24 h period. Daily, feces were weighed, thoroughly mixed, and sampled daily. Feces were packed in trays and were dried in a forced-air drying oven (55 °C) and were ground through a 1 mm sieve using a knife mill. For each animal, a composite sample was grouped for each collection period based on the dry weight of each day of collection for further

Table 1

Ingredient and chemical composition (% DM) of diets containing either 30% or 60% concentrates and supplemented with and without dicalcium phosphate (DP).

Item	With DP		Without DP	
	30% Conc.	60% Conc.	30% Conc.	60% Conc.
<i>Proportion</i>				
Sugarcane	68.60	39.80	68.50	39.80
Soybean hulls	13.67	27.67	13.67	27.67
Grounded corn	13.30	27.10	13.30	27.10
Soybean meal	1.10	2.60	1.10	2.60
Urea	2.25	1.35	2.25	1.35
Ammonium sulfate	0.15	0.15	0.15	0.15
Salt	0.20	0.40	0.20	0.40
Sodium bicarbonate	0.30	0.50	0.30	0.50
Magnesium oxide	0.10	0.30	0.10	0.30
Mineral mix	0.03	0.03	0.03	0.03
Dicalcium phosphate	0.30	0.10	–	–
Sand	–	–	0.30	0.10
<i>Chemical composition</i>				
Dry matter (% as fed)	46.37	63.91	46.37	63.91
Organic matter	95.59	95.41	95.59	95.41
Crude protein	11.82	11.76	11.82	11.76
Neutral detergent fiber	46.48	42.08	46.48	42.08
Indigestible NDF	18.68	11.74	18.68	11.74
Non-fiber carbohydrates	39.63	42.18	39.63	42.18
Calcium	0.46	0.40	0.38	0.37
Phosphorus	0.15	0.16	0.10	0.14

laboratory analyses. The difference between intake and excretion via feces and urine was considered the apparent retained.

Two hundred mL of 50% sulfuric acid was added to the urine collection containers. Urine collection was performed through of an adapted funnel, made by a flexible material. In the start moment of collection, the funnel was fixed in the animal with elastic ropes. The funnel had a tube to drive the urine to an individual container. Urine collected each day (over a 24 h period) was measured in a graduated cylinder, mixed and sampled. Two hundred mL samples of urine be mixed, sealed in plastic bottles, thoroughly, and stored frozen (–20 °C). The urine was grouped as a composite sample based on the daily urinary volume and was immediately frozen for further analyses.

Urine samples were analyzed for uric acid and allantoin. The analyses of uric acid were performed in an automatic biochemical analyzer (brand Mindray, model BS200E, Shenzhen, China), while the analysis of allantoin were performed as described by Chen and Gomes (1995).

The total excretion of purine derivatives (PD) was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine, expressed in mmol/d and obtained from the product of their concentration in the urine and the estimated urinary volume. The absorbed purines (AP, mmol/d) were calculated from the PD excretion (PD, mmol/d) by using the following equation:

$$AP = \frac{PD - 0.3 \times BW^{0.75}}{0.80}$$

where 0.80 is the recovery of absorbed purines as PD and $0.30 \times BW^{0.75}$ is the endogenous purine excretion (Barbosa et al., 2011).

The ruminal synthesis of nitrogen compounds (SNC, gN/d) was calculated as a function of AP (mmol/d) by using the following equation (Barbosa et al., 2011):

$$SNC = \frac{70 \times AP}{0.93 \times 0.137 \times 1000}$$

where 70 is the N content from purines (mgN/mmol), 0.137 is the purine N: total N ratio in bacteria, and 0.93 is the true digestibility of microbial purines.

Microbial protein synthesis (MPS) was obtained by multiplying the ruminal synthesis of nitrogen by 6.25.

A blood sample was collected via jugular venipuncture by using tubes with a coagulation accelerator. The samples were immediately centrifuged at 3600g for 20 min at room temperature. In blood serum, the concentrations of calcium, phosphorus, and alkaline phosphatase were quantified (Automatic biochemical analyzer – Autoanalyzer. Mark Mindray, model BS200E).

2.3. Requirements for maintenance and retention coefficients

The difference between intake and excretion through urine and feces for each mineral was considered as the retained mineral content in the body of the animal. At this point, maintenance and performance animals were considered, to reach different intake levels. The relationship between retention and intake of calcium and phosphorus was expressed through a linear equation using the following model:

$$RM_i = \beta_0 + \beta_1 \times CM_i$$

where RM_i =retained mineral “i” expressed as mg/kg EBW/d, CM_i =mineral intake “i” expressed as mg/kg EBW/d, β_0 was considered to be the net requirement for maintenance of the mineral “i”, in mg/kg EBW/d, and β_1 is the true retention coefficient for the mineral “i”, in percentage.

2.4. Body composition

All animals were slaughtered after 84 d. Prior to slaughter the animals were fasted by 16 h. Animals were slaughtered via captive bolt stunning followed by exsanguination. Post slaughter, the gastrointestinal tract of each animal was emptied, washed, and weighed. The weight of the gastrointestinal tract was summed with the weight of heart, lungs, liver, spleen, kidneys, diaphragm, mesentery, tail, esophagus, trachea, pelvic and heart fat, head, hide, limbs, blood, and carcass for the determination of EBW.

The organs and viscera were ground in an industrial cutter for 20 min to obtain a homogeneous sample. The hide, head and limbs of the carcass were removed. The hide was manually chopped and sampled and the head and legs were ground in a bone grinder to obtain a homogeneous sample. The blood was quantified and sub-sampled. All samples were packed in aluminum trays for further lyophilization.

After slaughter, the carcass of each animal was divided into two half-carcasses which were weighed and then cooled in a cold chamber at 5 °C for 18 h. After this period, the half-carcasses were removed from the cold chamber for weighing and to perform the complete dissection of the left half of the carcass. Bones, meat, and fat were ground separately, sampled, and lyophilized. All samples were ground in a Willey knife mill.

Due large number of samples, two samples per animal were reconstituted and named as either carcass or non-carcass. The sample of carcass consisted of the lyophilized and grounded samples of bone,

meat, and fat, which were grouped proportionally based on the dissection weights. The sample of non-carcass components consisted of lyophilized and grounded samples of blood, head and limbs, organs and viscera, and hide, which were also grouped proportionally based on the dissection weights. All samples were analyzed for dry matter, ash, Ca and P as described by AOAC (2012).

2.5. Requirements for weight gain

The mineral contents in the body, as a function of EBW of the animals, were estimated by using equations relating the body content of each mineral to the performance and baseline reference animals, according to the following exponential model (ARC, 1980):

$$C_i = \beta_0 \times EBW^{\beta_1}$$

where C_i =constituent “i” of the animal's body as either Ca or P, EBW=empty body weight, and ‘ β_0 ’=regression parameter and β_1 =proportion of deposition of constituent “i” in EBW.

From the regression parameters presented above, the net requirements for each mineral per kilogram of empty body weight gain were obtained by the derivative of the equation above, according to the model below (ARC, 1980):

$$Y = \beta_0 \times \beta_1 \times EBW^{\beta_1 - 1}$$

where Y =the net requirement for each mineral for weight gain (g/kg of EBW), EBW=empty body weight (kg), and ‘ β_0 ’ and ‘ β_1 ’ are the regression parameters. The net requirements for each mineral correspond to the sum of the requirements for maintenance and weight gain.

2.6. Statistical analysis

Since linear and nonlinear regressions are the main tool for determination of nutritional requirements, different intake levels are necessary. Like this, maintenance group was not considered in statistical analysis regarding intake, apparent retention, bone and serum concentrations of Ca and P. Regarding the requirements, true retention and absorption estimates, factorial design was not used as a way to analyze data for comparative differences among dietary levels, but to collect source data to generate the equations.

Animals were completely randomized divided among treatments. Data were submitted to variance analysis. When a statistical difference was verified among treatments, the Tukey test was utilized for the comparison of the means. The linear models were built in PROC REG of SAS (SAS Institute Inc., Cary, NC), while for the non-linear models the PROC NLIN of SAS was used. For all tests, $P < 0.05$ was utilized as the critical level of probability to verify the significance of the parameters of the models.

Table 2

Dry matter and organic matter intake, performance and efficiency of Holstein×Zebu steers fed diets containing either 30% or 60% concentrates and supplemented with and without dicalcium phosphate.

Parameter	With DP ^a		Without DP		P- value		
	30% Conc.	60% Conc.	30% Conc.	60% Conc.	C	DP	C×DP
Dry matter intake, kg/d	7.76	10.27	7.31	10.17	0.001	0.684	0.795
Organic matter intake, kg/d	7.40	9.76	6.98	9.67	0.001	0.687	0.796
Digestible organic matter, %	64.53	69.30	63.92	71.57	0.044	0.434	0.434
Microbial efficiency, g/kg dOM	119.15	111.38	118.27	114.16	0.738	0.726	0.795
Average daily gain, kg	0.60	1.13	0.52	1.03	0.001	0.400	0.963
Productive efficiency, kg/kg	0.0748	0.1095	0.0711	0.1002	0.001	0.104	0.429

^a DP=dicalcium phosphate.

3. Results and discussion

3.1. Dry matter intake, performance and efficiency

The lack of supplementation of dicalcium phosphate did not affect DM and OM intake, or OM digestibility (Table 2). These data are similar to data reported by Goetsch and Owens (1985) for dairy steers. They observed no differences in the digestibility when they increase Ca in the diet. Prados et al. (2015) did not observed differences in nutrient digestibility when animals were fed diets containing or not containing supplemental Ca and P.

Due to the close relationship between ruminal fauna and the animal, microbial requirements of P are not negligible (Bravo et al., 2000). Microbial protein efficiency from digestible OM were similar across treatments (Table 2). Furthermore, overall animal performance and the efficiency of gain were similar across treatments.

Increasing the concentrate in the diet improved DM and OM intake and DM digestibility. This may be related to the greater NFC and NDF levels in this diet (Table 1). There is a direct relationship between DM digestibility and the digestibility of other nutrients. Large amounts of soybean hulls in diets with a greater proportion of concentrate provide a high NDF digestibility (Bach et al., 1999).

In a production setting, the absence of dietary dicalcium phosphate implies a reduction of 19.52 g of dicalcium phosphate per day for each animal. Considering an average feedlot period of 100 days, 1.95 kg of dicalcium phosphate per animal can be saved, which represents a financial and natural resource savings for finishing beef cattle.

3.2. Intake and excretion of Ca and P

Phosphorus intake in diets supplemented with dicalcium phosphate was 14.82 g/d (92% of the requirements predicted by BR-Corte, Valadares Filho et al., 2010) compared to 11.21 g/ for non-supplemented diets (69.60%) (Table 3). In relation to the ARC (1980), these intakes were 84.2% and 65.5% for the supplemented and non-supplemented diets, respectively; while these values were 73.7% and 55.7% of AFRC (1991) recommendations. Phosphorus intake for BR-Corte, ARC and AFRC were calculated based on the average body weight and weight gain that were observed in this study. The inclusion of dicalcium phosphate resulted in higher P intakes.

In contrast, supplementary dicalcium phosphate did not increase

Ca intake compared to non-supplemented animals (Table 3). This may be related with ingredients used in the diets. Among the feedstuffs utilized, fresh sugarcane and soybean hulls are high in Ca when compared to other feedstuffs that were used in a lower proportion in the diets, such as grounded corn. Thus, the amount of dicalcium phosphate that is needed to balance the diet was low, and did not meet the amount needed to generate differences in intake of this mineral.

Diets containing a greater inclusion of concentrate provided greater Ca and P intake (Table 3). Concentrate feedstuffs have different mineral profile (Valadares Filho et al., 2015) than forages. Therefore, the supply of minerals in finishing diets with high concentrate inclusion has been regarded as unnecessary (Costa e Silva et al., 2015b; Prados et al., 2015). Two aspects can be considered to confirm this explanation. First, Erickson et al. (1999) reported that P present in the concentrate feedstuffs indicate no need of P supplementation because P from feedstuffs is adequate in supplying P to the animal (Erickson et al., 1999); and second, Block et al. (2004) show that P requirements for beef cattle are overestimated by the NRC (2000).

In general, the relationship between mineral intake and fecal excretion is positive (Geisert et al., 2010; Feng et al., 2015). Phosphorus excretion via feces was increased by increasing the concentrate level. Absence of dicalcium phosphate resulted in tendentially lower fecal P. For Ca excretion via feces, differences were not observed, both for dicalcium phosphate supply as for concentrate level (Table 3). These finding is similar to finding reported in the literature.

Urine excretion of Ca and P have been recently described as significant (Costa e Silva et al., 2015a). Until the publication by Costa e Silva et al. (2015a), urinary excretion of these minerals were considered negligible. Different behavior was observed to Ca and P fecal and urinary excretion. While fecal excretion is a direct response of intake, urinary excretion may be more controlled by hormones and metabolite concentrations (Horst et al., 1994; Goff, 2014) and not only by absorption mechanisms (Field, 1983). In this case, the treatments with greater P intake had similar P excretion via urine. However, those with similar Ca intake had different urinary Ca excretion across treatments. Concentrate level and absence of dicalcium phosphate affected Ca excretion via the urine. Lower Ca excretion was related to diets with greater P intake. This confirm the close relationship between these two minerals and the ideal relationship between Ca and P (Cohen, 1973; Challa and Braithwaite, 1988).

Table 3

Intake, excretion, apparent retention, bone and serum concentrations of calcium and phosphorus of Holstein×Zebu steers fed diets containing either 30% or 60% concentrates and supplemented with and without dicalcium phosphate.

Parameter	With DP ^a		Without DP		P- value		
	30% Conc.	60% Conc.	30% Conc.	60% Conc.	C	DP	C×DP
Ash, % bone DM	51.01	52.12	50.06	50.63	0.849	0.002	0.081
Total alkaline phosphatase, U/L	210.00	240.17	277.33	287.50	0.629	0.184	0.810
Phosphorus							
Intake, g/d	12.49	17.15	7.44	14.98	< 0.001	< 0.001	0.107
Feces, g/d	7.87	9.89	6.86	8.23	0.026	0.070	0.636
Urine, g/d	0.28	0.19	0.16	0.17	0.889	0.301	0.380
Apparent retained, g/d	4.34	7.07	0.42	6.58	< 0.001	0.010	0.352
Serum, mg/dL	6.47	6.55	4.18	5.72	0.040	< 0.001	0.059
Bone, %	9.57	8.47	8.60	8.52	0.268	0.418	0.334
% ash bone	18.76	16.25	17.18	16.83	0.256	0.992	0.548
Calcium							
Intake, g/d	37.31	44.08	29.58	41.27	0.003	0.059	0.350
Feces, g/d	17.58	20.11	15.79	19.09	0.066	0.350	0.796
Urine, g/d	1.78	0.51	4.30	0.95	0.001	0.011	0.059
Apparent retained, g/d	17.95	23.46	9.49	21.23	0.001	0.017	0.134
Serum, mg/dL	8.75	8.48	9.12	8.97	0.552	0.235	0.867
Bone, %	18.66	17.68	20.04	19.01	0.473	0.280	0.681
% ash bone	36.58	33.92	40.03	37.55	0.472	0.061	0.397

^a DP=dicalcium phosphate.

3.3. Ca and P body concentrations

Retention of Ca and P was improved by dicalcium phosphate addition in combination with the higher level of concentrate inclusion (Table 3). The main location of Ca and P reserves in animal tissues are bones; thus, the percentage of ash in bones was improved in treatments with dicalcium phosphate supplied. Treatments fed with non-supplemented diets had a reduction of 1.23% (50.34 vs 51.57%) of ash content in bone comparing with animals receiving dicalcium phosphate. A similar result has been reported by Prados et al. (2015) where animals fed with reduced dietary Ca and P content had a reduction of 3.05% in the ash content of the rib bones. This reduction of ash content in bones when Ca and P are not supplemented to feedlot diets should lead to a reduction in Ca and P concentration in bones. However, this was not observed. Calcium and P content across treatments were similar when expressed as defatted bone mass, or when expressed in relation to ash bone. Among factors that can affect bone development, nutrition can affect chemical and physical bone properties (Loveridge, 1999). Williams et al. (1991) reported increases in metacarpal strength in Angus heifers fed with adequate dietary P (0.20% of DM) when compared to heifers fed with low dietary P (0.12% of DM). It could be related to bone resorption; however, other parameters or markers were not measured in our study to support it.

Serum Ca concentrations were not affected by treatments. Serum concentrations of P were reduced in animals receiving the lower level of concentrate with the absence of dicalcium phosphate. However, all animals had serum P concentrations within adequate ranges for beef cattle (4.0–4.5 mg/dl; AFRC, 1991; Underwood and Suttle, 1999; McDowell and Arthington, 2005). If this reduction in P serum concentration affected bone formation, indicators of osteoblast activity would be reduced (Kerr, 2002). However, when serum alkaline phosphatase was measured, values were similar across treatments, indicating that P homeostasis did not affect bone deposition.

3.4. Requirements for maintenance

Maintenance requirements were obtained through of investigating the relationship between the daily retention and intake of Ca and P, expressed in mg/kg EBW/d (Fig. 1). The intercepts represent the requirements for maintenance for each mineral. Thus, the net requirements for maintenance that were calculated in this experiment were 28.18 mg/kg EBW/d for Ca and 10.31 mg/kg EBW/d for P.

Experiments that calculate mineral endogenous losses with Holstein×Zebu are limited (Silva et al., 2002). In comparison to the NRC (15.4 mg/kg BW/d), the Ca requirement for maintenance in the current experiment was greater (28.18 mg/kg EBW/d). The ARC reports a value of 16 mg/kg BW/d, which was lower than those found in this study. The value that was observed in this study is similar to those found by BR-Corte (Valadares Filho et al., 2010) of 26.5 mg/kg EBW/d for Nellore heifers and by Chizzotti et al. (2009) for Holstein×Zebu animals (26.1 mg/kg EBW/d); both studies were conducted in tropical conditions.

The maintenance requirement for P in the current experiment (10.31 mg/kg EBW/d) was lower than those described in the literature: 16 mg/kg BW (NRC, 2000), 12 mg/kg BW (ARC, 1980) and 17.6 mg/kg BW (BR-Corte, Valadares Filho et al., 2010). However, in this study, some of the animals were fed lower levels of P in relation to those predicted by the findings of previously discussed experiments. However, performance was similar across treatment in the current experiment which suggests that the P requirements published by others may overestimate P requirements for maintenance of Holstein×Zebu cattle. Prados et al. (2015) fed different levels of Ca and P to Holstein×Zebu bulls, concluded that the BR-Corte recommendations for phosphorus can be reduced by up to 14%.

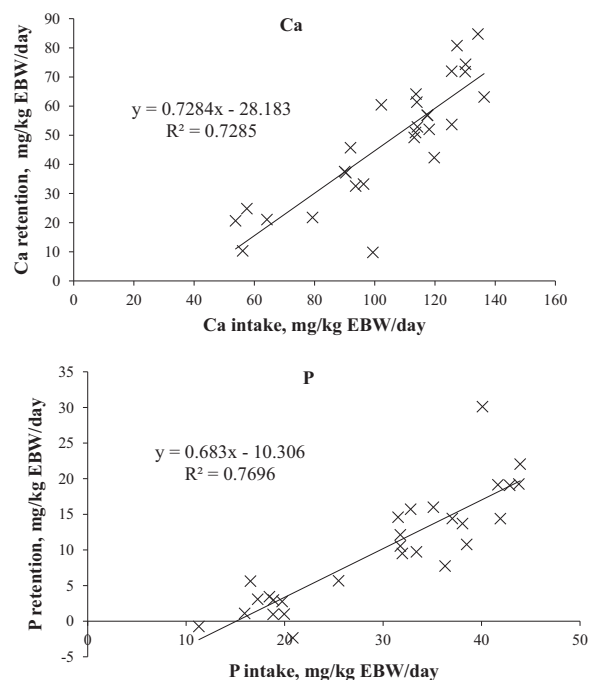


Fig. 1. Relationship between the intake and retention of calcium (Ca) and phosphorus (P).

3.5. Absorption and retentions coefficients

Similarly, there is a large variation in the reported data for mineral requirements for maintenance, as wide oscillations are found in values described in the literature for the absorption coefficients of each mineral. The values that were observed in this experiment are described in Fig. 2; the values for true absorption coefficients are 83.34% for Ca and 77.21% for P. In the literature, these coefficients vary from 30% (NRC, 2000) to 91% (Marcondes, unpublished data,

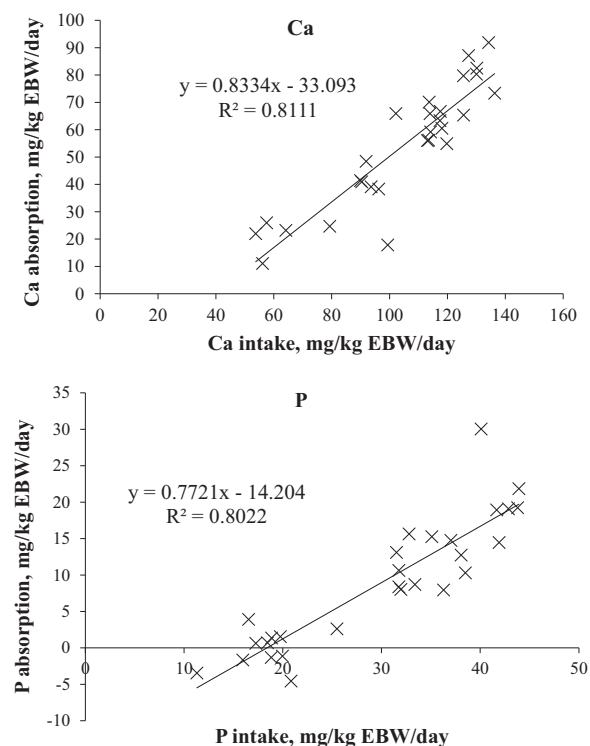


Fig. 2. Relationship between intake and absorption of calcium (Ca) and phosphorus (P).

Table 4

Equation to predict body content of calcium and phosphorus of Holstein×Zebu steers, in kg, and daily net requirements, in grams.

Mineral	Equation ^a
Calcium	$Ca_{EBW}=0.0742 \times EBW^{0.6977}$ $NRWG_{Ca}=EBG \times (51.77 \times EBW^{-0.3023})$
Phosphorus	$P_{EBW}=0.0472 \times EBW^{0.6541}$ $NRWG_P=EBG \times (30.87 \times EBW^{-0.3459})$

^a Ca_{EBW} =Body content of calcium. $NRWG_{Ca}$ =Net calcium requirement for weight gain. EBW =Empty body weight. EBG =Empty body gain. P_{EBW} =Body content of phosphorus. $NRWG_P$ =Net phosphorus requirements for weight gain.

cited in BR-Corte, Valadares Filho et al., 2010) for Ca, and from 56% (BR-Corte, Valadares Filho et al., 2010) to 80% (NRC, 2000; NRC, 2001) for P. For all cases, the values are within the interval of the reviewed literature.

Costa e Silva et al. (2015a), in study of mineral requirements for Nellore cattle, suggested adoption of retention coefficient instead of absorption coefficient, due to urinary excretion of minerals. In relation to the amount absorbed, urinary Ca and P excretion represent 9.46% and 4.16%, respectively. Therefore, we recommend adoption of retention coefficient. This coefficient was calculated in relationship to maintenance requirements, between the daily retention and intake of Ca and P, expressed in mg/kg EBW/d. These values were 72.84% and 68.30% for Ca and P, respectively.

3.6. Mineral requirements for weight gain

The requirements of each mineral per unit of weight gain are estimated from the first derivative of the relationship proposed between the mineral content in the empty body and the empty body weight (Table 4). The exponent observed in the equations for prediction of Ca and P body contents were 0.6977 and 0.6541, respectively. These coefficients infer that body contents of Ca and P are improved in lower proportion than body weight gain, once there are smaller than 1.0. These exponents are similar to reported in the literature of 0.60 and 0.71 to Ca and P, respectively (Valadares Filho et al., 2010).

The requirements for weight gain of calcium and phosphorus can be calculated respectively by the following equations: $Ca=EBG \times (51.77 \times EBW^{-0.3023})$ and $P=EBG \times (30.87 \times EBW^{-0.3459})$ (Table 4). Some authors suggest that the requirements of Ca and P for weight gain must be calculated according to the model described in Table 4 up to a determined body weight while there is bone growth, due to the 99% calcium and 80% phosphorus that are deposited in bone. From the bone tissue stabilization, we suggest that the increase in Ca and P in the EBW should be constant (AFRC, 1991; Chizzotti et al., 2009). However, Chizzotti et al. (2009) suggested that this stabilization occurs in steers after 420 kg of empty body weight.

Table 5

Net and dietary requirements of calcium and phosphorus for Holstein×Zebu steers and the requirements predicted by the BR CORTE, NRC Beef and Dairy Cattle, and ARC, in grams^a.

BW	ADG	NR _{maint}	NR _{gain}	RC	Total	BR CORTE	NRC beef cattle	NRC dairy cattle	ARC
<i>Calcium</i>									
300	0.6	7.3	7.0	0.7284	19.6	20.2	21.0	18.9	22
	1.1	7.3	12.8		27.6	30	32.0	28.3	34.4
400	0.6	9.7	6.4		22.1	21.7	22.0	20.8	24.3
	1.1	9.7	11.8		29.5	30.5	31.0	29.6	36.8
<i>Phosphorus</i>									
300	0.6	2.7	3.3	0.6830	8.7	12.8	12.0	13.1	15.3
	1.1	2.7	6.0		12.7	17.1	16.0	17.2	23.7
400	0.6	3.6	3.0		9.5	14.9	14.0	15.6	17.1
	1.1	3.6	5.4		13.2	18.9	18.0	19.5	25.5

^a BW=body weight. ADG=average daily gain. NR_{maint}=net requirement for maintenance. NR_{gain}=net requirements for gain. RC=retention coefficient.

3.7. Total requirements of Ca and P for Holstein×Zebu

In Table 5, the net and dietary requirements of Ca and P for cattle are shown based on the values and equations that were generated in this experiment in comparison to the values that were calculated through nutrient requirement systems (ARC, 1980; NRC, 2001; BR-Corte, Valadares Filho et al., 2010; NRC, 2016). Thus, the values that were observed for the dietary requirements of P are lower than those predicted by the three systems for two body weights and weight gain rates. As previously discussed, the literature shows that the estimated requirements for P from the referred councils are overestimated for Holstein×Zebu beef cattle (Erickson et al., 1999; Block et al., 2004; Prados et al., 2015). Coelho da Silva (1995) reported substantial variation in determination of mineral requirements. CSIRO (2007) attribute it to many factors such as: the difficulty in measuring microelements concentration in feeds and animal tissues; and the metabolic status of animal as determined age, breed, nutritional status, and physiologic status.

4. Conclusions

The lack of supplementation of dicalcium phosphate in diets of finishing cattle reduces serum levels of phosphorus, lower phosphorus excretion in the environment, and lower retention of calcium and phosphorus in the carcass without compromising intake, performance, and efficiency. The net requirements for maintenance of crossbred cattle are 28.18 mg/kg EBW/d for calcium and 10.31 mg/kg EBW/d for phosphorus. The requirements for weight gain of calcium and phosphorus can be calculated respectively by the following equations: $Ca=EBG \times (51.77 \times EBW^{-0.3023})$ and $P=EBG \times (30.87 \times EBW^{-0.3459})$. Retention coefficients are 72.84% and 68.30% for Ca and P, respectively. Dietary requirements of calcium and phosphorus for Holstein×Zebu cattle were lower than those described by the nutritional requirement systems from Brazil, the USA, and the UK.

Conflict of interest

Author declares that there is no conflict of interest.

Acknowledgement

The author gratefully acknowledges National Council of Scientific and Technological Development (CNPq; Grant number 471795/2012-7), National Institute of Science and Technology in Animal Science (INCT – Ciência Animal; Grant number 573592/2008-0), Coordination of Improvement of Personal Higher Education (CAPES), and Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for providing a research grant for this research.

References

- AFRC, 1991. Agricultural and food research council: a reappraisal of the calcium and phosphorus requirements of sheep and cattle (report 6). *Nutr. Abstr. Rev.* 61, 573–612.
- AOAC, 2012. Official Methods of Analysis 19th ed. AOAC International, Gaithersburg, MD.
- ARC, 1980. Agricultural Research Council: The Nutrient Requirements of Ruminant Livestock. The Gresham Press, London, UK.
- Bach, A., Yoon, I.K., Stern, M.D., Jung, H.G., Chester-Jones, H., 1999. Effects of type of carbohydrate supplementation to lush pasture on microbial fermentation in continuous culture. *J. Dairy Sci.* 82, 153–160. [http://dx.doi.org/10.3168/jds.S0022-0302\(99\)75219-7](http://dx.doi.org/10.3168/jds.S0022-0302(99)75219-7).
- Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nelore cattle. *J. Anim. Sci.* 89, 10–519. <http://dx.doi.org/10.2527/jas.2009-2366>.
- Block, H.C., Erickson, G.E., Klopfenstein, T.J., 2004. Review: re-evaluation of phosphorus requirements and phosphorus retention of feedlot cattle. *Prof. Anim. Sci.* 20, 319–329. [http://dx.doi.org/10.15232/S1080-7446\(15\)31321-8](http://dx.doi.org/10.15232/S1080-7446(15)31321-8).
- Bogstrand, J., Kristensen, P., Kronvang, B., 2005. Source Apportionment of Nitrogen and Phosphorus Inputs Into the Aquatic Environment. European Environment Agency, Copenhagen, Denmark.
- Bravo, D., Meschy, F., Bogaert, C., Sauvart, D., 2000. Ruminal phosphorus availability from several feedstuffs measured by the nylon bag technique. *Reprod. Nutr. Dev.* 40, 149–162. <http://dx.doi.org/10.1051/rnd:2000126>.
- Challa, J., Braithwaite, G.D., 1988. Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homeostasis: 1. Studies of the effect of changes in the dietary phosphorus intake on phosphorus and calcium metabolism. *J. Agr. Sci.* 110, 573–581. <http://dx.doi.org/10.1017/S0021859600082150>.
- Chen, X.B., Gomes, M.J., 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives—an overview of the technical details. *Int. Feed Resour. Unit.*
- Chizzotti, M.L., Valadares Filho, S.C., Tedeschi, L.O., Paulino, P.V.R., Paulino, M.F., Valadares, R.F.D., Amaral, P.M., Benedeti, P.D.B., Rodrigues, T.I., Fonseca, M.A., 2009. Net requirements of calcium, magnesium, sodium, phosphorus and potassium for growth of Nelore×Red Angus bulls, steers, and heifers. *Livest. Sci.* 124, 242–247. <http://dx.doi.org/10.1016/j.livsci.2009.02.004>.
- Coelho da Silva, J.F., 1995. Exigências de macroelementos inorgânicos para bovinos: O sistema ARC/AFRC e a experiência no Brasil. In: Pereira, J.C. (Ed.) International Symposium of Nutritional Requirements of Ruminants. Viçosa, Brazil
- Cohen, R.D.H., 1973. Phosphorus nutrition of beef cattle. 3. Effect of supplementation on the phosphorus content of blood and on the phosphorus and calcium contents of hair and bone of grazing steers. *Anim. Prod. Sci.* 13, 625–629. <http://dx.doi.org/10.1071/EA9730625>.
- Costa e Silva, L.F., Engle, T.E., Valadares Filho, S.C., Rotta, P.P., Valadares, R.F.D., Silva, B.C., Pacheco, M.V.C., 2015a. Intake, apparent digestibility, and nutrient requirements for growing Nelore heifers and steers fed two levels of calcium and phosphorus. *Livest. Sci.* 181, 17–24. <http://dx.doi.org/10.1016/j.livsci.2015.09.024>.
- Costa e Silva, L.F., Valadares Filho, S.C., Engle, T.E., Rotta, P.P., Marcondes, M.I., Silva, F.A.S., Martins, E.C., Tokunaga, A.T., 2015b. Macrominerals and trace element requirements for beef cattle. *PLoS One* 10, e0144464. <http://dx.doi.org/10.1371/journal.pone.0144464>.
- CSIRO, 2007. Nutrient Requirements of Domesticated Ruminants. Commonwealth Scientific and Industrial Research Organization Publications, Collingwood, AU.
- Erickson, G.E., Klopfenstein, T.J., Milton, C.T., Hanson, D., Calkins, C., 1999. Effect of dietary phosphorus on finishing steer performance, bone status, and carcass maturity. *J. Anim. Sci.* 77, 2832–2836. <http://dx.doi.org/1999.77102832x>.
- Feng, X., Ronk, E., Hanigan, M.D., Knowlton, K.F., Schramm, H., McCann, M., 2015. Effect of dietary phosphorus on intestinal phosphorus absorption in growing Holstein steers. *J. Dairy Sci.* 98, 3410–3416. <http://dx.doi.org/10.3168/jds.2014-9145>.
- Field, A.C., 1983. A review requirements of dairy and beef cattle for major elements. *Livest. Prod. Sci.* 10, 327–338. [http://dx.doi.org/10.1016/0301-6226\(83\)90017-9](http://dx.doi.org/10.1016/0301-6226(83)90017-9).
- Geisert, B.G., Erickson, G.E., Klopfenstein, T.J., Macken, C.N., Luebke, M.K., MacDonald, J.C., 2010. Phosphorus requirement and excretion of finishing beef cattle fed different concentrations of phosphorus. *J. Anim. Sci.* 88, 2393–2402. <http://dx.doi.org/10.2527/jas.2008-1435>.
- Goetsch, A.L., Owens, F.N., 1985. Effects of calcium source and level on site of digestion and calcium levels in the digestive tract of cattle fed high-concentrate diets. *J. Anim. Sci.* 61, 995–1003. <http://dx.doi.org/10.2134/jas1985.614995x>.
- Goff, J.P., 2014. Calcium and magnesium disorders. *Vet. Clin. North Am. Food Anim. Pract.* 30, 359–381. <http://dx.doi.org/10.1016/j.cvfa.2014.04.003>.
- Horst, R.L., Goff, J.P., Reinhardt, T.A., 1994. Calcium and vitamin D metabolism in the dairy cow. *J. Dairy Sci.* 77, 1936–1951. [http://dx.doi.org/10.3168/jds.S0022-0302\(94\)77140-X](http://dx.doi.org/10.3168/jds.S0022-0302(94)77140-X).
- Kerr, M.G., 2002. Veterinary Laboratory Medicine: Clinical Biochemistry and Hematology. Blackwell Science, Oxford, UK, Iowa.
- Khorasani, G.R., Janzen, R.A., McGill, W.B., Kennelly, J.J., 1997. Site and extent of mineral absorption in lactation cows fed whole-crop grain silage or alfalfa silage. *J. Dairy Sci.* 75, 239–248. <http://dx.doi.org/1997.751239x>.
- Loveridge, N., 1999. Bone: more than a stick. *J. Anim. Sci.* 77, 190. http://dx.doi.org/10.2527/1999.77suppl_2190x.
- McDowell, L.R., Arthington, J.D., 2005. Minerals for Grazing Ruminants in Tropical Regions. Minerals for Grazing Ruminants in Tropical Regions. Center for Tropical Agriculture, Gainesville, Florida, USA.
- Menezes, G.C.C., de campos Valadares Filho, S., Lopez-Villalobos, N., Ruas, J.R.M., Detmann, E., Zanetti, D., de Castro Menezes, A., Morris, S., Mariz, L.D.S., Duarte, M.D.S., 2015. Effect of feeding strategies on weaning weight and milk production of Holstein×Zebu calves in dual purpose milk production systems. *Trop. Anim. Health Prod.* 47, 1095–1100. <http://dx.doi.org/10.1007/s11250-015-0832-5>.
- Murphy, P.N.C., Mellander, P.E., Melland, A.R., Buckley, C., Shore, M., Shortle, G., Wall, D.P., Treacy, M., Shine, O., Mechan, S., Jordan, P., 2015. Variable response to phosphorus mitigation measures across the nutrient transfer continuum in a dairy grassland catchment. *Agric. Ecosyst. Environ.* 207, 192–202. <http://dx.doi.org/10.1016/j.agee.2015.04.008>.
- National Research Council, 2000. Nutrient Requirements of Beef Cattle 7th rev. ed.. Natl. Acad. Press, Washington, DC.
- National Research Council, 2001. Nutrient Requirements of Dairy Cattle 7th rev. ed.. Natl. Acad. Press, Washington, DC.
- National Research Council, 2016. Nutrient Requirements of Beef Cattle 8th rev. ed.. Natl. Acad. Press, Washington, DC.
- Prados, L.F., Valadares Filho, S.C., Santos, S.A., Zanetti, D., Nunes, A.N., Costa, D.R., Valadares, R.F.D., 2015. Reducing calcium and phosphorus in crossbred beef cattle diets: impacts on productive performance during the growing and finishing phase. *Anim. Prod. Sci.* 55, 1369–1374. <http://dx.doi.org/10.1071/AN14781>.
- Rotta, P.P., Valadares Filho, S.C., Gionbelli, T.R.S., e Silva, L.C., Engle, T.E., Marcondes, M.I., Machado, F.S., Villadiego, F.A.C., Silva, L.H.R., 2015. Effects of day of gestation and feeding regimen in Holstein×Gyr cows: I. Apparent total-tract digestibility, nitrogen balance, and fat deposition. *J. Dairy Sci.* 98, 3197–3210. <http://dx.doi.org/10.3168/jds.2014-8280>.
- Santana, M.L., Pereira, R.J., Bignardi, A.B., El Faro, L., Tonhati, H., Albuquerque, L.G., 2014. History, structure, and genetic diversity of Brazilian Gir cattle. *Livest. Sci.* 163, 26–33. <http://dx.doi.org/10.1016/j.livsci.2014.02.007>.
- Schoumans, O.F., Chardon, W.J., Bechmann, M.E., Gascuel-Oudoux, C., Hofman, G., Kronvang, B., Rubæk, G.H., Ulen, B., Dorjoo, J.M., 2014. Mitigation options to reduce phosphorus losses from the agricultural sector and improve surface water quality: a review. *Sci. Total Environ.* 468, 1255–1266. <http://dx.doi.org/10.1016/j.scitotenv.2013.08.061>.
- Silva, F.F.D., Valadares Filho, S.C., Ítavo, L.C.V., Veloso, C.M., Valadares, R.F.D., Cecon, P.R., Paulino, P.V.R., Moraes, E.H.B.K.D., 2002. Net and dietary energy, protein and macrominerals requirements of beef cattle in Brazil. *Rev. Bras. Zootec.* 31, 776–792. <http://dx.doi.org/10.1590/S1516-35982002000300029>.
- Underwood, E.J., Suttle, N.F., 1999. The Mineral Nutrition of Livestock 3rd ed. CABI Publishing, NY, USA.
- Valadares Filho, S.C., Marcondes, M.I., Chizzotti, M.L., Paulino, P.V.R., 2010. Nutrient requirements of Zebu beef cattle – BR-Corte. Suprema Ed. Visconde do Rio Branco, MG, Brazil
- Valadares Filho, S.C., Machado, P.A.S., Chizzotti, M.L., Amaral, H.F., Magalhães, K.A., Rocha Junior, V.R., Capelle, E.R., 2015. CQBAL 3.0. Tabelas Brasileiras de Composição de Alimentos para Bovinos. Suprema Ed. Visconde do Rio Branco, MG, Brazil
- Williams, S.N., Lawrence, L.A., McDowell, L.R., Wilkinson, N.S., Ferguson, P.W., Warnick, A.C., 1991. Criteria to evaluate bone mineralization in cattle: I. Effect of dietary phosphorus on chemical, physical, and mechanical properties. *J. Anim. Sci.* 69, 1232–1242. <http://dx.doi.org/10.2527/1991.6931232x>.
- Wu, Z., Satter, L.D., Sojo, R., 2000. Milk production, reproductive performance and fecal excretion of phosphorus in dairy cows fed three amount of phosphorus. *J. Dairy Sci.* 83, 1028–1041. [http://dx.doi.org/10.3168/jds.S0022-0302\(00\)74967-8](http://dx.doi.org/10.3168/jds.S0022-0302(00)74967-8).