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Clinical parameters and calf liveweight of Nellore breeders supplemented or not with phosphorus, during pregnancy and lactation – a case study

[*Parâmetros clínicos e peso de bezerros Nelore cujas matrizes foram suplementadas ou não com fósforo, durante a gestação e a lactação - estudo de caso*]

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ABSTRACT

The aim of this case study was to evaluate the clinical and productive data of Nellore cows during pregnancy and lactation, supplemented or not with P. Ninety-five pregnant heifers were divided into two groups (G_{40} and G_0). Until the date of calving animals from G_{40} received a mineral mixture composed of 224g of Na and 40g of P/kg, and G_0 received only NaCl. After calving heifers were divided into four treatments: from G_{40}, 28 first-calf cows continued to receive the mineral mixture containing 224g of Na and 40g of P/kg (group 40/40) and 12 started to receive only NaCl (group 40/0); from G_0, 26 continued to receive only NaCl (group 0/0) and 15 started to receive a mixture containing 224g of Na and 40g of P/kg (group 0/40). In the second experimental phase, 205 multiparous cows were divided into two groups: G_1 (40/40) consisting of 40 cows, receiving a mineral mixture containing 244g of Na and 40g/P per kg, during pregnancy and lactation, and group 2 (0/0) formed by 165 cows, which were supplemented only with NaCl. In both experiments, whether in pregnant or lactating breeders, there was not any sign of P deficiency or differences in calves LW at 120 and 210 days.

Keywords: beef cattle, Nellore breeders, phosphorus

RESUMO

Este estudo avaliou os aspectos clínicos e produtivos de matrizes Nelore, suplementadas ou não com P. Noventa, e cinco nulíparas prenhes foram distribuídas em dois grupos (G_{40} e G_0). Os animais do G_{40} receberam uma mistura mineral com 224g de Na e 40g de P/kg, até a data do parto, e o G_0 recebeu apenas NaCl. Após o parto, as primíparas foram divididas em quatro grupos: do G_{40}, 28 continuaram a receber a mistura contendo 224g de Na e 40g de P/kg (grupo 40/40), e 12 passaram a receber apenas NaCl (grupo 40/0); do G_0, 26 continuaram recebendo apenas NaCl (grupo 0/0), e 15 passaram a ser suplementadas com a mistura contendo 224g de Na e 40g de P/kg (grupo 0/40). Na segunda fase experimental, 205 vacas foram divididas em dois grupos: G_1 (40/40), composto por 40 vacas, suplementadas com a mistura mineral contendo 244g de Na e 40g de P/kg, durante a gestação e a lactação, e o G_2 (0/0) foi formado por 165 vacas, as quais foram suplementadas apenas com NaCl, durante a gestação e a lactação. Em ambos os experimentos, seja nas matrizes em gestação ou naquelas em lactação, não houve qualquer sinal da deficiência de P, e isso se refletiu na ausência de diferenças nos pesos dos bezerros aos 120 e 210 dias.

Palavras-chave: bovinos de corte, fósforo, Nelore

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INTRODUCTION

Tropical beef cattle production is an important economic activity across several countries and is present in a great variety of ecosystems. In Brazil, more than 90% of the herds are raised under extensive conditions on pastures of perennial forages (natives or cultivated) grown in soils with low or moderate fertility, what may result in low phosphorus (P) concentration in their diet (Malafaia, 2020). The low P concentration in soils may reflect on P levels in the grasses and, consequently, that low P diet may produce subclinical or clinical deficiencies in the herds (Tokarnia *et al*., 2010).

Phosphorus is a metabolically essential mineral, and its deficiency may cause economic losses to cattle producers if animal performance is impaired. Nonetheless, Malafaia *et al*. (2014); Malafaia (2020) highlight that there is a broadly spread misconception that this mineral should always be supplemented to cattle, even without a clinical-nutritional diagnosis of its deficiency in the herd. This simplistic view can result in an excessive intake of P in grazing herds, and lead to economic losses because no increments in cattle productivity are expected (Malafaia, 2020).

Particularly within the livestock industry of Brazil, there is much controversy regarding the effect of P on cattle LW performance and fertility, and wide dissemination of information based on non-scientific aspects (*i.e.,* purely commercial). However, cattle performance and fertility are affected by numerous factors and variability amongst results can be particularly confounded by the deficiencies of protein and energy and by the diseases of reproduction (Malafaia, 2020).

Worldwide research and development in agriculture can only be maximized through adequate extension methods to effectively reach the rural communities they serve (Thomson *et al*., 2019). Case studies are often conducted in partnership with rural producers, what represents an ideal approach to reach out to those rural communities and demonstrate the use of existing knowledge applied into real and practical contexts.

The aim of this case study was to evaluate the productive and clinical aspects of Nellore breeders, during the gestation and lactation phases, supplemented or not with P.

MATERIALS AND METHODS

The protocols used in this study were in accordance with the guidelines of Brazilian College of Animal Experimentation (COBEA) and was approved by the ethics, bioethics, and animal welfare committee of the UFRRJ – IZ, under protocol 23083.025632/2018-46.7741/14.

This study was carried out in a rural property situated in Valença (22º17’57”S; 43º46’56”W), state of Rio de Janeiro, Brazil. The region has a pronounced wet season from October to middle May and the annual rainfall is not highly variable, staying usually between 1200 – 1450 mm. The property conducted an extensive cow-calf system operation with Nellore cows raised under suitable sanitary control and livestock bookkeeping. The pastures were managed under a rotational grazing system with variable stock rates, *i.e.*, 0.9 – 1.3 animal unit (AU) per hectare, always set according to forage mass on offer. Clinical examination revealed adequate P status in the herd and there was no history of osteophagia; the fertility rate was 78% and the average liveweight at weaning (8 months of age) was 184 kg. Two composite soil samples from a minimum of replicates of 20 soil cores, collected at 0-10 cm depth, resulted in P levels of 3.2 and 2.6 mg P/dm3, and soil pH of 5.3 and 4.6, respectively. Throughout the experiment, all groups of breeders were kept in separate paddocks managed under continuous stocking, with adequate and similar forage allowance (pastures of *Urochloa decumbens*), water quality, and adequate shade.

The first experiment (E_1) was conducted from November 2015 to August 2017 only with pregnant nulliparous breeders (heifers) and the second (E_2), from May 2018 to September 2019, with pregnant multiparous cows (2^{nd} up to 5^{th} parity order). Before entering in their respective experimental groups, all breeders received a mineral mixture (MM) containing 12.5 g of P per kg and no animals showed clinical signs of mineral deficiency.

For the E_1, 100 heifers were selected, according to age (20 to 25 months) and LW (300 to 330 kg). These heifers were submitted to a mating season (MS) of approximately four months, from November 2015 till mid-March 2016, using one Nellore bull to 30 heifers. About 45 days after the end of the MS, heifers were submitted to a pregnancy diagnosis and to a general and specific clinical examination for mineral deficiencies, according to Tokarnia *et al*. (2010). Ninety-five heifers had confirmed pregnancy and were randomly divided into two (n = 45) experimental groups (G_{40} and G_0). G_{40} received a MM composed of 224 g of Na and 40 g of P per kg and G_0 only received NaCl. Both groups of heifers were submitted to these two treatments, from approximately 45 days of pregnancy until the calving date. After calving, the experimental animals were separated into four groups: 28 G_{40} primiparous (*i.e.* first-calf cows) were supplemented with the MM containing 224 g of Na and 40 g of P per kg (group 40/40) and 12 were supplemented only with NaCl (group 40/0); in contrast, the G_0 was divided into 26 primiparous receiving only NaCl (group 0/0) and 15 offered the MM containing 224 g of Na and 40 g of P per kg (group 0/40). Figure 1 shows the scheme used in the experimental phase E_1.

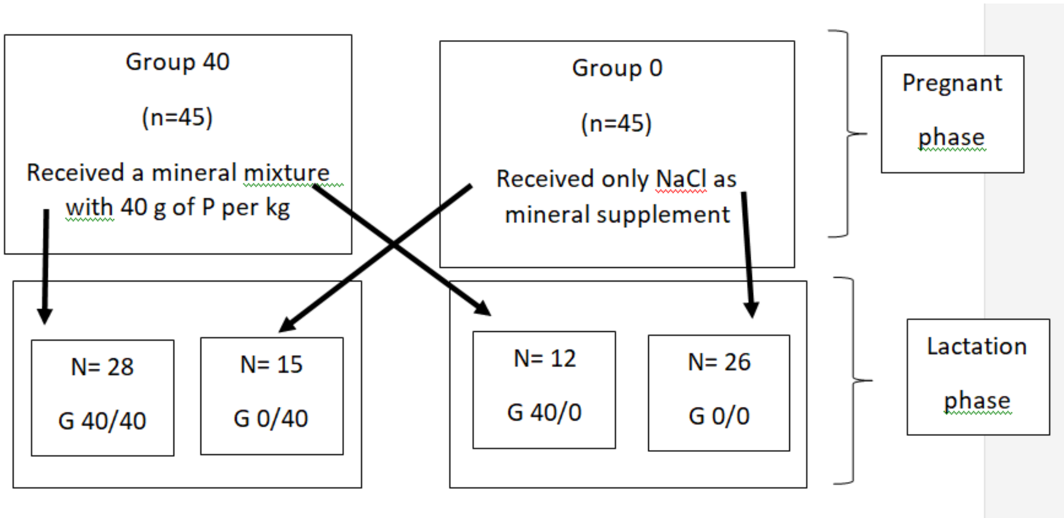

Figure 1. Scheme of mineral supplementation during the phase E_1. Four experimental treatments were tested: 40 g of P per kg of mineral mixture (MM) during pregnancy and lactation (group 40/40), 40 g of P per kg of MM during pregnancy and only NaCl during lactation (group 40/0), only NaCl during pregnancy and 40 g of P per kg of MM during lactation (group 0/40) and only NaCl during pregnancy and lactation of Nellore breeders (group 0/0).

During E_1, about 10% of breeders were subjected to the needle test (NT), according to Malafaia *et al*. (2018). The NT was performed when heifers were pregnancy-tested, then approximately 7 to 8 months of gestation and again 30 to 90 days after calving. Similarly, three sequences of rib biopsies were also performed, according to Malafaia *et al*. (2017), in four different heifers from G_{40} and G_0; on the pregnancy-test day, the second between the 7^{th} and 8^{th} month of pregnancy and the final biopsy, between 30 and 90 days postpartum. After performing the biopsies, the animals were evaluated daily for clinical status, expression of pain signals (Fitzpatrick *et al*., 2006) and wound healing; after 14 days, after assuring that no animals had problems to heal, the sutures were removed. Soon after being extracted from the animals, the bone fragments were stored in 10% formalin for further evaluations. The cortical thickness (CT, on the cranial border of the lateral side of the rib), the specific bone density (D), and bone mineral density (BMD) were measured according to Malafaia (2020). The bone fragments were also processed following the procedures described by Suvarna *et al*. (2013) and thereafter histology slides were microscopically examined.

Blood samples (collected in the coccigeal vein) were taken in four animals from each group, for Pi measurement, at the time of pregnancy diagnosis, between 7 and 8 months of gestation and between 30 and 90 days postpartum.

Subsamples (7 to 10 per paddock) of the forage grazed by animals from G_{40} and G_0 were collected monthly, using the hand-plucked technique (Johnson, 1978). On the same day as the forage sampling, fecal samples were collected (7 to 10 fresh fecal pies per paddock) from each experimental group. At the end of the collection, the individual samples of forage and feces were pooled to form composite samples and submitted, under refrigeration (3 – 5 ºC), to the laboratory where they were, dried at 55ºC for 72 hours, ground, and analyzed for dry matter (DM) and P. Liveweight of calves were measured at birth, at 120 days and at 210 days of age (at the time of weaning) and the individual values were adjusted for the respective ages.

In the second phase (E_2), a total of 205 multiparous cows with confirmed pregnancy were divided into two groups. Group 1 (40/40) comprising of 40 cows, was offered a MM containing 244 g of Na and 40 g of P per kg throughout pregnancy and lactation and group 2 (0/0), formed by 165 cows supplemented only with NaCl, during pregnancy and lactation. The clinical and nutritional examination of the cows and the NT were performed every two months. Calves were weighed at birth, at 120 days and 210 days (at weaning) and the individual LW values were adjusted according to the day of birth, for 120 and 210 days, respectively.

During the conduction of E_1 and E_2, the amounts of mineral supplements consumed by the different experimental groups were measured weekly. With the data of intake and the price of the kg of the supplements it was possible to estimate the costs with the different supplementation schemes (with or without P).

Case studies should be conducted to investigate complex environments, held in natural contexts, and applied to contemporary matters, trying always that possible to be combined with other scientific approaches, such as the experimental method utilized in the current paper. This approach called meta-method is ideal for applications in field-oriented studies (Malafaia *et al*., 2018). Since case study does not have true replications nor casualization, hypothesis tests were not conducted for any variables in this paper. To interpret the data of rib parameters, blood Pi concentration and the liveweight of the calves, the averages were described along with their respective confidence intervals (CI). The estimation of CI was presented as follows: $\mu x \pm (U - L)/2$; where μ is the mean of a variable, x; and U and L are the upper and lower limits of the 95% confidence interval, respectively.

RESULTS AND DISCUSSION

During the E_1 phase, 81 calves were weaned from 90 pregnant heifers, and during the E_2 phase, 200 calves were weaned from 205 cows.

In E_1, the 405kg of NaCl utilized were purchased at a cost of R$ 0.52 per kg; therefore, the total expenses with supplementation of groups that received only NaCl was R$ 211 and the average daily consumption was 25.4g per animal unit (1 AU = 450kg). The total consumption of the supplement containing 40g of P per kg was 585kg at a cost of R$ 0.81 per kg, with a total expense of R$ 474 and an average daily consumption of 38.4g per AU.

The liveweight of the calves, adjusted for 120 and 210 days, in phases E_1 and E_2, are shown in Table 1.

In the evaluation of bone resistance, during the E_1 phase, no breeder was positive in the NT, regardless of the experimental group, stage of pregnancy or lactation time. Cortical thickness (CT), specific bone density (D), and bone mineral density (BMD) of the 12^a rib are shown in Table 2.

Table 1. Nellore Calves liveweight, adjusted for 120 and 210 days. Values in parentheses refer to the $95\%_{CI}$

E_1 (First-calf cows)				
Experimental groups	0/0	0/40	40/0	40/40
Liveweight at 120d	128.9 (3.8)	128.5 (10.0)	131.8 (7.4)	132.2 (5.1)
liveweight at 210d	187.6 (6.3)	187.4 (13.8)	186.5 (12.6)	207.9 (8.2)
E_2 (multiparous cows)				
Experimental groups	0/0			40/40
Liveweight at 120d	136.3 (0.84)			147.9 (4.2)
Liveweight at 210d	196.7 (1.0)			193.3 (5.5)

Group 0/0 = NaCl supplementation during pregnancy and lactation, Group 0/40 = supplementation with NaCl during pregnancy and with MM containing 40 g of P per kg in lactation, Group 40/0 = supplementation with MM containing 40g of P per kg during pregnancy and with NaCl during lactation, and Group 40/40 = supplementation with MM containing 40g of P per kg, during pregnancy and lactation of Nellore breeders.

Table 2. Information about cortical thickness (CT), specific bone density (D), bone mineral density (BMD) sampled on 12[a] rib of Nellore breeders. Values in parenthesis refer to the $95\%_{CI}$.

	CT (mm)	D (g/cm^3)	BMD (mmAl)
Before the beginning of E_1 (heifers)	3.33 (0.49)	1.55 (0.18)	4.50 (0.69)
6 to 7 months of gestation G_{40}	3.37 (0.41)	1.58 (0.24)	5.42 (1.02)
6 to 7 months of gestation G_0	3.50 (0.29)	1.51 (0.39)	5.50 (0.98)
30 to 60 days postpartum (group 40/40)	4.77 (1.9)	1.56 (0.10)	4.83 (0.75)
30 to 60 days postpartum (group 0/0)	3.94 (1.6)	1.53 (0.11)	4.75 (0.49)

Group 0/0 = NaCl supplementation during pregnancy and lactation, Group 0/40 = supplementation with NaCl during pregnancy and with MM containing 40 g of P per kg in lactation, Group 40/0 = supplementation with MM containing 40 g of P per kg during pregnancy and with NaCl during lactation, and Group 40/40 = supplementation with MM containing 40 g of P per kg, during pregnancy and lactation.

In the evaluation of histological slides, both hematoxylin/eosin and Goldner's trichrome stains did not reveal significant histological changes in bone samples from any of the breeders, regardless of the experimental group and pregnancy stage and lactation period. In the medullary space, trabeculae with a normal degree of mineralization were observed in the middle of the adipose bone marrow. The endosteal surface presented multifocal areas with a thin layer of osteoid, and in some areas not covered by osteoid, a slight bone resorption by osteoclasts was observed. On the endosteal surface, the apposition and absorption were in equilibrium.

The mean serum Pi concentrations were 4.93 (± 0.21) mg per dL (at the beginning of phase E_1), 4.45 (± 0.16) mg per dL (G_0) and 4.22 (± 0.18) mg per dL (G_{40}), around the sixth / seventh month of gestation. After calving, around 30 to 60 days of lactation, the 40/40 group had an average phosphatemia of 5.10 (± 0.16) mg per dL and in the 0/0 group it was 4.75 (± 0.24) mg per dL.

The forage P concentrations were similar between the two both paddocks in all months evaluated (Figure 2). The highest P concentration in the forage was in March (3.48g per kg of DM); after May there was a substantial drop (0.97g of P per kg of DM in the group supplemented only with NaCl and 0.82g of P per kg of DM in the group that received the mineral mixture containing 40g of P per kg); this reduction was related to the naturally decrease in forage quality (crude protein and energy) and also in P concentration during the dry season (Malafaia, 2020).

The fecal P concentration showed slight variations between the experimental groups throughout the experiment (Fig. 2). The G_{40} had a higher concentration of P in the feces, a fact directly related to a higher intake, through mineral supplementation with P. The P content in the feces was positively related to the forage P content throughout the experimental periods (Figure 2).

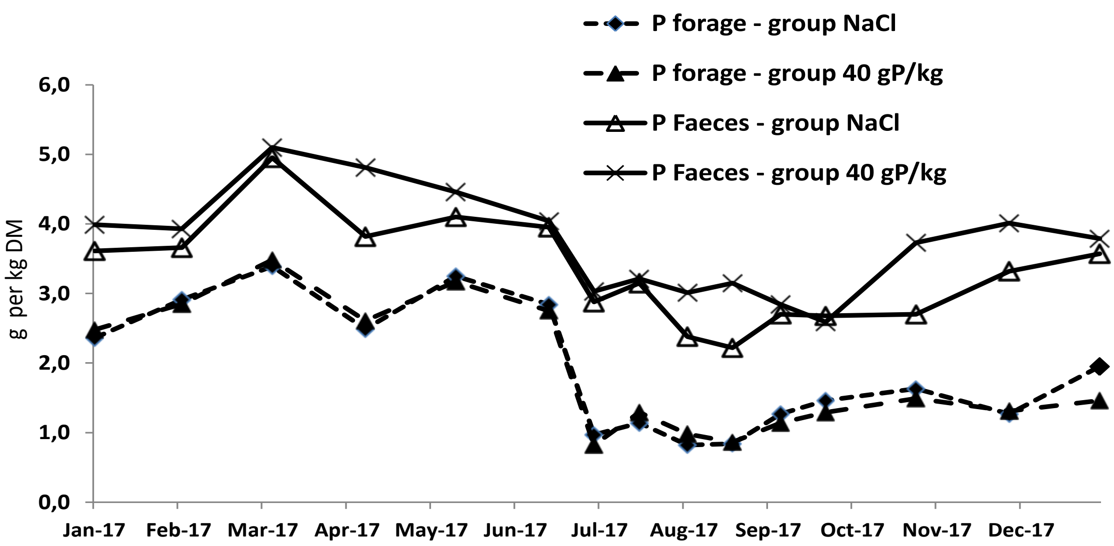

Figure 2. Annual variation in P concentration in the samples of forage (*Urochloa decumbens*) and feces of the Nellore breeders.

Case studies should be conducted to investigate complex environments, held in natural contexts, and applied to contemporary matters, trying, where possible, to be combined with other scientific approaches, such as the experimental methods used in the current experiment. As stated by Thomsom *et al*. (2019), there no extended value in agricultural research and innovation if there is no effective adoption by rural communities. Case studies like the one presented in this paper follow an approach called meta-method and are ideal for applications in farm-oriented studies (Johansson, 2007, Malafaia *et al*., 2018). Results presented in this paper were obtained during long-term experiments that followed the above considerations aiming to evaluate P status in breeders and to assist in decisions about different P supplementation schemes.

Breeders can mobilize their body P reserves to alleviate the effects of diet P deficiency (Dixon *et al*., 2017) and therefore, the correct diagnosis of P deficiency in herds must always be based on a detailed history and clinical-nutritional examination of the herd/property always in combination with ancillary tests (Tokarnia *et al*., 2010). The latter approach, recently revisited by Malafaia (2020) becomes of a greater important when subclinical deficiency is suspected. In Brazil, subclinical P deficiency is the most common form and occurs frequently on cattle properties; however, it often goes unnoticed, and causes great economic losses to livestock production systems because it leads to reductions of fertility rate, milk production and low liveweights at weaning (Tokarnia *et al*., 2010; Malafaia, 2020). The studied property is known to be in a region where often subclinical P deficiency occurs. Therefore, the present study aimed to investigate, in heifers, first-calf cows and multiparous cows, a mineral supplementation scheme without P versus a supplementation with 40 g of P per kg of MM.

The liveweight gain of Nellore calves, at 120 days of age, is greatly influenced by the breeder's milk production. After that period, calves can graze and thus LW is dictated by the grazing conditions on pastures and by the intake of milk which is drastically decreased at the end of the lactation. The highest milk production of Nellore cows occurs during the first 3 to 4 months after calving; therefore, LW of calves at 120 days indirectly measures cows' maternal ability (Pereira, 1994). As P deficiency can affect cows' milk production, measuring LW at 120 days could be a good indicator as to whether there was a P deficit during pregnancy and in the first 4 months of lactation or not. When comparing LW at 120 days in E_1, it was observed that there were no significant differences between the four experimental groups; therefore, there was no P deficiency in the gestational phase and in the first

4 months postpartum (Table 1). In E_2, performed with multiparous cows, there was an increase of approximately 12 kg in LW at 120 days in the 40/40 group in relation to the 0/0 group (Table 1); this increase could, indeed, be related to subclinical P deficiency, since multiparous are known to produce more milk (and consequently require more P; 1kg milk = 0.95 g of P) than the primiparous of E_1 group. Differences on forage allowance in this case study, could be what led to LW differences between the two experimental groups at 120 days in E_2 and not P itself; however, this was variable impractical to be measured in an extensive cow-calf operations such as the one in the current experiment and therefore, LW at weaning was an extremely important variable in the overall analysis.

In E_1, the LW at weaning of the calves of group 0/0 was 20.3 kg lower than the calves of the 40/40 group (Table 1); such difference could be explained by a P deficiency, or it could also be linked to the small number of calves weaned in each experimental treatment, which may have resulted in greater variation between the experimental groups. This difference in weaning LW, in the first experiment, was further evaluated on E_2 with a larger number of breeders with different order of parities, because the LW is a multifactorial variable being affected by genetics, nutritional and health status it is necessary for any result found in a experimental treatment, from a case study, to be evaluated in more detail and caution. Weaning LW in the E_2 was similar between the groups evaluated (Table 1). Therefore, regardless of the adopted mineral supplementation scheme, the LW at weaning was adequate and within the ideal range recommended for cow-calf systems of Nellore breeders raised exclusively on *Urochloa* pastures, under Brazilian conditions. Weaned calves, originating from breeders that received only NaCl, generated lower production costs and thus higher profitability as opposed to those from cows supplemented with P.

In any evaluated phase of E_1 (*i.e.*, pregnancy and lactation), the heifers of the two experimental groups were always negative in the NT, which means these animals had impenetrable transverse processes (from L_3 and L_4) and did not present bone fragility. Therefore, these were considered as having no P deficiency. It is also important to note that the test was performed every two months from about 45 days of gestation to 30 - 90 days after calving. Similar results were described by Lopes *et al*. (2018) when evaluating pregnant and lactating Nellore cows, supplemented with 12.5g of P per kg, in which it was found that the selective mineral supplementation, formulated for the studied property, was sufficient to meet the P requirements of cows and generated lower costs with mineral supplementation. Malafaia *et al*. (2018) reported a case of a cow-calf property with 430 Nellore cows that initially received a commercial mineral mixture that was incorrectly diluted in NaCl and had 13 g of P per kg; in which many cows performed osteophagia. In the latter study when the NT was conducted 53% of cows were positive and after the clinical-nutritional evaluation, the herd was submitted to a new mineral formula with 28 g of P per kg; months after the start of the new mineral supplementation, there were no more positive cows when subjected to the NT. The NT is a fast, very efficient, and practical way to evaluate in the field the diagnosis of different degrees of P deficiency (Malafaia *et al*., 2018).

The G_{40} heifers showed an increase in bone parameters (CT, D, and BMD) when compared to G_0 (Table 2). Malafaia (2020) mentioned that the CT may vary with age, LW and the physiological status of cattle and, therefore, should not be used as a single parameter to establish the diagnosis of P deficiency, especially in the subclinical form, in which the signs can be discrete. Therefore, it is necessary to associate this measurement with other clinical parameters, as performed in the present study. Malafaia *et al*. (2018) in a study with a positive herd in the NT and performing osteophagia, evaluated the CT and reported that the average CT was 2.3 mm, a thickness compatible with P deficiency. This value differs from the results of the present study, which revealed, at the beginning of the study, a 3.3 mm CT mean in the heifers. During the 7^{th} and 8^{th} month of gestation, the mean CT in the group supplemented only with NaCl was 3.50 mm and 3.37 mm in the group that received a MM with 40 g of P per kg. After the calving period, G_0 had an average CT of 3.94 mm and G_{40} of 4.77 mm. Malafaia (2020) suggested that cattle can be considered clinical P deficient when the CT falls below 2.5 mm, subclinical P deficiency as being 2.5 to 3.0 mm and adequate levels of P generate CT above 3 mm. The increase in CT, above the value of 3.0 mm, does not present greater benefit to the animal, it only means that there was a greater P intake and that it was deposited in the bones. Thicker cortical bone does not mean more health, greater fertility nor milk production.

Specific bone density (D), together with other bone and clinical parameters, can assist in the diagnosis of P deficiency. Malafaia (2020) evaluated results of bone biopsies from adult zebu cattle and concluded that densities less than 1.4 g per cm³ are suggestive of cattle with clinical P deficiency, between 1.40 and 1.50 g per cm³ are suggestive of subclinical deficiency and greater than or equal to 1.50 g per cm³ are indicative of healthy cattle. However, these variations that may occur according to the individual's physiological condition (age, pregnancy, LW, lactation). Therefore, specific bone density values should not be used solely, but in conjunction with the other results, as performed in the present study. Therefore, we demonstrated that heifers supplemented only with NaCl and 40 g of P per kg, even with a greater challenge had a specific mineral density greater than 1.50 g per cm³, which according to Malafaia (2020) can be considered as suitable for the specific mineral density.

Bone mineral density, associated with other clinical parameters, helps in the identification of P deficiency. Malafaia (2020) states that BMD values below 3.50 are from cattle with clinical P deficiency, values between 3.5 and 4.0 are from cattle with subclinical deficiency and cattle with values greater than 4.0 are from animals without P deficiency. However, it is important to emphasize that it is not recommended to perform the diagnosis based solely on this parameter, since variations may occur depending on the LW, age, and physiological status of the animals. When comparing the results of the present study (Table 2) with the values cited by Malafaia (2020), none of the heifers presented subclinical or clinical P deficiency.

According to Tokarnia *et al*. (2010) the histological changes in P-deficient bones are very characteristic and consist of the excessive presence of osteoid tissue (non-mineralized eosinophilic matrix), bordering the bone sheaths. Such changes were not seen in all fragments of rib biopsies, regardless of the experimental group, the stage of pregnancy and the lactation period. In the ribs of all heifers the findings of osteogenesis and modelling were compatible with bone from animals in homeostasis. Thus, what was found in the other bone parameters evaluated endorses these histological findings and reinforces that the P intake was sufficient to meet the requirements of the heifers. Therefore, there was no P deficiency in any group evaluated.

Serum Pi concentrations can fluctuate considerably in healthy animals, reflecting the balance between P “inlets” and “outlets”, as well as variation due to stress previous and during blood collection, the vein chosen for blood collection and problems during transport to the lab (Malafaia, 2020). The blood Pi values fluctuate according to the seasons (Malafaia, 2020). Thus, periods of drought, when the forage is in late vegetative stage, the levels of P are lower than those occurring in periods of rain, when the forage is in active growth and rich in crude protein and energy. The Pi concentration in the blood collected in August (dry season) was the lowest (4.45 mg per dL in the G_0 group and 4.22 mg per dL in the G_{40} group). Phosphorus concentration in forage was also the lowest in August (Figure 1). In August, there was also a great physiological demand of heifers, since, in this final period of gestation, the fetus requires much more P for its growth and the colostrogenesis starts (colostrum is rich in P). Therefore, heifers, not supplemented with P, were intensely challenged and had great chances of becoming P deficient if the P intake during the spring and summer were not adequate and guaranteed the P deposition in the bones and its subsequent release during the dry season, when the forage P contents are naturally reduced, and the animals are in the final third of gestation. According to Tokarnia *et al*. (2010) in deficient animals there is a reduction of inorganic P values in blood serum, being considered deficient animals presenting values below 3.5 mg per dL. During all phases of the study, the lowest serum P concentration was 4.22 mg per dL, referring to G_{40} in the pre partum period. Serum P concentrations in heifers were within the range considered normal (*i.e.*, between 4 – 6 mg/dL, Tokarnia *et al*., 2010).

When P intake is higher than required for cattle maintenance, this mineral is excreted, mainly through feces and in smaller amounts through urinary excretion (Malafaia, 2020). In Figure 1, we see that the group that received the MM containing 40 g of P kg had a greater amount of fecal P when compared to the group that received only NaCl, in which P was obtained only through forage intake. Regardless of the sampled paddocks, the concentration of P in the forage, throughout the experimental period was similar, between pastures over the evaluated months (Figure 2). According to Tokarnia *et al*. (2010) the forage analyses, to be valid, need to be repeated throughout the year, because over the months there is a variation in P concentrations, a fact corroborated in the present study (Figure 2). Malafaia (2020) mentioned that cattle ingest P from the forage, as well from ingestion of other plants that are correctly harvested using the hand plucked method and, possibly, by the ingestion of small amounts of soil. Therefore, with the use of simulated grazing, a discrepancy between the sample collected and the forage consumed may occur. In the case of the studied property, through the P concentration of the forage and in the feces, the complementary exams, and the clinical evaluation of the herd, it was possible to conclude that the P in forage was sufficient to meet the physiological requirements of the heifers.

From a practical point of view, as the value of P is the main contributor in the increase of cost of mineral supplementation, supplementation with high levels of P may lead to unnecessary expense, given that the excess of P ingested does not have any additional beneficial effects to animals (Malafaia *et al*., 2004). Therefore, it is essential to seek the rational use of P, leading to significant reductions in the operating cost of beef cattle production systems. A slight difference in the intake of mineral mixtures occurred between the groups in the first experimental phase and those in the second; this fact can be attributed to the age, size and LW of the cows in the second experimental period, as this group consisted of mature cows, which have greater LW than first-calf cows.

It is important to note that the data obtained through this long study cannot be applied to other properties, without prior nutritional clinical examination and technical monitoring of the herd. Through the knowledge generated, it is clear there is a need to perform a mineral supplementation with P levels as defined by the clinical-nutritional examination of the herd (Tokarnia *et al*., 2010; Lopes *et al*., 2018; Malafaia, 2020). The current case study illustrates how important a more complete approach is when it comes to the use of P in beef cattle production systems in Brazil.

CONCLUSIONS

Only through a serious and thorough experimentation, as conducted in the present study, P deficiency can be accurately diagnosed in beef cattle production systems. As observed in this case study, premeditated formulations, using high levels of P in the mineral formula can lead to unnecessary expenses.

In the current experiment, after being challenged, from about 45 days of gestation until the weaning of their calves, no breeder (heifers, first-calf cows, or cows) showed any sign of P deficiency nor failed to produce satisfactory calf weight at 120 and 210 days.

Nellore breeders supplemented with only NaCl and raised on pastures with P concentrations at adequate levels produced good results, had lower costs associated with mineral supplementation and generated greater profitability in the production system.

Supplementation with high P levels, where there is no deficiency, does not benefit cattle and increases the wastage of the mineral through feces.

The use of ancillary tests, such as the analysis of P in the feces and the needle test, are useful to confirm the diagnosis of subclinical P deficiency; however, they should not be used without prior nutritional and clinical examination of the herd and detailed history of the property.

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