FLÁVIO IGOR GAMA REBORDÕES

EFFECT OF PHOSPHATE NUTRITION DURING THE GROWING PHASE OF BEEF CATTLE ON SHORT AND LONG-TERM PERFORMANCE

LAVRAS – MG

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Master's thesis presented to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Animal Science, area of concentration in Ruminant Production and Nutrition, to obtain the title of Master.

Mateus Pies Gionbelli

Advisor

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EFFECT OF PHOSPHATE NUTRITION DURING THE GROWING PHASE OF BEEF CATTLE ON SHORT AND LONG-TERM PERFORMANCE

EFEITO DA NUTRIÇÃO FOSFATAFA DURANTE A FASE DE CRESCIMENTO DE BOVINOS DE CORTE SOBRE O DESEMPENHO DE CURTO E LONGO PRAZO

Master's thesis presented to the Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Animal Science, area of concentration in Ruminant Production and Nutrition, to obtain the title of Master.

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ABSTRACT

Our objective with this study was to evaluate the short and long-term effects that phosphorus supplementation during the growth phase could have on the performance, digestibility and carcass parameters of beef cattle. In this experiment, 90 recently weaned Nellore heifers weighing an average of 195kg were allocated as a trio in 30 stalls and divided into 3 treatments: P70 (70% of phosphorus requirements), P100 (100% of phosphorus requirements) and P130 (130% of phosphorus requirements). The animals received the treatments during the growth phase, for a period of 108 days, after which all animals entered the finishing phase, receiving the same diet, for a period of 115 days. The weight gain of animals during the growth phase tended to increase linearly according to the increase in phosphorus level ($P = 0.08$). There was no effect of treatments on animal performance in the finishing phase ($P \geq 0.1$). Phosphorus digestibility in the growth phase suffered a quadratic effect of treatments ($P = 0.045$). There was a tendency for a positive linear correlation between treatments and phosphorus digestibility at finishing ($P = 0.092$). There was no effect of treatments on blood phosphorus concentration during the growth phase ($P \geq 0.1$). The results show that increasing the level of phosphorus in the diet of animals in the growing phase does not directly alter the weight gain of animals at finishing, but has the potential to improve the efficiency of phosphorus use after the animals' growth phase.

Key-words: growing cattle; mineral nutrition; P requeriments; suplementation; muscle development.

RESUMO

Nosso objetivo com este estudo foi avaliar os efeitos de curto e longo prazo que a suplementação de fósforo durante a fase de crescimento poderia ter sobre o desempenho, digestibilidade e parâmetros de carcaça de bovinos de corte. Neste experimento, 90 novilhas Nelore recém-desmamadas, com peso médio de 195kg, foram alocadas em trio em 30 baias e divididas em 3 tratamentos: P70 (70% das exigências de fósforo), P100 (100% das exigências de fósforo) e P130 (130% das exigências de fósforo). das necessidades de fósforo). Os animais receberam os tratamentos durante a fase de crescimento, por um período de 108 dias, após os quais todos os animais entraram na fase de terminação, recebendo a mesma dieta, por um período de 115 dias. O ganho de peso dos animais durante a fase de crescimento tendeu a aumentar linearmente de acordo com o aumento do nível de fósforo (P = 0,08). Não houve efeito dos tratamentos sobre o desempenho dos animais na fase de terminação (P ≥ 0,1). A digestibilidade do fósforo na fase de crescimento sofreu efeito quadrático dos tratamentos (P = 0,045). Houve tendência de correlação linear positiva entre os tratamentos e a digestibilidade do fósforo na terminação (P = 0,092). Não houve efeito dos tratamentos na concentração sanguínea de fósforo durante a fase de crescimento (P ≥ 0,1). Os resultados mostram que o aumento do nível de fósforo na dieta de animais em fase de crescimento não altera diretamente o ganho de peso dos animais na terminação, mas tem potencial para melhorar a eficiência de utilização do fósforo após a fase de crescimento dos animais.

Palavras-chave: bovinos em crescimento; nutrição mineral; necessidades de P; suplementação; desenvolvimento muscular.

Phosphorus influence on animal development

The body development of beef cattle is very intense during the pre-pubertal phase. Phosphorus is a fundamental mineral for body development, both for its structural and physiological functions, such as its action on energy metabolism and muscle hypertrophy through the stimulation of the proliferation of satellite cells. Optimizing phosphorus supplementation during the growth phase has the potential to increase animal performance and reduce the duration of the rearing stage, which provides faster turnover of animals on the farm and improves the efficiency of phosphorus use by the organism, also reducing the excessive excretion of phosphorus into the environment. These factors promote productive and financial gains and significantly contribute to more sustainable and environmentally friendly meat production. Higher levels of phosphorus in the diet increased the animals' weight gain during growth, promoting increases of up to 57g in the animals' ADG. In practice, this potentially reduces 35 days in a rearing carried out over 12 months with a total weight gain of 7@.

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SUMÁRIO

1. INTRODUCTION

The growth phase is the period in which cattle have the greatest potential for muscle mass gain throughout their postnatal life. This is because at this stage there is a physiological priority focused on bone development and the deposition of muscle tissue about the deposition of adipose tissue (Berg et al., 1978). For this reason, the postnatal growth rate of bovines, in general, presents itself in the form of a sigmoid curve, considering weight and age, where after birth there is an accelerated growth until puberty and from that point onwards there is a great growth deceleration and a favoring process of adipose tissue deposition (Owens et al., 1993). Therefore, the ideal is that during the rearing phase there is an intense stimulus to weight gain so that this physiological window of accelerated growth is taken advantage of to the maximum and thus obtain heavier animals at the beginning of termination and consequently decrease the slaughter age.

However, due to the seasonality of forage production, typical in tropical countries, animals raised in extensive systems end up being periodically subjected to nutritional restrictions, especially during rearing (Paulino et al., 2004; Vieira et al., 2005). Animals that suffer nutritional neglect during this growth phase are unable to express their real potential for weight gain. And this reflects in practice a large increase in the time required for the animals to reach the ideal weight for finishing. Thus, the rearing phase, which should be an opportunity to take advantage of the animals' high capacity to gain weight, is a great challenge, being the longest period of the complete cycle system.

One of the nutritional factors that limit the performance of cattle in the growing phase is a mineral deficiency, with phosphorus (P) deficiency being a highlight because phosphorus is the most deficient mineral in the soil and consequently in the pastures, in most regions of Brazil (Moraes, 2001). The lack of phosphorus is even more pronounced for these animals, due to the cut in milk consumption, which until weaning was the main source of minerals, associated with the fact that most producers neglect mineral supplementation, especially phosphorus because of the cost. (Giacomel et al., 2022).

Recent studies have pointed out that phosphorus can be an important factor influencing muscle mass gain after birth. Alexander et al. (2010); Alexander et al. (2012); Zhang et al. (2020) presented evidence that phosphorus can drive the proliferation of satellite cells in

skeletal muscle tissue. According to Gonzalez et al. (2020), satellite cells are myogenic stem cells and progenitor population of skeletal muscle, responsible for supporting muscle regeneration or growth. These cells are undifferentiated myoblasts that remain attached to muscle fibers, in a quiescent state until they receive stimuli that trigger their activation, and from that point on, they begin to proliferate and fuse with muscle fibers, making them multinucleated cells and thus increasing their capacity. of protein synthesis and consequently the volume of muscle fibers and muscle tissue, in a process called hypertrophy (Silva & Carvalho, 2007). Considering the fact described by Maltin et al. (2001) that postnatal muscle growth occurs through hypertrophy (increase in the volume of muscle fibers) and no longer by hyperplasia (increase in the number of muscle fibers), it is important to explore the hypertrophic capacity of the body to the fullest. The muscle tissue of production animals.

Given this, further studies must be carried out to elucidate the biological role that phosphorus exerts on the activity of bovine satellite cells and the influence that the restriction of this mineral during rearing can exert on the subsequent phase, since that potentiating muscle mass gain is essential to ensure success within the meat production chain and the optimization of phosphorus supplementation can be an important tool to explore the ascending pattern of the growth curve of cattle during the rearing phase, through the influence on the activities of satellite cells.

2. HYPOTHESIS

Our hypothesis is that the provision of diets with deficient or excessive levels of phosphorus can reduce or accelerate muscular hypertrophy in young cattle in the growth phase, and that this would also have an impact on the performance of the animals in subsequent phases.

3. OBJECTIVE

The objective is to explore a gap in phosphorus deficiency in recently weaned cattle since these animals have a high requirement of this mineral during this stage of life and, in practice, face a lack of it in pastures and most cases do not have frequent access to adequate supplementation.

3.1 Specificific Objectives

- Evaluate the average daily gain, carcass weight, carcass yield, and measures of

animal feed efficiency during the period of phosphorus supplementation in the growing and finishing phases.

- Evaluate muscle tissue deposition parameters, such as longissimus muscle area (AML) and picanha depth (PP8), throughout the growth and finishing phase and after slaughter.
- Evaluate meat quality parameters such as pH, color, shear force, water loss during cooking, and proximate composition.

4. BACKGROUND

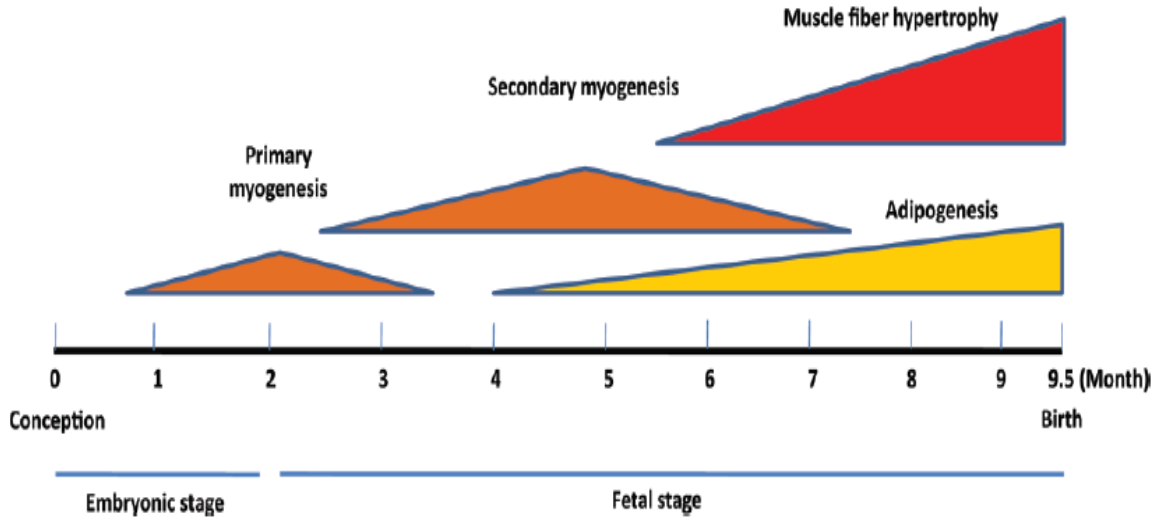
4.1 Muscle building and growth

Skeletal muscle plays an extremely important role within the agricultural chain, as in the field of animal production it is the provider of meat for human consumption (Du, Tong, et al., 2010; Zhao et al., 2019). The formation of skeletal muscle is composed of three distinct processes: myogenesis, adipogenesis, and fibrogenesis, which give rise to muscle fibers and adipose and connective tissues, respectively, which are the components of this muscle (Costa et al., 2021).

Muscle fibers are the main components of muscles, therefore, the myogenesis process is the most important in the formation of muscle tissue (Rehfeldt et al., 2000). This process begins during the embryonic phase (primary myogenesis) when primary myofibers are formed from the differentiation of a group of mesenchymal stem cells (MSC); and continues during the fetal phase (secondary myogenesis), when secondary myofibers are formed, which in turn originate from primary myofibers (Du, Tong, et al., 2010; Swatland, 1973).

During primary myogenesis, MSCs differentiate into myoblasts, which will fuse and form a limited amount of primary myofibers (Buckingham et al., 2003). During secondary myogenesis, an intense process of proliferation and fusion of myoblasts with the primary myofibers begins, which will give rise to secondary myofibers (Beermann et al., 1978). Subsequently, primary and secondary myofibers undergo differentiation and become primary and secondary fibers, which in turn will represent the vast majority of muscle cells in adult animals (Beermann et al., 1978; Silva & Carvalho, 2007).

Figure 1. Stages of development of muscle and adipose tissue during pregnancy. Source: Du, Tong, et al. (2010).



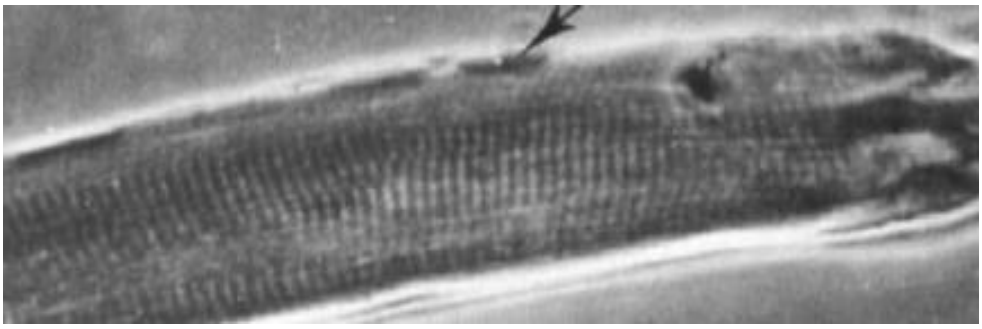
The muscle fibers of cattle are formed almost entirely by the 7th month of gestation (Du, Tong, et al., 2010). This process of muscle fiber formation is called hyperplasia and only happens during the prenatal phase (Maltin et al., 2001). Hyperplasia is the main form of muscle growth in the gestational phase and has extreme relevance to the postnatal muscle mass gain capacity of mammals (Du, Yan, et al., 2010; Maltin et al., 2001).

During the late fetal myogenic phase, a part of the myoblasts do not continue the process of secondary myogenesis and enter a state of quiescence to form a cell lineage that is called satellite cells (SC) (Zhao et al., 2019). These cells are essential for the next stage of muscle growth, the increase in the volume of muscle fibers, called hypertrophy (Silva & Carvalho, 2007). Muscle cell hypertrophy begins in the second third of pregnancy and continues throughout postnatal life as the only muscle growth mechanism (Maltin et al., 2001).

4.2 Importance and activity of satellite cells

Satellite cells were first described by Mauro (1961), after finding a distinct population of cells that were located adjacent to the muscle fibers of frogs. Based on the work of Mauro (1961), Shafiq et al. (1968) suggested that satellite cells would act as myonucleus donors for muscle fibers during the muscle growth phase. This hypothesis was later confirmed by Moss and Leblond (1971), in an experiment that aimed to identify the source of myonuclei in muscle fibers. The work by Shafiq et al. (1968) found that the myonuclei of the satellite cells had functional mitotic activity, but after being integrated into the muscle fibers, they lost their mitotic capacity and from that point on, they no longer contribute to the accumulation of myonuclei within the fiber.

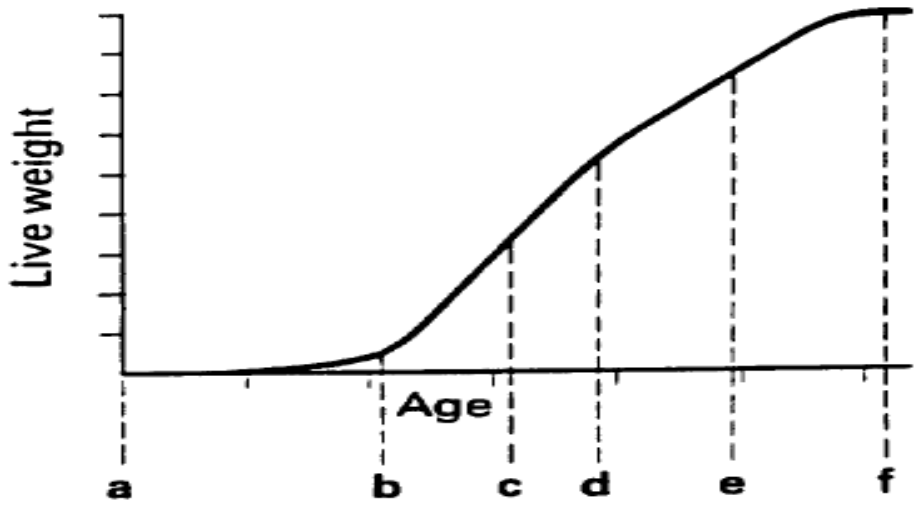
Figure 4. Satellite cell adjacent to the muscle fiber. Source: Cardassis and Cooper (1975).



Postnatal muscle growth occurs only through cell hypertrophy and no longer through hyperplasia (Du, Tong, et al., 2010; Maltin et al., 2001; Silva & Carvalho, 2007). In this context, satellite cells play an extremely important role, as they are cells responsible for promoting hypertrophy and muscle fiber regeneration (Dhawan & Rando, 2005). These cells remain in a quiescent state from their formation until they receive some stimulus that causes their activation (Silva & Carvalho, 2007; Zhao et al., 2019). When activated, satellite cells begin to proliferate and fuse with preexisting muscle fibers (Zhao et al., 2019). After fusion with the satellite cell, the muscle fiber has one more myonucleus and this factor enables greater protein synthesis for the formation of new sarcomeres, which consequently increases the volume of the muscle fiber (Silva & Carvalho, 2007).

In adult animals, the activation of satellite cells occurs mainly in response to external stimuli such as exercise and injuries, which shows an activity more related to the regeneration of muscle fibers (Silva & Carvalho, 2007; Zhao et al., 2019). On the other hand, prepubertal animals present a very intense rhythm of body growth during the post-pubertal phase, which manifests itself in the form of a sigmoid curve (Owens et al., 1993). During the ascending phase of prepubertal growth, satellite cells have as their main activity the promotion of muscle fiber hypertrophy (Gonzalez et al., 2020).

Figure 9. Sheep growth curve. a) Conception, b) Birth, c) Rapid growth phase, d) Puberty, e) Decelerated growth phase, f) Maturity. Source: Owens et al. (1993).



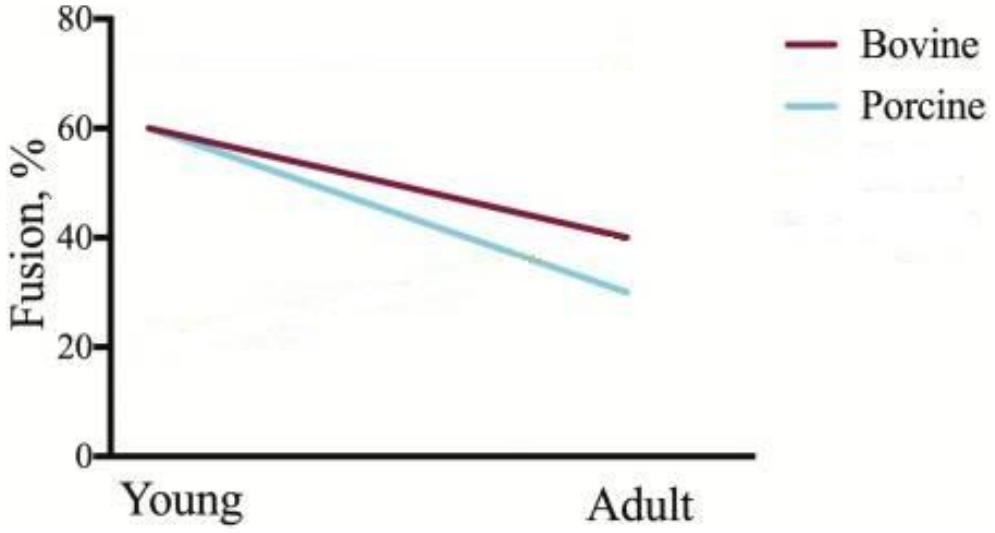
A study with mutant adult rats, with an almost total absence of satellite cells, showed a great capacity for muscle fiber hypertrophy even with the absence of satellite cells, suggesting that muscle hypertrophy is not dependent on these cells after physiological maturity (McCarthy et al., 2011). In another similar experiment, but with young and adult rats, he reinforced the idea that hypertrophy in adult animals is not dependent on satellite cells, but young animals showed a strong need for the mediation of satellite cells for hypertrophy, showing that the dependence of cells satellites for muscle fiber hypertrophy is age dependent (Murach et al., 2017).

Age is also a determining factor in the fusion capacity of satellite cells in bovine species (Gonzalez et al., 2020). Results found by Brandt et al. (2018); Ge et al. (2012) show a decrease in the fusion rate of satellite cells in adult bovines compared to young bovines. Similar data were also found in studies with young and adult pigs, showing a drop in the fusion ability of

satellite cells throughout life (Chen et al., 2017; Zhu et al., 2013). These facts show that the involvement of satellite cells with muscle hypertrophy is probably limited to prepubertal growth in these species (Gonzalez et al., 2020).

Another influential factor for satellite cell activity is nutrition (Gonzalez et al., 2020). Kao et al. (2016) found less proliferation of satellite cells in piglets fed a low-protein diet than in piglets fed a high-protein diet. Results consistent with this statement were also found by Xu et al. (2018) who identified an increase in the fusion rate of these cells in cattle treated with palmitic, oleic, and docosahexaenoic acid.

Figure 14. Bovine and swine satellite cell fusion rate over time. Source: Gonzalez et al. (2020).



Nutrient restriction in early postnatal life is a critical feature of satellite cell activity, as has been shown in some studies. Halevy et al. (2000) evaluated the effects of starvation on chicks immediately after hatching and found that feed restriction dramatically reduces the number and activity of satellite cells. In addition, the results of this same work suggest that the prolongation of the restriction period increases the severity of the damage caused to the activity of these cells. Similar results were found by Moore et al. (2005), who showed that nutritional restriction immediately after hatching decreases body weight and mitotic activity of satellite cells in turkeys.

The damage caused by nutritional restriction to the activity of satellite cells is not limited to energy and protein deficits, but also the lack of micronutrients, such as minerals. Calcium deficiency significantly decreased mesenchymal stem cell proliferation in young pigs in a study conducted by Mahajan et al. (2011). From this work, further studies were carried out to explore

the effects of mineral restriction on the activity of mesenchymal and muscle stem cells. Zhang et al. (2020) evaluated the deficiency and excess of calcium and phosphorus on the activity of these cells. The results of the work showed a 19% decrease in the proliferation of satellite cells in animals that were restricted, compared to those that received adequate levels of calcium and phosphorus.

4.3 Phosphorus in the animal organism

Phosphorus is a mineral of extreme nutritional importance for animals and is the most abundant mineral in the body after calcium (McDowell, 2000). Along with calcium, phosphorus plays a very important structural role in the composition of bones and teeth, which compromises 80 to 85% of all body phosphorus (McDowell, 2000; Ternouth, 1990). This large amount of phosphorus present in skeletal tissue also constitutes an important temporary reserve of this mineral to serve physiological functions in cases of nutritional deficit (Minson, 1990).

The remaining 20% of body phosphorus is present in soft tissues and body fluids, where it is essential for metabolism, acting on several enzymatic reactions, especially those related to energy transfer, such as ATP, ADP, AMP, and Phosphocreatine (Karn, 2001). Furthermore, phosphorus is an important component of the structure of nucleic acids (DNA and RNA), of plasma membranes in the form of phospholipids; they are factors that regulate osmotic pressure and body acid-base balance and are necessary for the processes of cell growth and differentiation (Saraiva et al., 2009).

In ruminant animals, there is still an additional factor related to phosphorus requirements, which is the P requirement by rumen microorganisms, which must be met for a ruminal activity to occur (Bryant et al., 1959). However, ruminal P levels, to some extent, are maintained at acceptable levels for the proper functioning of microbial activity in the rumen, even in cases of nutritional P deficiency, although this deficiency affects the s bone composition and ADG (Williams et al., 1991; Williams et al., 1989; Witt & Owens, 1983). According to (Karn, 2001), the blood P limit for maintaining acceptable levels of ruminal P is up to 20 mg/l at least.

Despite the great importance of P for the animal organism, in Brazil, it is very common for cattle herds kept on pasture to experience a nutritional deficiency of phosphorus. This is because the phosphorus level in approximately 80% of Brazilian arable land is low and consequently the phosphorus level in pastures is also low (Leonel & Araújo, 2023; Moraes,

2001; Teixeira et al., 2011). Another factor that contributes to phosphorus deficiency in grazing cattle is infrequent mineral supplementation, especially P supplementation, which is neglected by most producers due to the high cost of this mineral and the false impression that efficient P supplementation would not bring significant economic returns (Leonel & Araújo, 2023; Giacomel et al., 2022; Bowen et al, 2020).

In this context, animals may show clinical signs of deficiency such as a drop in bone density, weight loss, retarded growth, low immunity, and stiffening of the joints (Dantas & de Mattos Negrão, 2010). In more severe cases of deficiency, it is common for animals to have a depraved appetite and get used to gnawing bones from old carcasses. Conrad et al. (1985) reported frequent deaths of animals that contracted botulism by gnawing on the bones of carcasses contaminated with *Clostridium botulinum*.

The performance of cattle on pasture, which undergo phosphorus deficiency is negatively affected. MORAES et al. (1985) found differences ranging from 44 to 62% less in ADG of bovines without phosphorus supplementation than in supplemented bovines. Similar results were found by Nicodemo et al. (2000), who in an experiment that evaluated three levels of phosphorus in supplementation 5, 11, and 15 g/day, showed an average decrease of 64% in ADG of animals that received supplementation deficient in phosphorus, about the other two treatments; in addition, the animals of the deficient treatment also presented lower dry matter intake and feed conversion. Recent studies also show the direct impact of P deficiency on females intended for reproduction, such as low pregnancy weight, low ADG during pregnancy, lower fertility rate, lower milk production during lactation and lower calf weight at weaning (Leonel & Araújo, 2023; Schatz et al., 2023).

However, studies evaluating phosphorus deficiency on bovine performance are mostly old and present controversial results, similar to those found by Winter et al. (1990) and McLean et al. (1990), which did not present differences between deficient and non-deficient treatments in phosphorus, differing from the works mentioned above.

More recent works evaluating dietary phosphorus restriction in other species of agricultural interest can be used as parameters to understand the effects caused in cattle. In an experiment that evaluated the influence of phosphorus restriction during the growth phase on the final phase performance of swine, Varley et al. (2011) found that animals that are restricted during the growth phase cannot keep up with the performance of animals that are not restricted,

even if after the restriction phase, the animals are fed adequate levels of phosphorus.

The same effect occurred in laying birds that received phosphorus-deficient diets during the initial phase. Li et al. (2021) tested the effect of phosphorus restriction during the first 8 weeks of bird life on body weight and laying rate after the growth phase. The birds that received restricted diets in the initial phase had both body weights and laying rates lower than those that were not restricted. This effect was the same in birds that received more than needed phosphate supplementation after the restriction period. The results of this work showed that adequate phosphorus supplementation after a prolonged period of restriction of this mineral during growth, cannot repair the damage caused by a restriction in the previous phase.

4.4 Phosphorus relationship with satellite cell activity

Despite the great importance of satellite cells for muscle development, few studies have explored the influence of phosphorus on the activity of these cells (Alexander et al., 2010). Therefore, to try to understand the biological role that phosphorus plays in muscle development and performance of animals during the growth phase, some studies have been carried out (Alexander et al., 2010; Alexander et al., 2012; Zhang et al., 2012; Zhang et al., 2020).

Therefore, Alexander et al. (2010) initially evaluated the effect of phosphorus deficiency on the performance and activity of satellite cells in newborn piglets. The experiment simulated a phosphorus deficiency situation, in which one group of piglets received a diet that met only 75% of the phosphorus requirement of the animals and another group of piglets received a diet that met 100% of the phosphorus requirements. As a result, piglets that had phosphorus-restricted nutrition suffered a reduction in weight gain, which was equivalent to almost half the weight gain of piglets that received adequate nutrition. This also caused a 39% decrease in feed efficiency. In addition to the effects on performance, the piglets that received a restricted diet showed a value 2.2 times lower in the proliferation rate of satellite cells than those that received the adequate diet.

Later, the same authors proposed another study to evaluate the effects of phosphorus deficiency or moderate excess on the performance and activity of satellite cells in newborn piglets. Three treatments were evaluated: one simulating a marginal deficiency (0.7% of total P), one with adequate levels (0.9% of total P), and one simulating a marginal excess of phosphorus in the diet (1.2% of total P). Regarding weight gain, there was no significant

difference, but the results tended to be greater with the increase in the dose of phosphorus in the diet. Feed efficiency was higher in piglets with diets above adequate phosphorus (Alexander et al., 2012).

The results regarding the activity of satellite cells showed a reduction in the in vivo proliferation of satellite cells in piglets that received deficient diets about other treatments and an increase in the in vitro proliferation of satellite cells in piglets that received diets above adequate about the other treatments. These data suggest that this change in satellite cell activity can cause consequences for muscle development throughout the life of the animals since the decrease in satellite cell proliferation also decreases the number of cells that continue in the myogenic lineage and consequently minimizes the ability to hypertrophy of muscle tissue. Furthermore, possibly a marginal increase boosts the proliferation of satellite cells, which can cause an opposite effect to what happens in cases of deficiency and thus improve the hypertrophic capacity of the muscle (Alexander et al., 2012).

REFERENCES

Alexander, L. S., Mahajan, A., Odle, J., Flann, K. L., Rhoads, R. P., & Stahl, C. H. (2010, Mar). Dietary phosphate restriction decreases stem cell proliferation and subsequent growth potential in neonatal pigs. *J Nutr, 140*(3), 477-482. https://doi.org/10.3945/jn.109.117390

Alexander, L. S., Seabolt, B. S., Rhoads, R. P., & Stahl, C. H. (2012, Jun). Neonatal phosphate nutrition alters in vivo and in vitro satellite cell activity in pigs. *Nutrients, 4*(6), 436-448. https://doi.org/10.3390/nu4060436

Beermann, D., Cassens, R., & Hausman, G. (1978). A second look at fiber type differentiation in porcine skeletal muscle. *Journal of Animal Science*, *46*(1), 125-132. https://academic.oup.com/jas/article-abstract/46/1/125/4700799?redirectedFrom=fulltext

Berg, R. T., Butterfield, R. M., García de Siles, J., & Luca de Tena, E. (1978). *Nuevos conceptos sobre desarrollo de ganado vacuno*.

Bowen, M.K., Chudleigh, F., Dixon, R.M., Sullivan, M.T., Schatz, T., Oxley, T., (2020). Evaluating the economics f phosphorus supplementation of beef cattle grazing northern Australian rangelands. *Animal Production Science* 60, 683-693. doi:10.1071/AN19088

Brandt, A. M., Kania, J. M., Reinholt, B. M., & Johnson, S. E. (2018, Jan). Human IL6 stimulates bovine satellite cell proliferation through a Signal transducer and activator of transcription 3 (STAT3)-dependent mechanism. *Domest Anim Endocrinol*, *62*, 32-38. https://doi.org/10.1016/j.domaniend.2017.08.004

Bryant, M. P., Robinson, I. M., & Chu, H. (1959, 1959/11/01/). Observations on the Nutrition of Bacteroides Succinogenes—A Ruminal Cellulolytic Bacterium. *Journal of Dairy Science*, *42*(11), 1831-1847. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(59)90815-X

Buckingham, M., Bajard, L., Chang, T., Daubas, P., Hadchouel, J., Meilhac, S., Montarras, D., Rocancourt, D., & Relaix, F. (2003). The formation of skeletal muscle: from somite to limb. *Journal of anatomy*, *202*(1), 59-68. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1571050/pdf/joa0202-0059.pdf

Cardasis, C. A., & Cooper, G. W. (1975). An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: A satellite cell-muscle fiber growth unit. *Journal of Experimental Zoology*, *191*(3), 347-357.

Chen, Y., Zhu, H., McCauley, S. R., Zhao, L., Johnson, S. E., Rhoads, R. P., & El-Kadi, S. W. (2017, Feb). Diminished satellite cell fusion and S6K1 expression in myotubes

derived from skeletal muscle of low birth weight neonatal pigs. *Physiol Rep, 5*(3). https://doi.org/10.14814/phy2.13075

Costa, T. C., Gionbelli, M. P., & Duarte, M. S. (2021, Dec). Fetal programming in ruminant animals: understanding the skeletal muscle development to improve meat quality. *Anim Front, 11*(6), 66-73. https://doi.org/10.1093/af/vfab061

Dantas, C. C. O., & de Mattos Negrão, F. (2010). Funções e sintomas de deficiência dos minerais essenciais utilizados para suplementação dos bovinos de corte. *UNICiências, 14*(2).

Detmann, E., & Valadares Filho, S. (2010). On the estimation of non-fibrous carbohydrates in feeds and diets. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62*, 980-984.

Dhawan, J., & Rando, T. A. (2005, Dec). Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation, and replenishment. *Trends Cell Biol, 15*(12), 666-673. https://doi.org/10.1016/j.tcb.2005.10.007

Du, M., Tong, J., Zhao, J., Underwood, K. R., Zhu, M., Ford, S. P., & Nathanielsz, P. W. (2010). Fetal programming of skeletal muscle development in ruminant animals1. *Journal of Animal Science, 88*(suppl_13), E51-E60. https://doi.org/10.2527/jas.2009-2311

Du, M., Yan, X., Tong, J. F., Zhao, J., & Zhu, M. J. (2010, Jan). Maternal obesity, inflammation, and fetal skeletal muscle development. *Biol Reprod, 82*(1), 4-12. https://doi.org/10.1095/biolreprod.109.077099

Ge, X., Yu, J., & Jiang, H. (2012, Apr). Growth hormone stimulates protein synthesis in bovine skeletal muscle cells without altering insulin-like growth factor-I mRNA expression. *J Anim Sci, 90*(4), 1126-1133. https://doi.org/10.2527/jas.2011-4358

Giacomel, A., Freitas, T. C. d., Costa, A. L. B. d., Sbardelotto, E. M., Bergmann, E., & Debortoli, E. d. C. (2022, 03/01). Mineral supplementation for beef cattle – a meta-analysis. *Research, Society and Development, 11*(3), e39211326616. https://doi.org/10.33448/rsd-v11i3.26616

Gonzalez, M. L., Busse, N. I., Waits, C. M., & Johnson, S. E. (2020, May 1). Satellite cells and their regulation in livestock. *J Anim Sci, 98*(5). https://doi.org/10.1093/jas/skaa081

Halevy, O., Geyra, A., Barak, M., Uni, Z., & Sklan, D. (2000). Early Posthatch Starvation Decreases Satellite Cell Proliferation and Skeletal Muscle Growth in Chicks. *The Journal of Nutrition, 130*(4), 858-864. https://doi.org/10.1093/jn/130.4.858

Kao, M., Columbus, D. A., Suryawan, A., Steinhoff-Wagner, J., Hernandez-Garcia, A., Nguyen, H. V., Fiorotto, M. L., & Davis, T. A. (2016, Jun 1). Enteral β-hydroxy-β-methyl butyrate supplementation increases protein synthesis in the skeletal muscle of neonatal pigs. *Am J Physiol Endocrinol Metab, 310*(11), E1072-1084. https://doi.org/10.1152/ajpendo.00520.2015

Karn, J. F. (2001, 2001/02/15/). Phosphorus nutrition of grazing cattle: a review. *Animal Feed Science and Technology, 89*(3), 133-153. https://doi.org/https://doi.org/10.1016/S0377-8401(00)00231-5

Knudsen, K. E. B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology, 67*(4), 319-338.

Leonel, F. de P., & Araújo, D. B. (2023). *Advances and challenges in mineral nutrition for ruminants* (1º ed, Vol. 1). Lux.

Li, L., Zhang, X., Zhao, L., Zhang, J., Ji, C., & Ma, Q. (2021, Dec 14). Phosphorus Restriction in Brooding Stage Has Continuous Effects on Growth Performance and Early Laying Performance of Layers. *Animals (Basel), 11*(12). https://doi.org/10.3390/ani11123546

Mahajan, A., Alexander, L. S., Seabolt, B. S., Catrambone, D. E., McClung, J. P., Odle, J., Pfeiler, T. W., Loboa, E. G., & Stahl, C. H. (2011). Dietary Calcium Restriction Affects Mesenchymal Stem Cell Activity and Bone Development in Neonatal Pigs. *The Journal of Nutrition, 141*(3), 373-379. https://doi.org/10.3945/jn.110.131193

Maltin, C. A., Delday, M. I., Sinclair, K. D., Steven, J., & Sneddon, A. A. (2001). Impact of manipulations of myogenesis in utero on the performance of adult skeletal muscle. *REPRODUCTION-CAMBRIDGE-*, *122*(3), 359-374. https://rep.bioscientifica.com/downloadpdf/journals/rep/122/3/359.pdf

Mauro, A. (1961). Satellite cells of muscle skeletal fibers. *J. Biophys. Biochem, 9*, 493-495.

McCarthy, J. J., Mula, J., Miyazaki, M., Erfani, R., Garrison, K., Farooqui, A. B., Srikuea, R., Lawson, B. A., Grimes, B., Keller, C., Van Zant, G., Campbell, K. S., Esser, K. A., Dupont-Versteegden, E. E., & Peterson, C. A. (2011, Sep). Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development*, *138*(17), 3657-3666. https://doi.org/10.1242/dev.068858

McDowell, L. R. (2000). *Vitamins in animal and human nutrition*. John Wiley & Sons.

McLean, R., Hendricksen, R., Coates, D., & Winter, W. (1990). Phosphorus and beef production in northern Australia. 6. Dietary attributes and their relation to cattle growth. *Tropical grasslands, 24*(3), 197-208.

Minson, D. (1990). *Forage in ruminant nutrition*. Harcourt Brace Jovanovich.

Moore, D. T., Ferket, P. R., & Mozdziak, P. E. (2005, 2005/05/01/). The effect of early nutrition on satellite cell dynamics in the young turkey. *Poultry Science, 84*(5), 748-756. https://doi.org/https://doi.org/10.1093/ps/84.5.748

MORAES, E. d., Italiano, E., & Pieniz, L. (1985). Efeito de níveis de fósforo no crescimento e engorda de bovinos mantidos em pastagem de quicuio da Amazônia.

Moraes, S. d. S. (2001). *Principais deficiências minerais em bovinos de corte*. Campo Grande, MS: Embrapa Gado de Corte, 2001.

Moss, F., & Leblond, C. (1971). Satellite cells as the source of nuclei in the muscles of growing rats. *The Anatomical Record, 170*(4), 421-435.

Murach, K. A., White, S. H., Wen, Y., Ho, A., Dupont-Versteegden, E. E., McCarthy, J. J., & Peterson, C. A. (2017, Jul 10). Differential requirement for satellite cells during overload-induced muscle hypertrophy in growing versus mature mice. *Skeletal Muscle, 7*(1), 14. https://doi.org/10.1186/s13395-017-0132-z

Nicodemo, M. L. F., Moraes, S. d. S., Rosa, I. V., Macedo, M. C. M., Thiago, L. R. L. d. S., & Anjos, C. R. d. (2000). Avaliação de níveis de fósforo na dieta de novilhos Nelore em crescimento: efeitos no desempenho. *Revista Brasileira de Zootecnia, 29*, 1191-1195.

Owens, F. N., Dubeski, P., & Hanson, C. F. (1993). Factors that alter the growth and development of ruminants. *Journal of Animal Science, 71*(11), 3138-3150. https://academic.oup.com/jas/article-abstract/71/11/3138/4719016?redirectedFrom=fulltext

Paulino, M. F., Figueireido, D. d., Moraes, E., Porto, M. O., Sales, M. F., Acedo, T. S., Villela, S. D., & Valadares Filho, S. d. C. (2004). Suplementação de bovinos em pastagens: uma visão sistêmica. *Simpósio de produção de gado de corte, 4*(2004), 93-139.

Rehfeldt, C., Fiedler, I., Dietl, G., & Ender, K. (2000, 2000/10/01/). Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. *Livestock Production Science, 66*(2), 177-188. https://doi.org/https://doi.org/10.1016/S0301-6226(00)00225-6

Saraiva, A., Donzele, J. L., Oliveira, R. F. M. d., Abreu, M. L. T. d., Silva, F. C. d. O., & Haese, D. (2009). Níveis de fósforo disponível em rações para suínos de alto potencial genético para deposição de carne dos 30 aos 60 kg. *Revista Brasileira de Zootecnia, 38*, 1279-1285.

Schatz, T. J., McCosker, K. D., Heeb C. (2023). Phosphorus supplementation improves the growth and reproductive performance of female Brahman cattle grazing phosphorus-deficient pastures in the Victoria River District, Northern Territory, Australia. *Animal*

Production Science, 63(6), 544-559. https://doi.org/10.1071/AN21565

Shafiq, S. A., Gorycki, M. A., & Mauro, A. (1968, Jun). Mitosis during postnatal growth in skeletal and cardiac muscle of the rat. *J Anat, 103*(Pt 1), 135-141.

Silva, M. D. P., & Carvalho, R. F. (2007). Mecanismos celulares e moleculares que controlam o desenvolvimento e o crescimento muscular. *Revista Brasileira de Zootecnia, 36*, 21-31.

Swatland, H. J. (1973). Muscle Growth in the Fetal and Neonatal Pig. *Journal of Animal Science, 37*(2), 536-545. https://doi.org/10.2527/jas1973.372536x

Teixeira, S., Branco, A. F., Granzotto, F., Barreto, J. C., Roma, C. F. d. C., & Castañeda, R. D. (2011). Fontes de fósforo em suplementos minerais para bovinos de corte em pastagem de Cynodon nlemfuensis Vanderyst. *Revista Brasileira de Zootecnia, 40*, 190-199.

Ternouth, J. (1990). Phosphorus and beef production in northern Australia. 3. Phosphorus in cattle-a review. *Tropical grasslands, 24*(3), 159-169.

Van Soest, P. v., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides about animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597.

Varley, P. F., Sweeney, T., Ryan, M. T., & O'Doherty, J. V. (2011). The effect of phosphorus restriction during the weaner-grower phase on compensatory growth, serum osteocalcin, and bone mineralization in gilts. *Livestock Science, 135*(2-3), 282-288. https://doi.org/10.1016/j.livsci.2010.07.025

Vieira, A., Lobato, J. F. P., Torres Junior, R. A. d. A., Cezar, I. M., & Correa, E. S. (2005). Recria de machos Nelore em pastagens cultivadas com suplementação na seca nos Cerrados do Brasil Central. *Revista Brasileira de Zootecnia, 34*, 1349-1356.

Williams, S., McDowell, L., Warnick, A., Wilkinson, N., & Lawrence, L. (1991). Phosphorus concentrations in blood, milk, feces, bone, and selected fluids and tissues of growing heifers as affected by dietary phosphorus. *Livestock Research for Rural Development, 3*(2).

Williams, S. N., McDowell, L., Warnick, A., Lawrence, L., & Wilkinson, N. (1989). Influence of dietary phosphorus level on growth and reproduction of growing beef heifers. *Florida beef cattle research report-Florida Cooperative Extension Service, University of Florida (USA)*.

Winter, W., Coates, D., Hendricksen, R., Kerridge, P., McLean, R., & Miller, C. (1990).

Phosphorus and beef production in northern Australia. 4. The response of cattle to fertilizer and supplementary phosphorus. *Tropical grasslands, 24*(3), 170-184.

Witt, K. E., & Owens, F. N. (1983). Phosphorus: Ruminal Availability and Effects on Digestion. *Journal of Animal Science, 56*(4), 930-937. https://doi.org/10.2527/jas1983.564930x

Xu, J., Liu, D., Yin, H., Tong, H., Li, S., & Yan, Y. (2018, Aug). Fatty acids promote bovine skeletal muscle satellite cell differentiation by regulating ELOVL3 expression. *Cell Tissue Res, 373*(2), 499-508. https://doi.org/10.1007/s00441-018-2812-3

Zhang, W., Kroscher, K. A., Murray, R. L., Gagliardi, R., Guiltinan, C., Rhoads, R. P., & Stahl, C. H. (2020, May 1). Dietary Calcium and Phosphorus Amounts Affect Development and Tissue-Specific Stem Cell Characteristics in Neonatal Pigs. *J Nutr, 150*(5), 1086-1092. https://doi.org/10.1093/jn/nxaa011

Zhao, L., Huang, Y., & Du, M. (2019, Jul). Farming animals for studying muscle development and metabolism has dual purposes for animal production and human health. *Anim Front, 9*(3), 21-27. https://doi.org/10.1093/af/vfz015

Zhu, H., Park, S., Scheffler, J. M., Kuang, S., Grant, A. L., & Gerrard, D. E. (2013). Porcine satellite cells are restricted to a phenotype resembling their muscle origin. *Journal of Animal Science, 91*(10), 4684-4691. https://doi.org/10.2527/jas.2012-5804

SECOND SECTION – ARTICLE

ARTICLE 1: Effect of phosphate nutrition during the growing phase of beef cattle on short and long-term performance

Article formatted according to Animal guidelines

ABSTRACT

Our objective with this study was to evaluate the short and long-term effects that phosphorus supplementation during the growth phase could have on the performance, digestibility and carcass parameters of beef cattle. In this experiment, 90 recently weaned Nellore heifers weighing an average of 195kg were allocated as a trio in 30 stalls and divided into 3 treatments: P70 (70% of phosphorus requirements), P100 (100% of phosphorus requirements) and P130 (130% of phosphorus requirements). phosphorus requirements). The animals received the treatments during the growth phase, for a period of 108 days, after which all animals entered the finishing phase, receiving the same diet, for a period of 115 days. The weight gain of animals during the growth phase tended to increase linearly according to the increase in phosphorus level ($P = 0.08$). There was no effect of treatments on animal performance in the finishing phase ($P \geq 0.1$). Phosphorus digestibility in the growth phase suffered a quadratic effect of treatments ($P = 0.045$). There was a tendency for a positive linear correlation between treatments and phosphorus digestibility at finishing ($P = 0.092$). There was no effect of treatments on blood phosphorus concentration during the growth phase ($P \geq 0.1$). The results show that increasing the level of phosphorus in the diet of animals in the growing phase does not directly alter the weight gain of animals at finishing, but has the potential to improve the efficiency of phosphorus use after the animals' growth phase.

1. INTRODUCTION

During the growth phase, cattle express their highest potential for muscle mass accumulation. This occurs because there is a physiological prioritization for bone development and muscle tissue deposition over adipose tissue deposition (Berg et al., 1979). This developmental stage typically follows a sigmoid growth curve, with a significant intensity in muscular and skeletal growth until puberty, followed by a deceleration in this growth and a shift towards adipose tissue deposition (Owens et al., 1993). Therefore, optimizing weight gain during this phase is essential to capitalize on this window of accelerated growth and produce heavier animals for finishing, potentially reducing the age at slaughter.

However, challenges arise, especially in tropical regions where seasonal forage production leads to periodic nutritional limitations, especially during the growth phase (Mario Fonseca et al., 2004; Vieira et al., 2005). Nutritional deficiencies during this phase prevent cattle from expressing their full potential for weight gain, consequently prolonging the time required to reach ideal finishing weights. Among nutritional limitations, phosphorus deficiency stands out, especially in tropical regions like Brazil where it is commonly deficient in soil and pastures (Moraes, 2001). The reduction in milk consumption after weaning exacerbates this deficiency, as milk is a primary source of minerals, coupled with the fact that producers often neglect mineral supplementation, especially phosphorus, due to its high cost (Giacomel et al., 2022).

Recent research highlights the fundamental role of phosphorus in influencing postnatal muscle mass gain. Studies by Alexander et al., (2010, 2012); Zhang et al., (2020) have shown evidence that phosphorus stimulates the proliferation of satellite cells in skeletal muscle tissue. Satellite cells, as described by Gonzalez et al. (2020), are essential for muscle regeneration and hypertrophy, being directly responsible for the growth of this tissue (Dhawan & Rando, 2005).

These studies indicate an important indirect relationship of phosphorus to muscle hypertrophy, which has been underexplored, as few studies on the subject have been conducted in recent years.

In this regard, aiming to explore the information gap regarding the influence of phosphorus on muscle cell hypertrophy, we evaluated whether deficient or excessive phosphorus supply during the growth phase of beef cattle would impact muscle development and animal performance, and whether these impacts would influence subsequent phases after the growth phase.

2. MATERIALS AND METHODS

All procedures involving the care and management of the animals in this research followed the guidelines established by the Ethics Committee for the Use of Animals at the Federal University of Lavras.

2.1. Animals and Experimental Design

The test was carried out at the facilities of the Beef Cattle Sector of DZO/FZMV/UFLA. Ninety recently weaned Nellore heifers, with an initial body weight of 195kg and seven months of age, were housed in groups of three animals in 30 pens with concrete and earth floors, measuring 4m x 10m, equipped with drinking troughs.

Before starting the experimental period, the heifers were dewormed with Dectomax (Zoetis, Campinas, SP, Brazil), weighed, blocked and randomly distributed into three groups, which were fed a basal diet (73% sorghum and grass stalklage + 27% of corn silage, DM basis) plus protein-energy supplementation (level of 3.5g/kg of BW), the level of phosphorus in the supplement differing between groups: deficient P (P70) with 0.28% of phosphorus in the supplement , ideal P (P100) with 0.67% phosphorus in the supplement and excessive P (P130)

with 1.07% phosphorus in the supplement meeting respectively 70,100 and 130% of nutritional phosphorus requirements. The animals were fed for 108 days during the growth phase in confinement to better control diet intake and digestibility. After this period, all animals were fed an initial finishing diet, 50% sorghum panicle silage and 50% concentrate (DM basis) for 40 days and then started receiving a final finishing diet with 35% silage. of corn and 65% concentrate (DM basis) for a period of 75 days, until they are slaughtered. All diets and supplements were formulated in accordance with the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016). The feed was offered to the animals in both periods (growth and finishing) ad libitum, twice daily (07:00 and 16:00) with free access to water. Every day before the first meal, the trough score was evaluated to adjust the amount of feed, which were weighed daily to calculate the animals' dry matter intake (DMI).

2.2 Performance Measurements

The initial and final body weights of each growth phase (day one and day 108) and finishing phase (day 115) were collected with the animals fasting with food restriction for 16 hours, other weightings were carried out without fasting. We use this methodology, with the aim of improving the accuracy of weight gain data, considering adjustments related to gastrointestinal content. The average daily gain (ADG) for each phase (growth and finishing) were calculated based on the recorded body weight of each animal (ADG = final weight – initial weight/days of the experimental period) and feed efficiency were calculated based on average daily gain and dry matter intake (Feed efficiency = kg ADG / kg DMI).

2.3. Digestibility Test

Two spot collections of feces were carried out during the experimental period (one during growth and another during finishing). In each period, fecal samples were collected daily at different times, at 7:00 am, 12:00 pm and 4:00 pm, respectively, on the first, second, and third

days of each period. Fresh feces were collected from each animal in the pen and a composite sample was subsequently taken per pen. Samples of diets and leftovers were also collected daily during the period, with a composite sample being reconstituted at the end. After collection, all composite samples were stored at -20 ºC until chemical analyzes were carried out. All samples were pre-dried in a forced ventilation oven at 65 ºC for 72 hours. The partially dried samples were ground in a knife mill with a 1 and 2 mm sieve and stored at room temperature.

All samples were chemically analyzed according to method No. G-003/1 for measuring humidity, M-001/1 for measuring ash, N-001/1 for measuring nitrogen and (FDNap; N-002/1) and (F-009/2), to measure ash- and protein-free neutral detergent fiber and NDFi respectively. All methods mentioned are based on the analytical guidelines of the National Institute of Science and Technology (INCT-CA) (Detmann et al., 2012). P and Ca were also determined in the samples, according to the methodology described by (Malavolta et al., 1989).

Nutrient digestibility coefficients were estimated through the difference between consumption and fecal content divided by consumption. To measure fecal production, indigestible neutral detergent fiber (NDFi) was used as an internal marker. To determine NDFi, autoclaved 2 mm samples were used after in situ incubation (288 h) using beef animals cannulated in the rumen (Detmann et al., 2012).

2.4.Blood collection and assessments

On days 60 of the growing phase and 70 of finishing, blood samples were collected by puncture of the jugular vein in vacutainer tubes (First Lab, São José dos Pinhais, PR, Brazil), and plasma was collected by centrifugation (1,500 × g for 15 min at +4°C) and stored at -20°C until processing. The concentration of inorganic phosphorus was determined by the ammonium molybdate method using a commercial kit (Labtest, Lagoa Santa-MG, Brazil).

2.5.Slaughter, carcass evaluation, and sample collection

The animals were slaughtered in a slaughterhouse with Federal Inspection (SIF), as chosen by the borrower. The animals were slaughtered using the technique of cerebral concussion and section of the jugular vein, followed by removal of the hide and evisceration, according to Normative Instruction 4 of 03/31/2000 (MAPA, 2000).

At the end of the growth phase, a demonstrative slaughter of 12 heifers was carried out, taken from four random stalls from each treatment, to estimate the carcass yield at the beginning of the finishing phase and subsequently obtain the average carcass gain at the end of the phase.

After slaughter, the carcasses were divided into two halves to obtain hot carcass weight, carcass daily gain and carcass yield. During deboning of the carcasses, the thickness of the subcutaneous fat between the 12th and 13th rib of the left half carcass was measured, using a caliper graduated at ¾ of the length of the lumbar eye from the cranial portion. The rib eye area, also measured between the 12th and 13th ribs, was outlined on transparent paper and subsequently analyzed using ImageJ® software (National Institutes of Health, Bethesda, Maryland, USA).

From the carcass of one animal from each pen, four one-inch-thick steaks of the Longissimus muscle were removed from the first lumbar vertebra in the caudal direction, vacuum packed and transported at -2°C for subsequent meat analysis.

2.6.Meat quality analysis

• Centesimal composition

The centesimal composition was determined on 100 grams of each sample with subcutaneous fat previously removed and ground for near-infrared analysis using a

FoodScanTM (AOAC method: 2007-04; FOSS, Hillerod, Denmark

• Color and pH

Color indices were estimated on the surface of steaks previously removed from vacuum packaging and exposed to oxygen for 30 minutes for blooming. Meat surface reflectance data (390 to 710 nm, with a 10 nm interval) were recorded from the average of five consecutive measurements using a CM-700 spectrophotometric colorimeter (Kônica Minolta Sensing Inc., Osaka, Japan), with 8 mm aperture, illuminant A, 10° observation angle and specular component excluded (SCE) mode. From the readings obtained in SCE mode, the values of brightness (L*), red index (a*), yellow index (b*), saturation (, C*) and hue angle (h) were determined. Along with the color analysis, the same steaks were used to evaluate the pH, using a previously calibrated portable pH meter (Model HI 99,163; Hanna, Woonsocket, RI, USA). Three pH readings were taken for each steak and an average pH was obtained from them.

• Shear force

From the same steaks used for color measurement, five rectangular cubes (1.0 × 1.0 × 2.5 cm) were obtained in the direction of the muscle fibers. Each cube were cut transversely at 200 mm/min by a Warner-Bratzler blade coupled to a TA.XTplus Texturometer (Stable Micro Systems Ltd., Godalming, Surrey, United Kingdom). The shear strength results were measured in Newtons from the average value of the five rectangular cubes of each steak.

2.7.Statistical analysis

Data were analyzed using a completely randomized design model through the MIXED procedure of SAS 9.0 Software (SAS System, Cary, NC, USA). Dietary phosphorus level was treated as the main fixed effect, treated as a quantitative variable, allowing for the estimation

and testing of both linear and quadratic parameters for regression. When only the linear effect was significant, the quadratic parameter estimate was removed from the model. The initial weight of each animal was incorporated as a covariate within the model. Each pen served as the experimental unit, and variation among animals within the same pen was also accounted for in the model, with the pen nested within the treatment being treated as a random effect.

The foundational statistical model for this analysis was expressed as follows:

$$y_{ijk} = \mu + P_i + p_{j:i} + \varepsilon_{ijk}$$

where y_{ijk} represents the observed values, μ is the overall population mean, P_i denotes the effect of the i_{th} phosphorus level, $p_{j:i}$ represents the random effect of the j_{th} pen within the i_{th} treatment, and ε_{ijk} is the random error, which is assumed to be independently and identically distributed as $N(0, \sigma^2_{\varepsilon})$. Notably, ε_{ijk} considers the effect of the animal nested within the interaction of treatment and pen. This methodological approach follows the guidelines set forth by St-Pierre (2007).

Before conducting the analyses, we evaluated residuals to ensure they met distributional assumptions. Studentized residuals exceeding ±3 standards deviations were individually removed, with their homogeneity across fixed effects and normality being continuously assessed through visual inspections and Shapiro-Wilk's test, respectively, ensuring normality with a significance level of $P > 0.05$ (Osborne & Overbay, 2004). Statistical significance was established at $P < 0.05$, and observations were discussed as trends when $0.10 > P \geq 0.05$.

3. RESULTS

3.1 Performance

At the beginning of the experimental period, the weight of the animals showed no differences between treatments ($P = 0.998$). At the end of the growth phase, there was a

tendency towards a positive linear effect for final weight (P = 0.08), weight gain (P = 0.08) and average daily gain (P = 0.081). During the finishing phase there was no effect of phosphorus level on final weight (P = 0.637), weight gain (P = 0.826) and average daily gain (P = 0.730).

3.2 Voluntary feed intake, feed efficiency and digestibility

Dry matter intake in the growing phase increased linearly as a function of the increase in the level of phosphorus in the diet (P = 0.033) but had no significant effect in the finishing phase (P = 0.672). Feed efficiency also did not change depending on phosphorus levels either in the growing phase (P = 0.203) or in the finishing phase (P = 0.520).

The digestibility of crude protein and NDF was not affected in any of the phases ($P \geq 0.1$). Phosphorus digestibility suffered a linear (P = 0.019) and quadratic (P = 0.045) effect due to the increase in phosphorus levels in the diet during the growth phase. Throughout the finishing phase, phosphorus digestibility tended to be greater according to the increase in phosphorus in the diet (P = 0.092).

3.3 Phosphorus blood concentration

There were no effects of dietary phosphorus level on blood inorganic phosphorus concentration during the growth phase ($P \geq 0.1$). During the finishing phase, the concentration of inorganic phosphorus in the blood suffered a quadratic effect depending on the level of phosphorus in the diet (P = 0.011).

3.4 Carcass parameters and meat quality

No effect of dietary phosphorus levels on the parameter's hot carcass weight, total carcass gain, final carcass yield, degree of finishing carcass and ribeye area were detected ($P \geq 0.1$). For the backfat thickness parameter there was a significant quadratic effect (P = 0.021).

All color parameters (L*, a*, b*), shear force (WBSF) and pH were similar between treatments, without suffering any significant effect due to the different levels of phosphorus in the diet ($P \geq 0, 1$). There were also no significant effects of the treatments on the centesimal composition of the steaks. The moisture, collagen, ether extract and protein parameters were similar between treatments ($P \geq 0.1$).

4. DISCUSSION

In line with our hypothesis, the results obtained for the weight gain variable show that the level of phosphorus in the diet tends to correlate positively with the performance of the animals during the growth phase. Our results showed that every 1% increase in phosphorus in the diet, in relation to the animals' requirements, increased approximately 1g in ADG, that is, by purchasing the P70 treatment with P130, we left a scenario of 30% phosphorus deficiency. in relation to the requirements, for a scenario of 30% excess phosphorus in relation to the requirements, which resulted in an increase of 57g in the ADG of the animals. Schatz et al. (2023) recently presented similar results, obtained with females grazing under phosphorus deficiency for long periods. This positive correlation once again indicates a direct relationship between P and weight gain, which may be related to the action of this mineral on satellite cells and consequently on muscle cell hypertrophy, as suggested by Alexander et al. (2010). Furthermore, this linear growth in performance due to the increase in phosphorus in the diet suggests that there is a lag in relation to the P requirement levels currently predicted and shows the need for more studies to be carried out on this topic.

On the other hand, there was no significant effect of phosphorus level on animal performance during the finishing phase, which is contrary to our hypothesis. We expected that the different levels of phosphorus in the growth phase would alter the dynamics of proliferation and fusion of satellite cells during the physiological period of greatest activity of these cells in

the body, which would increase or decrease the hypertrophy capacity of muscle cells and consequently the performance at work. throughout the animal's life, since the fusion capacity of satellite cells practically ends when the animals reach adulthood (Silva & Carvalho, 2007; Zhao et al., 2019; Gonzalez et al., 2020).

In this sense, we can propose three justifications for this result: firstly, the period of application of the treatments may have been too short to cause long-term effects on the animals. Dixon et al. (2020) e Malafaia et al. (2023) explain that the magnitude of the manifestation of phosphorus deficiency symptoms is fundamentally determined, among other factors, by the duration of the period of exposure to the deficiency, that is, long periods increase the magnitude of the symptoms. Therefore, we can theorize that the period of application of the treatments was not long enough to cause long-term effects on the performance of the animals. The second factor that we can use as an answer to these results is the efficiency of phosphorus homeostasis, through physiological adaptations such as bone reabsorption, phosphorus recycling via saliva and regulation of phosphorus excretion mainly via feces. (Challa et al., 1989; Karn, 2001). According to Dixon et al. (2020) growing cattle submitted to diets deficient in phosphorus can delay the adverse effects of the deficiency, through the mobilization of phosphorus from body reserves, which reduces bone density and can lead the animal to develop rickets but can partially compensate for the deficiency of phosphorus in the diet for long periods. This may have mitigated the long-term effects of treatments. Third, we suggest that the window of more intensive satellite cell activity may occur earlier than we thought, which may make sense since, according to Gonzalez et al. (2020) the fusion capacity of satellite cells gradually decreases throughout life, and this raises the possibility that weaned animals have already missed this window and that in fact even younger animals are more susceptible to these effects than those we used in this work.

The effect of different levels of phosphorus on dry matter consumption has been well documented for a long time in research studies. Bass et al. (1981); Gartner et al. (1982) e Milton & Ternouth (1985) and has also been reinforced recently by Dixon et al. (2020). Ternouth (1990) stated that the reduction in DMI in animals exposed to phosphorus deficiency is mainly due to disturbances in cellular metabolism, caused by the scarcity of the mineral. This statement raises the hypothesis that animals that receive adequate phosphorus supplementation are encouraged to increase DMI, which corroborates our findings. On the other hand, we can consider that the lack of effect of phosphorus level on feed efficiency indicates that changes in DMI are reflections of the effects on performance. In other words, the change in performance depending on the dose of phosphorus also changes the DMI, since there is a direct relationship between performance and nutritional requirements and consequently DM requirements.

Our results for phosphorus digestibility show a positive correlation with phosphorus intake, as previously indicated by Bortolussi et al. (1996); Dixon et al. (2020) e Karn (2001). The explanation presented by Bortolussi et al. (1996) is that animals that receive diets with adequate levels of nitrogen, but deficient in phosphorus, have a potentiated phosphorus deficiency, as the organism starts to use body reserves to compensate for the increase in demand, which leads to an increase in loss endogenous fecal content of P, possibly due to the greater supply of phosphorus in the rumen through recycling via saliva, which ends up reducing the digestibility coefficient in cases of deficiency of this mineral. There is also another possibility that was described by Challa et al. (1989), which consists of the idea that the lower the concentration of phosphorus in the diet, the higher the Ca: P ratio, which results in greater precipitation of phosphate in the gastrointestinal tract, reducing its bioavailability.

Another analysis that we must do is regarding the quadratic effect on phosphorus digestibility during growth. The observed effect shows that the digestibility coefficient

apparently increases depending on the level of phosphorus in the diet, but reaches a plateau, which can be numerically observed in the averages of treatments P100 and P130. This effect can be explained by the work of Challa et al. (1989) e Challa & Braithwaite (1988c, 1988b, 1988a), which make it clear that the absorption of phosphorus increases as the consumption of the mineral increases, but once the phosphorus requirements are met, there is also an increase in its excretion.

The trend found regarding phosphorus digestibility at finishing is an interesting finding, which indicates that animals that receive adequate phosphate nutrition in the growth phase tend to have greater phosphorus absorption efficiency in the following phases. This is a very positive hypothesis, as it opens room for discussion regarding the supplementation strategies currently used, to adjust phosphorus requirements, in contexts in which animals have received adequate supplementation during growth. However, it would be interesting if more studies were carried out on this topic.

Some variables evaluated in our work presented surprising results, including two of the few residual effects detected in termination. The results regarding phosphorus concentrations in the blood during the growth phase did not suffer significant effects from the treatments, going against the results found by Bortolussi et al. (1996); Challa et al. (1989), which show that the concentration of phosphorus in the blood increases, along with the increase in phosphorus consumption. On the other hand, Anderson et al. (2017) shows that although blood phosphorus is generally a good marker of phosphorus status, it may not be a good indicator for animals that are undergoing great mobilization of phosphorus from body reserves, which may explain the results obtained in our study. We still observed residual quadratic effects for phosphorus concentration in the blood and backfat thickness at the finishing, for which we did not find any work in the literature that presents results that could provide support to explain such effects.

Despite the low variation, these effects may have appeared due to good uniformity between animals. However, it is important that new studies are carried out to better investigate the causes of these effects.

The hypothesis that phosphorus could alter muscle metabolism raised the idea that meat quality characteristics would also be altered depending on the level of phosphorus in the diet. However, the lack of significant effects for these parameters is in line with results found by Lima et al. (2020), which did not observe effects of phosphorus supplementation at finishing on meat quality characteristics.

Overall, our work brought interesting information that reinforces the importance of phosphorus on the performance of animals in the growth phase, but in addition, it raises a topic that needs to be further explored, regarding the impact that phosphorus supplementation in the early stages of the animal's life can have a long-term impact on both performance and the efficiency of phosphorus use. The vast majority of work we have on phosphate nutrition for cattle is very old, in addition to the fact that there are practically no recent studies investigating the influence of phosphorus on the muscle development of beef cattle. Therefore, it is very important that this area of knowledge is revisited and that new studies are carried out, to evaluate, for example, the effects of phosphate nutrition on males in the long term, younger animals and heavier animals or with longer periods of treatment application.

5. CONCLUSION

The results of this study show that higher levels of phosphorus in the diet of growing beef cattle improve performance and phosphorus digestibility during this phase, without causing long-term residual effects on performance, but improves phosphorus digestibility during finishing phase. Future experiments need to be carried out to better investigate the long-term

effects that can be caused by phosphorus supplementation in the early stages of the life of beef cattle, to optimize the use of phosphorus in the nutrition of these animals

REFERENCES

Alexander, L. S., Mahajan, A., Odle, J., Flann, K. L., Rhoads, R. P., & Stahl, C. H. (2010). Dietary phosphate restriction decreases stem cell proliferation and subsequent growth potential in neonatal pigs. *Journal of Nutrition*, *140*(3), 477–482. https://doi.org/10.3945/jn.109.117390

Alexander, L. S., Seabolt, B. S., Rhoads, R. P., & Stahl, C. H. (2012). Neonatal phosphate nutrition alters in vivo and in vitro satellite cell activity in pigs. *Nutrients*, *4*(6), 436–448. https://doi.org/10.3390/nu4060436

Anderson, S. T., Kidd, L. J., Benvenutti, M. A., Fletcher, M. T., & Dixon, R. M. (2017). New candidate markers of phosphorus status in beef breeder cows. *Animal Production Science*, *57*(11), 2291–2303. https://doi.org/10.1071/AN17363

Bass, J. M., Fishwick, G., Hemingway, R. G., Parkins, J. J., & Ritchie, N. S. (1981). The effects of supplementary phosphorus on the voluntary consumption and digestibility of a low phosphorus straw-based diet given to beef cows during pregnancy and early lactation. Em *J. agric. Sci., Camb* (Vol. 97).

Berg, R. T., Butterfield, R. M., García de Siles, J., & Luca de Tena, E. (1979). *Nuevos conceptos sobre desarrollo de ganado vacuno.*

Bortolussi, G., Ternouth, J. H., & Mcmeniman, N. P. (1996). Dietary nitrogen and phosphorus depletion in cattle and their effects on liveweight gain, blood metabolite concentrations and phosphorus kinetics. Em *Journal of Agricultural Science* (Vol. 126).

Challa, B. J., Braithwaite, G. D., & Dhanoa, M. S. (1989). Phosphorus homoeostasis in growing calves. Em *J. agric. Sci., Camb* (Vol. 112).

Challa, J., & Braithwaite, G. D. (1988a). Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homoeostasis: 1. Studies of the effect of changes in the dietary phosphorus intake on phosphorus and calcium metabolism. *The Journal of*

Agricultural Science, *110*(3), 573–581. https://doi.org/DOI: 10.1017/S0021859600082150

Challa, J., & Braithwaite, G. D. (1988b). Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homoeostasis: 2. Studies of the effect of different levels of phosphorus, infused abomasally, on phosphorus metabolism. *The Journal of Agricultural Science*, *110*(3), 583–589. https://doi.org/DOI: 10.1017/S0021859600082162

Challa, J., & Braithwaite, G. D. (1988c). Phosphorus and calcium metabolism in growing calves with special emphasis on phosphorus homoeostasis: 3. Studies of the effect of continuous intravenous infusion of different levels of phosphorus in ruminating calves receiving adequate dietary phosphorus. *The Journal of Agricultural Science*, *110*(3), 591–595. https://doi.org/DOI: 10.1017/S0021859600082174

Detmann, E., Valadares, S. F., Queiroz, A. D., Berchielli, T. T., Saliba, E. D. O., & Azevedo, J. A. G. (2012). *Métodos para análise de alimentos*. Visconde do Rio branco: Suprema.

Dhawan, J., & Rando, T. A. (2005). Stem cells in postnatal myogenesis: Molecular mechanisms of satellite cell quiescence, activation and replenishment. Em *Trends in Cell Biology* (Vol. 15, Número 12, p. 666–673). https://doi.org/10.1016/j.tcb.2005.10.007

Dixon, R. M., Anderson, S. T., Kidd, L. J., & Fletcher, M. T. (2020). Management of phosphorus nutrition of beef cattle grazing seasonally dry rangelands: a review. *Animal Production Science*, *60*(7), 863–879. https://doi.org/10.1071/AN19344

Gartner, B. J. W., Murphy, G. M., & Hoey, W. A. (1982). Effects of induced, subclinical phosphorus deficiency on feed intake and growth of beef heifers. *The Journal of Agricultural Science*, *98*(1), 23–29. https://doi.org/DOI: 10.1017/S002185960004106X

Giacomel, A., Freitas, T. C., Costa, A. L. B., Sbardelotto, E. M., Bergmann, E., & Debortoli, E. C. (2022). Mineral supplementation for beef cattle – a meta-analysis. *Research, Society and Development*, *11*, 3.

Gonzalez, M. L., Busse, N. I., Waits, C. M., & Johnson, S. E. (2020). Satellite cells and their regulation in livestock. Em *Journal of Animal Science* (Vol. 98, Número 5). Oxford

University Press. https://doi.org/10.1093/jas/skaa081

Karn, J. F. (2001). Phosphorus nutrition of grazing cattle: a review. *Animal Feed Science and Technology*, *89*, 133–153.

Leonel, F. de P., & Araújo, D. B. (2023). *Advances and challenges in mineral nutrition for ruminants* (1º ed, Vol. 1). Lux.

Lima, L. de O., de Souza, V. C., Messana, J. D., Castagnino, P. de S., Lima, A. R. C., & Berchielli, T. T. (2020). Phosphorus supplementation does not affect the intake, digestibility, and meat quality of nellore young bulls fed with high-grain diets. *Acta Scientiarum - Animal Sciences*, *43*(1), 1–8. https://doi.org/10.4025/actascianimsci.v43i1.50347

Malafaia, P., Barbosa, J. D., Brito, M. F., Souza, V. C. de, & Costa, D. F. A. (2023). Phosphorus for Cattle and Buffaloes in Brazil: Clinical Signs and Diagnosis of Its Deficiency and Relevance, and Recommended Strategies to Alleviate Issues Observed under Grazing Conditions. *Ruminants*, *3*(1), 55–75. https://doi.org/10.3390/ruminants3010006

Malavolta, E., Vitti, G. C., & Oliveira, S. A. (1989). *Avaliação do estado nutricional das plantas ; princípios e aplicações*. Potafos.

Mario Fonseca, P., Figueiredo, D., Henrique Bevitori Kling de Moraes, E., Oliveira Porto, M., Franklin Lima Sales, M., Sabela Acedo, T., Villela, S., & Valadares, S. (2004). Suplementação de bovinos em pastagens: uma visão sistêmica. *Simpósio de produção de gado de corte*, *4*, 93–139. https://www.researchgate.net/publication/281637635

Milton, J. T. B., & Ternouth, J. H. (1985). Phosphorus metabolism in ruminants. 2. Effects of inorganic phosphorus concentration upon food intake and digestibility. *Australian Journal of Agricultural Research*, *36*(4), 647–654. https://doi.org/10.1071/AR9850647

Moraes, S. da S. (2001). Principais Deficiências Minerais em Bovinos de Corte. *Embrapa gado de corte*. http://www.cnpgc.embrapa.br

Osborne, J. W., & Overbay, A. (2004). *The power of outliers (and why researchers should ALWAYS check for them) - Practical Assessment, Research & Evaluation* (Vol. 9, Número 6).

Owens, F. N., Dubeski, P., & Hansont, C. F. (1993). Factors that Alter the Growth and Development of Ruminants. *Journal of Animal Science*, *71*, 3138–3150.

Schatz, T. J., McCosker, K. D., & Heeb, C. (2023). Phosphorus supplementation improves the growth and reproductive performance of female Brahman cattle grazing phosphorus-deficient pastures in the Victoria River District, Northern Territory, Australia. *Animal Production Science*, *63*(6), 544–559. https://doi.org/10.1071/AN21565

St-Pierre, N. R. (2007). Design and analysis of pen studies in the animal sciences. *Journal of Dairy Science*, *90*(S), E87–E99. https://doi.org/10.3168/jds.2006-612

Ternouth, J. H. (1990). Phosphorus and beef production in northern Australia. 3. Phosphorusin cattle - a review. *Tropical Grasslands*, *24*, 159–169.

Vieira, A., Fernando Piva Lobato, J., Augusto de Almeida Torres Junior, R., Martins Cezar, I., & Simões Correa, E. (2005). Recria de Machos Nelore em Pastagens Cultivadas com Suplementação na Seca nos Cerrados do Brasil Central. *Revista Brasileira de Zootecnia*, *34*(4), 1349–1356.

Zhang, W., Kroscher, K. A., Murray, R. L., Gagliardi, R., Guiltinan, C., Rhoads, R. P., & Stahl, C. H. (2020). Dietary calcium and phosphorus amounts affect development and tissue-specific stem cell characteristics in neonatal pigs. *Journal of Nutrition*, *150*(5), 1086–1092. https://doi.org/10.1093/jn/nxaa0

Table 1. Ingredients and chemical composition of feedstuffs

Item	Basal Diet	P70	P100	P130
		Ingredients, *g/kg*		
Corn silage	270	-	-	-
Sorghum silage	730	-	-	-
Ground corn	-	718.3	718.3	718.3
Soybean meal	-	121.2	121.2	121.2
Urea	-	73.8	73.8	73.8
Sodium chloride	-	19.3	19.3	19.3
Limestone	-	8.2	8.2	8.2
Mineral Premix	-	1.9	1.9	1.9
Kaolin	-	29.7	15.2	-
Magnesium Oxide	-	27.6	14.2	-
Magnesium Phosphate	-	-	27.9	57.3
		Chemical composition		
Dry matter, *%*	32.99	88.56	88.56	88.56
Crude protein, *% of DM*	6.66	33.4	33.4	33.4
Organic matter, *% of DM*	94	89.68	89.68	89.68
Ca, *% of DM*	0.53	0.38	0.37	0.38
P, *% of DM*	0.19	0.28	0.67	1.07

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016).

Table 2. Ingredients and chemical composition of experimental diets.

Item	P70	P100	P130
	Ingredients, *g/kg*		
Basal Diet	850	850	850
Suplement	150	150	150
	Chemical composition		
Dry matter (%)	36,42	36,42	36,42
Crude protein (% of DM)	11,32	11,32	11,32
Organic matter (% of DM)	93,15	93,15	93,15
Ca (% of DM)	0,52	0,51	0,52
P (% of DM)	0,21	0,27	0,34

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016).

Table 3. Ingrediens and composition of finishing diets

Item	Finishing Diet (Sorghum Toplage)	Finishing Diet (Corn Silage)
	Ingredients, *g/kg*	
Corn silage	-	350,5
Sorghum Toplage	500	-
Ground corn	391,2	540
Soybean meal	79,4	79,7
Urea	11,1	11,6
Sodium Chloride	1,0	1
Limestone	5,0	5
Mineral Premix	10,2	10,1
Magnesium Phosphate	2,1	2,1
	Chemical composition	
Dry matter (%)	58,51	56,92
Crude protein (% of DM)	13,92	15,49
FDN(% of DM)	25,59	26,91
Organic matter (% of DM)	95,31	94,65
Ca (% of DM)	0,68	0,45
P (% of DM)	0,31	0,38

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016).

Table 4. Performance variables of Nellore heifers fed different phosphorus levels in the diet during the growing phase.

Item	Treatment			SEM	Parameter estimate		*P value*	
	P70	P100	P130		Linear	Quadratic	Linear	Quadratic
Initial weight[1], *kg*	193	195	197	7.31	.	.	0.998	0.969
Weight at the end of growing phase, *kg*	234	236	240	2.52	0.099 ± 0.055	.	0.080	0.691
Weight at the end of finishing phase, *kg*	341	343	344	4.90	.	.	0.637	0.916
Weight gain during growing phase, *kg*	33.5	35.3	39.5	2.40	0.099 ± 0.055	.	0.080	0.691
Weight gain during finishing phase, *kg*	117	118	116	4.60	.	.	0.826	0.737
ADG during growing phase, *kg/day*	0.259	0.291	0.316	0.023	0.000952 ± 0.00053	.	0.081	0.895
ADG during finishing phase, *kg/day*	1.001	1.008	0.984	0.034	.	.	0.730	0.703

[1] Initial weight was not adjusted for initial weight, while all other measurements were estimated considering initial weight as a covariate.

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016); SEM = standard error of the mean.

Table 5. Voluntary feed intake, feed efficiency and apparent total tract digestibility of Nellore heifers fed different phosphorus levels in the diet during the growing phase.

Item	Treatment[1]			SEM	Parameter Estimate		*P-value*	
	P70	P100	P130		Linear	Quadratic	Linear	Quadratic
Voluntary feed intake, *kg of DM*								
Growing phase	4.30	4.68	4.63	0.101	0.005509 ± 0.002448	.	0.033	0.100
Finishing phase	7.65	8.11	7.78	0.215	.	.	0.672	0.150
Feed efficiency, *g/day of BW per kg of DMI/day*								
Growing phase	0.061	0.061	0.069	0.0045	.	.	0.203	0.519
Finishing phase	0.132	0.125	0.128	0.0041	.	.	0.520	0.259
Apparent total tract digestibility, *g/day*								
Growing phase								
CP	761	757	742	9.61	.	.	0.160	0.630
NDF	455	447	443	11.4	.	.	0.439	0.911
Phosphorous	874	897	900	3.84	0.2594 ± 0.1032	-0.00109 ± 0.000514	0.019	0.045
Finishing phase								
CP	671	702	694	15.4	.	.	0.238	0.286
NDF	444	521	480	29.5	.	.	0.364	0.107
Phosphorous	382	372	440	23.1	0.09425 ± 0.05383	.	0.092	0.184

Abbreviations: P70, P100, and P130 = meeting 70%, 100% and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016); SEM = standard error of the mean; CP = crude protein; NDF = neutral detergent fiber.

Table 6. Phosphorus blood concentration of Nellore heifers fed different phosphorus levels in the diet during the growing phase (expressed in mg/dl).

Item	Treatment mean[1]			SEM	Parameter Estimate		*P-value*	
	P70	P100	P130		Linear	Quadratic	Linear	Quadratic
Growing phase	5.01	5.58	5.52	0.324	.	.	0.263	0.380
Finishing phase	6.01	7.68	6.70	0.396	0.3052 ± 0.1083	-0.00147 ± 0.000539	0.0089	0.011

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016); SEM = standard error of the mean.

Table 7. Carcass parameters and meat quality of Nellore heifers fed different phosphorus levels in the diet during the growing phase.

Item	Treatment mean[1]			SEM	Parameter Estimate		*P-value*	
	P70	P100	P130		Linear	Quadratic	Linear	Quadratic
Carcass Parameters								
Hot carcass weight, *kg*	179	176	177	2.549	.	.	0.733	0.489
Total carcass gain, *kg/day*	0.589	0.564	0.570	0.018	.	.	0.481	0.461
Final Carcass yield, *%*	48.9	49.7	49.6	0.377	.	.	0.212	0.362
Degree of carcass finishing, *arbitrary units*	2.54	2.24	2.46	0.189	.	.	0.812	0.242
Ribeye area, cm^2	55.7	55.7	58.4	2.154	.	.	0.375	0.601
Backfat thickness, *mm*	3.37	3.99	3.50	0.187	0.1249 ± 0.04936	-0.00061 ± 0.000246	0.019	0.021
Meat quality parameters								
Redness (a*)	15.2	14.8	14.8	0.494	.	.	0.614	0.725
Yellowness (b*)	12.0	11.5	12.4	0.330	.	.	0.377	0.106
Lightness (L*)	41.3	40.5	41.9	0.579	.	.	0.461	0.147
WBSF, *N*	7.58	6.91	7.64	0.801	.	.	0.958	0.481
pH	5.48	5.50	5.49	0.220	.	.	0.884	0.634
Centesimal Composition, *%*								
Moisture	72.4	72.0	72.3	0.300	.	.	0.784	0.354
Collagen	2.27	2.24	2.25	0.083	.	.	0.872	0.876
Ether extract	2.32	2.59	2.35	0.250	.	.	0.925	0.407
Protein	22.7	22.9	23.0	0.176	.	.	0.190	0.877

Abbreviations: P70, P100, and P130 = meeting 70%, 100%, and 130% of the daily phosphorus requirements according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016); SEM = standard error of the mean; WBSF = Warner-Bratzler Square Shear Force.