GABRIEL DE OLIVEIRA DAMÁSIO

SUPPLEMENTATION WITH A SLOW-RELEASE NITROGEN INGREDIENT ASSOCIATED OR NOT WITH MONENSIN ON PERFORMANCE, METABOLISM, AND MEAT QUALITY OF FINISHING BEEF CATTLE FED HIGH STARCH DIETS

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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SUPLEMENTAÇÃO COM INGREDIENTE NITROGENADO DE LIBERAÇÃO LENTA ASSOCIADO OU NÃO A MONENSINA NO DESEMPENHO, METABOLISMO E QUALIDADE DA CARNE DE BOVINOS DE TERMINAÇÃO DE CORTE ALIMENTADOS COM DIETAS DE ALTO AMIDO

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

This study aimed to assess the effects of a time-released nitrogen (N) supplement (Timafeed Boost®, Roullier Group, Saint-Malo, France) in association or not with monensin on the performance, metabolism, and meat quality of finishing steers. To investigate these outcomes, one hundred and twelve Nellore steers (380 kg ± 16.2) were used in a 2 × 2 factorial arrangement. Steers were allocated in 28 pens (four animals per pen) and the following treatments were randomly assigned to the experimental units: (1) Control (CON, n = 7) - finishing diet without additives; (2) Monensin-enriched diet (MON, n = 7) - monensin (Rumensin®, Elanco Animal Health, Greenfield, IN) provided at level of 30 mg per kg of dry matter (DM); (3) Gradual-N-release enriched diet (GRN, n = 7) - gradual-N-release supplement (Timafeed Boost®, Roullier Group, Saint-Malo, FR) provided at dose of 250 g per animal per day; or (4) Monensin + Gradual-N-release diet (MON + GRN, n = 7) - monensin (30 mg per kg of DM) associated to gradual-N-release product (250 g per animal per day). The experimental period comprised 102 days, being the first 15 days designated for the diet adaptation. The average daily gain (ADG) over the experimental period was reduced for steers fed GRN + MON (MON × GRN: P = 0.02) compared to those fed only GRN. Steers fed MON had greater DMI during the finishing phase than the other treatments (MON × GRN: $P \leq 0.03$). Overall, steers fed CON diet had a greater day-by-day dry matter intake (DMI) variation than other treatments (MON × GRN: $P \leq 0.05$). The GRN inclusion in the diet improved the feed efficiency in 5.7% (P = 0.04). Steers fed diets without GRN inclusion tended to had 17.8% additional blood urea concentration (P = 0.06). D-lactato and glucose levels were similar between treatments ($P \geq 0.21$). The GRN use tended to increase (P = 0.09) the DM digestibility in ~5.6%. The microbial crude protein was reduced by MON + GRN association (MON × GRN: P = 0.02). The hot carcass weight was greater for GRN group compared to others (MON × GRN: P = 0.02). The use of GRN in the diet increased ($P \leq$ 0.04) the total and daily carcass gain, carcass yield and biological efficiency. In summary, these data indicate that the associated use of monensin with GRN should be avoided in finishing diets. The GRN technology showed be a promising technology to be used to boost muscle hypertrophy and carcass production of beef cattle.

Key-words: biological efficiency, feedlot, hypertrophy, nitrogen metabolism, ruminal pH.

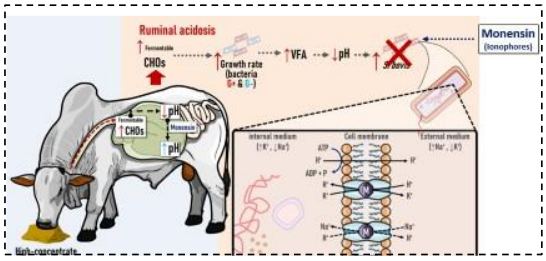
RESUMO

Este estudo teve como objetivo avaliar os efeitos de um suplemento de nitrogênio (N) liberado ao longo do tempo (Timafeed Boost®, Grupo Roullier, Saint-Malo, França) associado ou não à monensina sobre o desempenho, metabolismo e qualidade da carne de novilhos em terminação. Para investigar esses resultados, cento e doze novilhos Nelore (380 kg ± 16,2) foram utilizados em arranjo fatorial 2 × 2. Os novilhos foram alocados em 28 baias (quatro animais por baia) e os seguintes tratamentos foram distribuídos aleatoriamente nas unidades experimentais: (1) Controle (CON, n = 7) - dieta de terminação sem aditivos; (2) Dieta enriquecida com monensina (MON, n = 7) - monensina (Rumensin®, Elanco Animal Health, Greenfield, IN) fornecida no nível de 30 mg por kg de matéria seca (MS); (3) Dieta enriquecida com liberação gradual de N (GRN, n = 7) - suplemento de liberação gradual de N (Timafeed Boost®, Roullier Group, Saint-Malo, FR) fornecido na dose de 250 g por animal por dia; ou (4) Monensina + dieta de liberação gradual de N (MON + GRN, n = 7) - monensina (30 mg por kg de MS) associada a produto de liberação gradual de N (250 g por animal por dia). O período experimental compreendeu 102 dias, sendo os primeiros 15 dias destinados à adaptação à dieta. O ganho médio diário (GMD) ao longo do período experimental foi reduzido para novilhos alimentados com GRN + MON (MON × GRN: $P = 0,02$) em comparação com aqueles alimentados apenas com GRN. Novilhos alimentados com MON tiveram maior CMS na terminação do que os demais tratamentos (MON × GRN: $P \leq 0,03$). No geral, os novilhos alimentados com a dieta CON tiveram uma variação diária maior do consumo de matéria seca (CMS) do que os outros tratamentos (MON × GRN: $P \leq 0,05$). A inclusão de GRN na dieta melhorou a eficiência alimentar em 5,7% ($P = 0,04$). Novilhos alimentados com dietas sem inclusão de GRN tenderam a ter 17,8% de concentração adicional de uréia no sangue ($P = 0,06$). Os níveis de D-lactato e glicose foram semelhantes entre os tratamentos ($P \geq 0,21$). O uso de GRN tendeu a aumentar ($P = 0,09$) a digestibilidade da MS em ~5,6%. A proteína microbiana bruta foi reduzida pela associação MON + GRN (MON × GRN: $P = 0,02$). O peso da carcaça quente foi maior para o grupo GRN em relação aos demais (MON × GRN: $P = 0,02$). A utilização de GRN na dieta aumentou ($P \leq 0,04$) o ganho de carcaça total e diário, o rendimento de carcaça e a eficiência biológica. Em resumo, esses dados indicam que o uso associado de monensina com GRN deve ser evitado em dietas de terminação. A tecnologia GRN mostrou ser uma tecnologia promissora a ser utilizada para aumentar a hipertrofia muscular e a produção de carcaça de bovinos de corte.

Palavras-chave: eficiência biológica, confinamento, hipertrofia, metabolismo do nitrogênio, pH ruminal.

Similarities of monensin and slow-release N

In Brazil, sodium monensin is the main additive used in feedlot diets. Several studies have shown the effects of monensin on the N metabolism. In general, monensin use is associated to reduced N-NH3 concentration in the rumen, buffer power capacity, reduction in the concentration of ammonia-producing bacteria, decreases acetate: propionate ratio, decreases protozoa in the rumen. The slow-release N supplementation and monensin can provide some similar benefits for the ruminant animals through the modes of action of these supplements/additives, such as reduction of N-NH3 concentration, buffering capacity building, reduction in acetate: propionate ratio.



Effects on metabolism	Monensin	N slow release
Buffer power capacity	+	+
Reduces N-NH_3 concentration	+	+
Reduction in the concentration of ammonia-producing bacteria	+	+
Decreases acetate: propionate ratio	+	+
Decreases protozoa in the rumen	+	+

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SUMARY

1. Introduction

In the last two decades, the productivity of Brazilian beef cattle increased from 4.9 to 9,7 million tons of carcass equivalent (TCE). Of the total produced, 2.4 million TCE were destined for the foreign market and 7.2 million TCE for the domestic market (Athenagro/IBGE/ABIEC, 2022). The increase in Brazilian productivity was associated with an increased static capacity of feedlots and greater production system efficiency. Due to the current rise in the static capacity of feedlots for beef cattle production, the use of diets with a high proportion of forage became unfeasible due to its low energy content and due to the great land needs for planting (Machado et al., 2012).

On the other hand, the increase in energy density from the high inclusion of concentrate in feedlot diets improved feed efficiency in beef cattle (Keane et al., 2006). In this context, approximately 63.9% of Brazilian feedlots use diets with concentrate inclusions ranging between 81 and 90% (Silvestre & Millen, 2021). However, the risk of animals suffering metabolic disorders in such scenarios as clinical and subclinical acidosis increases (Valente et al., 2017). The acidosis onset is related to the limited ability to remove volatile fatty acids (VFA) from the ruminal fermentation process, which in turn causes their accumulation in the ruminal environment and leads to a pH reduction (Pan et al., 2016). As a way to control the negative effects of VFA production on the rumen pH, ionophores have been included in high concentrate levels diets, aiming to modulate the rumen environment by directing and altering the bacterial metabolism of gram-positive bacteria, such as cellulolytic, proteolytic, methanogenic, and lactate producing microorganisms (Ensley, 2020).

Feed additives are an important dietary tool to increase efficiency and profitability in grazing and feedlot systems (Bretschneider et al., 2008). Ionophores can provide rumen dynamics with a more efficient fermentation route, altering the rumen environment and ecosystem and reducing the substrate for methanogenic archaea (Marques & Cooke, 2021). Another notable effect of ionophores is the mitigation of ruminal proteolysis and the subsequent reduction in ammonia synthesis (Marques & Cooke, 2021).

Soybean meal is the most protein source in feedlot diets (Silvestre & Millen, 2021). However, due to the high soybean meal cost, the use of non-protein nitrogen (NNP) sources has been increased in the feedlot diets. The use of NNP sources can provide economic benefits based on its low cost to increase the synthesis of ruminal

microbial crude protein (MCP) (Strom & Øskov, 1984). The ruminants are the only species that, through the rumen microbiota, can convert NNP into a protein of high biological value (Rennó et al., 2000). However, the rapid release of ammonia nitrogen ($N\text{-}NH_3$) from NNP compared to the rate of its utilization by microorganisms depends on the availability of energy in the rumen (Russell et al., 1992). Excess ruminal $N\text{-}NH_3$ can negatively affect animal performance, besides the risk of ammonia toxicity (Huntington et al., 2006). Thus, the use of slow-release N supplements can improve the synchrony between the point of highest carbohydrate fermentation and $N\text{-}NH_3$ concentration in the rumen, which leads to maximized microbial growth and efficiency of feed utilization. Therefore, the replacement of conventional urea by controlled-release urea may be an alternative to improve the synchronization of $N\text{-}NH_3$ and energy in the rumen, from the reduction of $N\text{-}NH_3$, and by assigning favorable conditions to ruminal fermentation, microbial protein synthesis, pH, digestibility, and intake (Benedeti et al., 2014).

On the other hand, the ionophores inclusion in the feedlot diets can modify the animals performance due to the decrease in ruminal proteolysis (Chalupa et al., 1980). In Brazil, sodium monensin is the main additive used in feedlot diets, followed by virginiamycin, salinomycin, and the association between monensin and virginiamycin (Silvestre & Millen, 2021). Several studies have shown the effects of monensin on the N metabolism (Russell & Strobel, 1989; Schelling, 1984). In general, monensin use is associated to reduced $N\text{-}NH_3$ concentration in the rumen, buffer power capacity, reduction in the concentration of ammonia-producing bacteria, decreases acetate: propionate ratio, decreases protozoa in the rumen.

The slow-release N supplementation and monensin can provide some similar benefits for the ruminant animals through the modes of action of these supplements/additives, such as reduction of $N\text{-}NH_3$ concentration, buffering capacity building, reduction in acetate: propionate ratio (**Table 1**).

Table 1. *Similarities of monensin and slow-release N.*

Effects on metabolism	Monensin	N slow release
Buffer power capacity	Dennis et al., 1983; Duffield et al., 2012; Tedeschi et al., 2003	Benedeti, 2012; Puga et al., 2001
Reduces N-NH_3 concentration	Schelling, 1984	Taylor-Edwards et al., 2009; Marques & Cooke, 2021
Reduction in the concentration of ammonia-producing bacteria	Strobel and Russell, 1986	Ferme et al., 2004
Decreases acetate: propionate ratio	Nagaraja, 1995; Azzaz et al., 2015	Seal & Parker, 1994; Puga et al., 2001
Decreases protozoa in the rumen	Dinius et al., 1976; Richardson et al., 1976	Roullier, 2021

Hypothesis

Was hypothesized that there is an associative effect between the use of monensin and gradual-N-release products in the ruminant nutrition. Therefore, this study aimed to evaluated if the effects of a gradual N release ingredient (Timafeed Boost®, Roullier Group, Saint Malo, France) on the performance, metabolism and meat quality of beef cattle might be affected by the presence or absence of the monensin in the diet.

Objective

To evaluate the performance, metabolism, carcass characteristics and meat quality in Nellore steers fed diets containing a standard additive used in most beef production systems in Brazil (Monensin) and/or a source of gradual release of N, or no additive.

2.Background

2.2 Nitrogen metabolism in cattle

From the single crude (CP) fraction, more complex systems were developed by nutritional requirements models based on rumen-degradable (RDP) and non-degradable (RUP) protein (AFRC, 1989; NRC, 1996). N metabolism can be divided into two distinct events: protein degradation and microbial protein production, and the rate and extent to which protein degradation occurs will be dependent to the proteolytic activity of ruminal microorganisms and the type of protein (Bach et al., 2005).

The degradation of TP in the rumen is dependent of several microorganisms that contribute to the hydrolysis of peptide bonds by enzymes (Walker et al., 2005). Subsequently, oligopeptides are released for the catabolization of smaller peptides and amino acids (AAs). Amino acids are degraded and can be incorporated into microbial crude protein (MCP) or deactivated in VFAs, CO_2, and ammonia (Tamminga, 1979).

Amino acids can be transaminated or used directly for microbial protein synthesis when energy is available. Otherwise, when there is an energy deficiency, the AAs are deaminated and the carbon skeletons are fermented into VFAs (Bach et al., 2005) After protein hydrolysis, the free oligopeptides are catabolized into peptides and AAs, the latter being deaminated releasing NH_3, or incorporated directly into the microbial protein (Batista, 2015.). Other nitrogen sources used by microorganisms are NNP extracted from NH_3, AAs, small peptides, urea, DNA, and RNA (Bach et al., 2005).

The characteristics of the protein source for rumen microorganisms and the method of urea supplementation result in the amount of N-NH_3 varying over a wide range. N-NH_3 concentrations in the rumen can be expected to change rapidly, even when animals have continuous access to feed (Nolan & Leng, 1972). Satter & Slyter, 1974 suggested that the maximum rate of microbial synthesis occurs at concentrations of N-NH_3 between 5 and 8 mg N / 100 ml, while other researchers have suggested that diet influences the optimal level of N-NH_3. Studies by Leng; Nolan, 1984 observed that the value can be as high as 15-20 mg N/100 mL depending on the diet. In case of excess N by ruminal microorganisms, the protein is degraded to ammonia, absorbed, metabolized to urea in the liver, and lost in the urine, or it can be recycled, contributing to inefficient N retention and dietary N utilization (Walker et al., 2005; Bach et al., 2005).

The requirements for good N metabolism of rumen microorganisms can be summarized as follows: Necessary ruminal ammonia concentrations of 5 to 11 mM were

to maximize rumen microbiota N fluxes depending on diet and fermentation conditions at optimal ammonia concentration ruminal appears to be diet-dependent and influenced by factors such as N source and carbohydrate fermentability. Possibly factors affecting pass rates (eg CMS); increasing ruminal ammonia concentrations result in increased ruminal N losses; ammonia concentrations needed to maximize ruminal organic matter (OM) digestion were at least as high as those needed to maximize microbial protein synthesis; not only was the average ammonia concentration important, but also the time the concentration dropped below some critical level; and higher ruminal ammonia concentrations may be required if more easily fermentable carbohydrates were fed (Schwab et al., 2005).

Two interconnected pathways are used for N-urea metabolism. The first route is hydrolysis, which is necessary for the release of N from the compounds to the rumen environment. Second, assimilative and biosynthetic pathways produce amino acids and peptides used by the cell (Arriaga et al., 2009).

The evolutionary advantage of ruminant animals is the recycling of urea to the rumen. Part of the urea in the blood can be recycled to the rumen through saliva and mainly by the ruminal epithelium, which can also be used for microbial growth (Batista, 2015). This process plays a vital role in N utilization and metabolism in ruminants (Reynolds & Kristensen, 2008; Wang et al., 2011; Zhou et al., 2017). Transport of hepatic urea to the rumen through the intestinal epithelium occurs where ureases are located (Hailemariam et al., 2021).

The protein microbial contributes between 50 and 80% of the protein absorbed in the small intestine, although it is characterized as a relatively high proportion of non-protein nitrogen (NNP), which represents two-thirds of the amino acids absorbed by ruminants (AFRC 1992). The factors that most affect microbial protein degradation are the type of protein, interactions with other nutrients, and the predominant microbial population, which is dependent on the composition of diet ingredients, ruminal passage rate, and rumen pH (Bach et al., 2005).

The limitation of microbial growth through diets with a high energy density that induces a decrease in ruminal pH may be a result of low concentrations of peptides and AAs (Demeyer & Fievez, 2004). Cardozo et al., 2005, comparing diets with high forage content and one with high concentrate content, observed that protein degradation was reduced as the pH decreased in both diets.

Amylolytic bacteria tend to degrade more proteins than cellulolytic ones (Siddons & Paradine, 1981;Wallace et al., 1997). However, in the study by Cardozo et al., 2005, proteolysis was lower for diets with high concentrate content. Diet pH and composition directly affect rumen protein degradation (Lana et al., 1998; Bach et al., 2005). Total microbial N flux is negatively correlated with rumen pH, but there is no relationship between rumen pH and microbial efficiency synthesis (EMPS) (Bach et al., 2005).

In addition to bacteria, other microorganisms such as protozoa have a direct role in the digestion of proteins and carbohydrates. The supply of protozoan proteins to the small intestine is limited, being around 11% of the total flow of CP (Shabi et al., 2000). The result of defaunation causes a decrease in proteins and concentrations of peptides and AAs in the rumen (Ivan et al., 1992).

Dietary needs in the NRC (2001) are expressed in RPD and RUP, and metabolic needs are expressed in Metabolizable Protein (MP) (Schwab et al., 2005). The balance between the proportions of RUP and RPD, can control the degradation of proteins and supply of fermentable energy, or modify the profile of AA that arrives in the intestine from the diet to optimize the microbial fermentation and the flow of N to the intestine has been researching results (AFRC, 1993; NRC, 2001; INRA, 2007). It is understood that the AA composition and the intestinal digestibility of the RUP will determine its nutritional value for the animal (Schwab & Broderick, 2017).

2.2 Ionophore additives

Ruminant nutritionists and microbiologists have been challenged to modify the rumen microbial ecosystem to maximize productivity (Nagaraja & Taylor, 1987) Ionophores are the most studied and used feed additives in the diet of cattle, mainly to modify the rumen environment (Weimer et al., 2008). In general, these highly lipophilic molecules (Pressman, 1976) have a greater ability to adhere to the membranes of gram-positive bacteria (absence of the outer membrane) and protozoa, which determines the vulnerability of microorganisms in the gastrointestinal tract (Russell & Houlihan, 2003).

The modification of the movement of ions across biological membranes, and consequently the responses of animals, are the result of the basic mode of action of ionophores (Schelling, 1984). In more detail, ionophores insert themselves into the lipid membrane of rumen bacteria through the exchange of H^+ between Na^+ and K^+, interrupting intracellular and extracellular ionic balance, decreasing intracellular K^+ and pH, in addition to increasing intracellular Na^+ (Chen & Russell, 1989) (**Figure 1**).

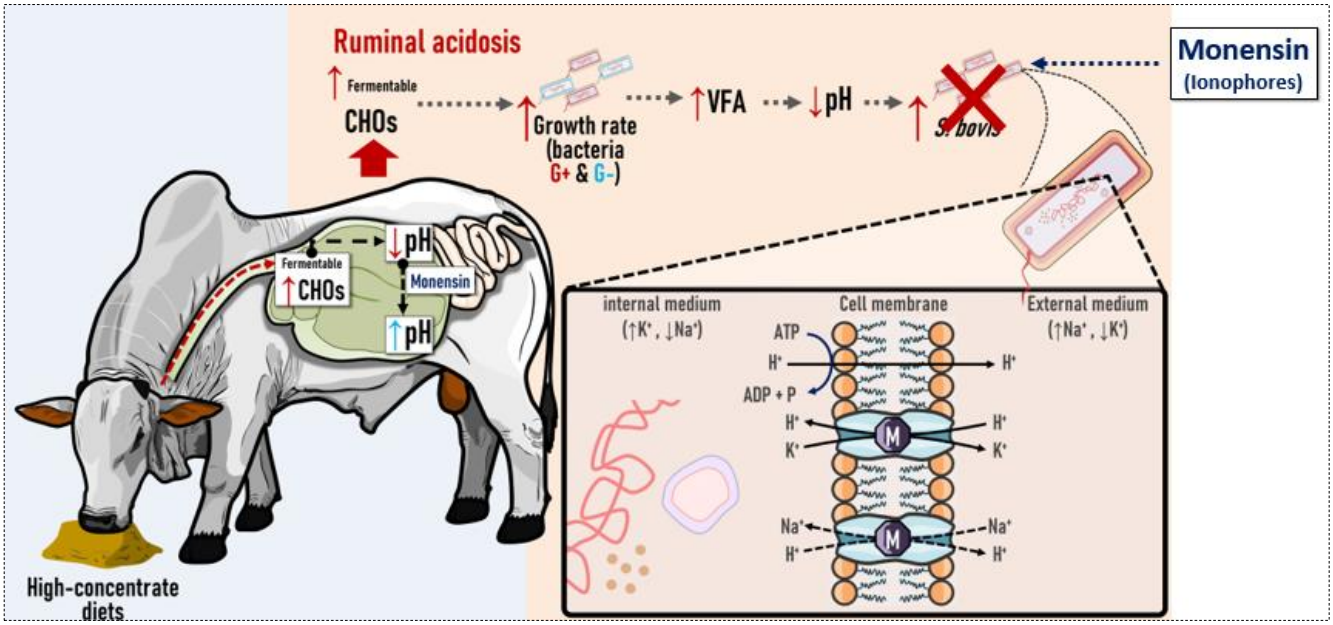

Figure 1. Monensin effects on the ruminal bacteria.

However, each molecule of the ionophore group has an affinity for specific ions, having a preference index for ion binding (Painter & Pressman, 1982) (**Table 2**).

Table 2. Characteristics of ionophores and selectivity in ion binding. Source: Marques and Cooke (2021) (adapted from Nagaraja, 1995)

Ionophore	Made by	Molecular weight	Ion selectivity sequence
Monensin	*Streptomyces cinnamonensins*	671	$Na^{+}> K^{+}, Li^{+}> Rb^{+}> Cs^{+}$
Lasalocide	*Streptomyces lasaliensis*	591	Ba^{++}, $K^{+}> RB^{+}> Na^{+}> Cs^{+}> Li^{+}$
Narasin	*Streptomyces aureofaciens*	765	$Na^{+}> K^{+}, Rb^{+}, Cs^{+}, Li^{+}$
Salinomycin	*Streptomyces albus*	751	Rb^{+}, $Na^{+}> K^{+}> Cs^{+}$, Sr^{+}, Ca^{++}, Mg^{+}

Additives have been commonly classified as optimizing propionate production or causing a decrease in methane production, deamination, and lactic acid production (Schelling, 1984). These modes of action occur through altering the rumen microbiome,

optimizing fermentation pathways, and reducing metabolic disorders (Marques & Cooke, 2021).

Feed additives such as lasalocid, monensin, salinomycin, laylomycin, virginiamycin, and narasin are available on the market, showing similar mechanisms of action in the rumen, while animal performance is dose, animal, and diet-dependent (Marques & Cooke, 2021). In this theoretical framework, we will focused on the Monensin action mode.

Richardson et al., 1976 observed that monensin and rumen microorganisms readily demonstrated a decrease in the acetate:propionate ratio. This mode of action increases the use of feed energy, as it has greater efficiency in the use of propionate, which is a precursor for gluconeogenesis, and a decrease in acetate and butyrate, responsible for the formation of methane (Bretschneider et al., 2008). The NASEM nutritional requirements model (2016) points out that the metabolizable energy (ME) of the diet increases by 2.3% and 1.5% when monensin or lasalocid is included in the diets of beef cattle, respectively.

Chen & Russell, 1989 observed that monensin reduced the concentration of ammoniacal N, through the inhibition of a small group of rumen bacteria called hyper-producing ammonia. Two species of gram-positive microorganisms, Peptostreptococcus and Clostridium, which produce high concentrations of ammonia in the rumen, are limited by ionophores, thus reducing the deamination of dietary protein (Russell & Strobel, 1988). This ensures that monensin directly influences rumen nitrogen metabolism by decreasing rumen proteolysis (Goodrich et al., 1984). Monensin decreases the contribution of bacterial N and increases the amount of non-degraded dietary N in the rumen, destined for the abomasum (Faulkner et al., 1985). The decrease in RPD by RUP can potentially have negative effects on rumen microorganisms, reducing the availability of N for microbial growth. However, the replacement of RPD by RUP may affect the recycling of urea to the rumen, which may compensate for the reduced availability of N (Carneiro de Souza et al., 2021; Titgemeyer, 2017).

Linear growth of rumen bacteria in response to carbohydrate fermentation may be a consequence of the increased availability of peptides and ammonia (Argyle et al., 1989). Consequently, the lower ammonia synthesis due to the reduction of ruminal proteolysis caused by ionophores increases the protein flow to the small intestine, which can bring improvements in the productive efficiency of beef cattle (Marques & Cooke, 2021).

2.3 Slow-release nitrogen

Strategies for cost reduction in diets and the optimization between the energy and protein balance of the feed have been widely studied. Soybean meal (SM) is the main source of protein for ruminant animals, however, the high cost of this ingredient and the margin of profitability of production systems increasingly limits its inclusion in the diet (CHALUPA, 2007). On the other hand, feedlot diets containing high starch content with high rates of rumen fermentation and urea hydrolysis to form ammonia may result in asynchrony by rumen microorganisms (Bourg et al., 2012).

The use of non-protein nitrogen (NNP) sources such as urea can be good alternatives compared to protein feeds with high degradability (Wanapat et al., 2009); (Xin et al., 2010). However, when the rate of degradation to ammonia exceeds the rate at which rumen bacteria transform N into MICP, this compound accumulates and escapes from the rumen (Satter & Slyter, 1974). This fact is because fibrolytic bacteria are more efficient in the degradation of urea into ammonia (Tedeschi et al., 2002). Additionally, urea can only be used as a source of N when there is the availability of fermentable carbohydrates in the rumen for MICP synthesis (Abreu, 2010).

Controlling the rate of ammonia release in a way that is similar to the fermentation of carbohydrates is a strategy to reduce N excretion and improve nutrient utilization (Pinos-Rodríguez et al., 2010). In this context, developing products that delay ruminal N-NH_3 release without limiting the extent of urea degradation in the rumen is a challenge (Cherdthong & Wanapat, 2010).

The development of slow-release urea compounds for ruminants initially started from urea compounds with the inclusion of biuret, ammonia, urea phosphate, oil-based coatings, formaldehyde-treated urea, and polymer-coated urea (Taylor-Edwards et al., 2009). However, these compounds were not effective, due to a substantial part of the NNP escaping from the rumen without being converted into NH_3, reducing the synthesis of MICP (Galina et al., 2003). Urea bound to substrates such as calcium chloride was proposed by the studies by (Huntington et al., 2006) to control the rate of NH_3 released from urea. The mixture of urea and calcium sulfate reduced ruminal ammoniacal nitrogen concentrations and improved the microbial population compared to conventional urea (Cherdthong & Wanapat, 2010).

Some authors have investigated the substitution of slow-release nitrogen sources for conventional urea and vegetable protein sources. With the addition of 10, 20, and 30% of controlled-release urea (**CRU**) in the study by (Puga et al., 2001) it was observed that

there was a determination between pH and molar concentrations of volatile fatty acids (**VFA**) in the rumen, together with the N-NH_3 concentration.

In a study performed by Taylor-Edwards et al. (2009) with multicatheterized steers, the intraluminal dosage of slow-release urea avoided the peak of ammoniacal N concentration about conventional urea dosage. The controlled and progressive release of protected NNP compared to urea decreases rumen N-NH_3 concentrations and may prolong microbial utilization of additional N sources during fermentation, allowing better synchrony with carbohydrate availability resulting in the greater MICP synthesis (Cherdthong & Wanapat, 2010).

Galina et al., 2003 a study with 60 Zebu steers fed with sugar cane and corn tips, and supplemented with slow-release urea, found increases in ADG, DM intake, DM digestibility, and reduction in the acetate: propionate compared to the control treatment. Additionally, Galina et al. (2003) using polymer-coated slow-release urea (Optigen; CPG Nutrients, Syracuse, NY), and also Xin et al., (2010) with polyurethane-coated urea, obtained higher results in digestibility of MS compared to conventional urea. On the other hand, supplementation with slow-release urea can improve MICP synthesis capacity, improving its conversion efficiency into milk (Galina et al., 2003); Broderick et al, 2009). Moretti (2010) observed that the optimal point of substitution aiming at the maximum performance of Nellore cattle reared on pasture was 35% of the crude protein source of vegetable origin.

In a meta-analysis, Salame et al., (2020) showed that slow-release N (Optigen) supplementation increased live weight gain and feed efficiency under several study factors. Supplementation of U-cas at 180 g/kg DM improved N utilization, total CP, and PGlu, while it did not adversely affect on hematological parameters (Cherdtong et al., 2013).

The use of slow-release nitrogen supplements such as Timafeed Boost can improve the use of the diet, reducing losses because it is a homogeneous organic-inorganic matrix, containing yeast, releasing N in a controlled and progressive way. In in vitro assays (Roullier, 2021) with Timafeed Boost carried out by CMI Roullier[1], a 44% decrease in the production of N-NH_3 was observed, reducing the risk of hepatic damage

[1] The Centre Mondial de l'InnovationRoullier (CMI Roullier) is a global innovation and research center of the Roullier group dedicated to the development and prospection of industrial and scientific solutions in the areas of animal and plant nutrition. CMI Roullier is headquartered in the city of Saint Malo, France.

and also the reduction of protozoa population responsible for the formation of methane and ammonia in the rumen fluid. Additionally, there was a significant increase in the proportion of propionate and pH of the rumen fluid by 0.73 points compared to soybean meal. This increase in pH under in vitro conditions was observed from the greater presence of basic NH_4^+.

2.4 Use of yeasts in ruminant nutrition

Yeasts have been used as feed additives for ruminants to promote better production performance to generate products (increased growth rate, meat and milk) and to decrease the risk of acidosis, thus improving animal health and welfare (Chaucheyras-Durand et al. 2012 ; Vohra et al. 2016).

Stabilization of the rumen microbiota, maintaining an optimal pH, increasing the formation of ruminal fermentation end products and improving the use of ammonia by rumen bacteria are contributions of the use of yeast as a natural food additive (Chaucheyras-Durand et al. 2012). The inclusion of yeast in the diet of beef cattle has been associated with increases in digestibility (Ovinge et al., 2018, Cagle et al., 2019, Peng et al., 2020).

The addition of the yeast product may increase blood urea nitrogen (BUN), due to the greater digestibility of CP (Batista et al., 2022). Schen et al., (2019) observed a linear increase in BUN with the addition of the fermentation product of S. cerevisiae and suggested an increase in intestinal absorption of the amino acid (AA). In a study evaluating the effects of Saccharomyces cerevisiae and monensin with different concentrations of starch Monnerat et al (2013) observed an increase in the concentration of volatile fatty acids for monensin and yeast (1g), and a higher concentration of ruminal N-NH3 for a rich diet in concentrate when the combination was used.

In a meta-analysis proposed by Sartori et al. (2017) suggested that the addition of yeast to rations fed to beef cattle could reduce dry matter intake (DMI), but it does not have significant effects on average daily gain, which could increase feed efficiency. The use of Saccharomyces cerevisiae fermentation products for feedlot beef cattle increased final body weight, average daily gain (ADG), dry matter intake (DMI) and feed efficiency (FE) (Wagner et al. 2016).

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SECOND SECTION - ARTICLE

ARTICLE 1: Supplementation with a slow-release nitrogen ingredient associated or not with monensin on performance, metabolism, and meat quality of finishing beef cattle fed high starch diets

Article formatted according to Animal guidelines

ABSTRACT

This study aimed to assess the effects of a time-released nitrogen (N) supplement (Timafeed Boost®, Roullier Group, Saint-Malo, France) in association or not with monensin on the performance, metabolism, and meat quality of finishing steers. To investigate these outcomes, one hundred and twelve Nellore steers (380 kg ± 16.2) were used in a 2 × 2 factorial arrangement. Steers were allocated in 28 pens (four animals per pen) and the following treatments were randomly assigned to the experimental units: (1) Control (CON, *n* = 7) - finishing diet without additives; (2) Monensin-enriched diet (MON, *n* = 7) - monensin (Rumensin®, Elanco Animal Health, Greenfield, IN) provided at level of 30 mg per kg of dry matter (DM); (3) Gradual-N-release enriched diet (GRN, *n* = 7) - gradual-N-release supplement (Timafeed Boost®, Roullier Group, Saint-Malo, FR) provided at dose of 250 g per animal per day; or (4) Monensin + Gradual-N-release diet (MON + GRN, *n* = 7) - monensin (30 mg per kg of DM) associated to gradual-N-release product (250 g per animal per day). The experimental period comprised 102 days, being the first 15 days designated for the diet adaptation. The average daily gain (ADG) over the experimental period was reduced for steers fed GRN + MON (MON × GRN: *P* = 0.02) compared to those fed only GRN. Steers fed MON had greater DMI during the

finishing phase than the other treatments (MON × GRN: $P \leq 0.03$). Overall, steers fed CON diet had a greater day-by-day dry matter intake (DMI) variation than other treatments (MON × GRN: $P \leq 0.05$). The GRN inclusion in the diet improved the feed efficiency in 5.7% ($P = 0.04$). Steers fed diets without GRN inclusion tended to had 17.8% additional blood urea concentration ($P = 0.06$). D-lactato and glucose levels were similar between treatments ($P \geq 0.21$). The GRN use tended to increase ($P = 0.09$) the DM digestibility in ~5.6%. The microbial crude protein was reduced by MON + GRN association (MON × GRN: $P = 0.02$). The hot carcass weight was greater for GRN group compared to others (MON × GRN: $P = 0.02$). The use of GRN in the diet increased ($P \leq 0.04$) the total and daily carcass gain, carcass yield and biological efficiency. In summary, these data indicate that the associated use of monensin with GRN should be avoided in finishing diets. The GRN technology showed be a promising technology to be used to boost muscle hypertrophy and carcass production of beef cattle.

Key-words: biological efficiency, feedlot, hypertrophy, nitrogen metabolism, ruminal pH.

1. INTRODUCTION

The microbial protein is the main source of metabolizable protein for cattle, and both, true protein or non-protein nitrogen (NPN) can be used for microbial crude protein (MCP) synthesis (Lu et al., 2019). In this sense, the formulation of diets considering the use of NPN sources, such as urea, is economically attractive, representing an opportunity to reduce the dietetic (Salami et al., 2021). In the ruminal environment, NPN sources are metabolized and the available ammonia can be used for intracellular synthesis of amino acids (Dijkstra et al., 2005). Carbohydrates are the most essential factor in the nitrogen use in the rumen, once they provide the carbon skeletons and energy necessary for

ammonia fixation (Bach et al., 2005). Nevertheless, there is an asynchrony between carbohydrate and urea degradation rates, which in turn, impair the NPN incorporation into MCP and reduce the nitrogen use efficiency (Taylor-Edwards et al., 2009). While dietary urea is quickly solubilized and rapidly hydrolyzed into ammonia, ruminal carbohydrate degradation occurs in a slower manner, resulting in a greater ammonia absorption through the rumen wall and in lower MCP synthesis (Gallo et al., 2022). Therefore, the use of commercial products with gradual-N-release properties may optimize the nitrogen conversion into bacterial protein, because ammonia is released more closely parallels to carbohydrate (Cherdthong and Wanapat, 2010). Moreover, its use promotes additional benefits, such as improved animal performance, enhanced profitability and reduced environmental impacts (Salami et al., 2020).

Nevertheless, despite the use of gradual-N-release products representing a promising technology for livestock industry, little efforts were done concerning how these products interact with dietary ionophores, such as monensin. Monensin is widely used to prevent digestive upsets in diets rich in rapidly fermentable carbohydrates (Lemos et al., 2016), routinely used in feedlots. This antimicrobial feed additive presents a selective bacteriostatic effect, modulating the ions transport across the bacteria cell wall, which impairs the gram positive bacteria survival (such as cellulolytic, proteolytic, methanogenic and lactate-producing bacteria) in detriment to gram negative bacteria (Duffield et al., 2008; Marques and Cooke, 2021). In addition, it is well-established that this ionophore decreases the ruminal proteolysis (Marques and Cooke, 2021), and the ruminal NH_3 concentration (Rezaei Ahvanooei et al., 2023), increasing the protein flow to the small intestine.

In this sense, aiming to simulate a real condition verified in beef cattle production, it was hypothesized that there is an associative effect between the use of monensin and

gradual-N-release products in the ruminant nutrition. Therefore, this study aimed to evaluated if the effects of a gradual N release ingredient (Timafeed Boost®, Roullier Group, Saint Malo, France) on the performance, metabolism and meat quality of beef cattle might be affected by the presence or absence of the monensin in the diet.

2. MATERIAL AND METHODS

This work was performed in a commercial feedlot (Confinamento Nosso Pai, Extrema, Minas Gerais, Brazil). All experimental procedures followed the guidelines established by the Ethics Committee on Animal Use of Animal Nutri Ciência e Tecnologia. Additionally, this study was performed in accordance with Brazilian legislation for scientific animal use (law nº 11.794).

2.1 Animals, housing and diets

One hundred and twelve Nellore steers (*Bos taurus indicus*), with an initial body weight (BW) of 380 kg (± 16.2 kg) were used. After arriving at the feedlot, animals were dewormed with Iver-Vet® 3.5% (Biovet, Vargem Grande Paulista, SP, Brazil), allocated in groups of 4 animals per pen, and adapted to the feedlot facilities. Pens (4 × 4 × 14 m) were individually delimited with wooden stakes and wire and equipped with individual troughs and drinkers. Subsequently, each experimental unit (pen) was randomly assigned to one of the following treatments: (1) Control (CON, $n = 7$) – finishing diet without ionophore or gradual-N-release inclusion; (2) Monensin-enriched diet (MON, $n = 7$) - finishing diet plus monensin (Rumensin®, Elanco Animal Health, Greenfield, IN) provided at the level of 30 mg per kg of dry matter; (3) Gradual-N-release enriched diet (GRN, $n = 7$) - finishing diet plus a commercial product with gradual-N-release (Timafeed Boost®, Roullier Group, Saint-Malo, FR) provided at dose of 250 g per animal per day;

or (4) Monensin + Gradual-N-release diet (MONGRN, *n = 7*) – finishing diet plus monensin (30 mg per kg of DM) associated to gradual-N-release product (250 g per animal per day).

The experimental period comprised 102 days. The finishing diet used during the experimental period was formulated according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho, 2016), and was based on 29% of corn silage and 71% of concentrate [on a dry matter basis (DM)] provided as total mixed ration (Table 1). Timafeed Boost consisted of urea added to an organic matrix with industrial treatment for gradual release in the rumen, plus distillers' dried grains and solubles, concentrated distillers solubles, sodium sulfate, ammonium sulfate and yeasts. The Timafeed Boost and Monensin doses were established according to manufacturer's recommendations of use. The first 15 days of the experimental period were designated to the animal's adaptation to the experimental diets. The diet adaptation was performed gradually through the step-up protocol (three intermediate diets with 25%, 50% and 75% of finishing diet concentrate level, supplied for 5 days each). During all experimental periods, steers were fed twice daily, at 0730 a.m. and 0400 p.m.

2.2. Performance measurements

The first and last measurements of the beef steers' body weight (performed at day one and day 102 of the experimental period) were performed considering a 16 hours of fasting, to determine the steers shrunk body weight. The other BW measurements were performed without fasting. This approach was adopted aiming to improve the experimental accuracy of performance data related to the gastrointestinal content adjustments on the final BW.

2.3 Intake and feed efficiency

Every day before feeding, the refusals of each pen were visually evaluated to assign a score from - 1 to 2, in which: (- 1) trough without orts and with saliva evidence;

(0) trough containing orts around 2% and 5% of the diet offered in the previous day; (1) trough containing orts around 10% of the diet offered in the previous day; (2) trough containing orts around 20% of the diet offered in the previous day. This score was used as an adjustment factor of the amount of diet provided daily. In each case, the following management was adopted: (-1) 10% increase in the daily TMR amount supplied; (0) maintenance of the supplied TMR; (1) 5% reduction in the daily TMR amount and (2) 20% reduction in the daily TMR amount.

The daily dry matter intake per pen was calculated as the differences between TMR provided and orts obtained. Also, a day-to-day variation in the matter intake (DMI_{var}) per pen was estimated as proposed by Bevans et al. (2005), as follows:

$$DMIvar\ (\%) = \frac{(CMS\ current\ day - CMS\ previous\ day)}{DMI\ previous\ day} \times 100 \qquad \text{Eq. (1)}$$

The feeding efficiency was calculated as the ratio between the average daily gain (ADG) and the dry matter intake.

2.4. Blood parameters

Blood samples was taken before the morning feeding (0600 a.m.) from the jugular vein from the heaviest animal of each pen on day 65 of the experimental period. For blood collection, commercial tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, USA) with anticoagulant were used. After blood collection, samples were centrifuged (2,500 × g for 30 min at 4°C), transferred to Eppendorf tubes and stored at -80°C until analysis. Blood samples were analyzed for D-lactate, urea and glucose concentration. D-lactate concentration was measured by calorimetric assay using the Sigma-Aldrich kit (number MAK058; Sigma-Aldrich®, St Louis, MO, USA). Briefly, in this assay, D-Lactate was oxidized by D-Lactate hydrogenase, generating a proportional colorimetric product, which in turn was measured at 450 nm. Glucose concentration was determined

by the colorimetric method according to the manufactures recommendation, using a Labtest® commercial kit (number 133, Labtest®, Lagoa Santa, Brazil). In summary, the principle of glucose determination consisted in the catalysis of glucose oxidation by glucose oxidase. The hydrogen peroxide formed when reacting with 4-aminoantipyrine and phenol formed a red antipyrilquinonimine, whose color intensity was proportional to the glucose concentration in the sample. Urea blood concentration was determined by the enzymatic method, using the Bioclin commercial kit (number K056, Química Básica Ltda, Belo Horizonte, MG, Brazil). In this analysis, urea was hydrolyzed by urease, releasing ammonia and CO_2. Ammonia, in turn, reacted with 2-oxoglutarate and NADH (via catalysis by glutamate dehydrogenase) with the oxidation of NADH and NAD+, being the absorbance proportional to the urea concentration.

2.5. Nutrient accretion

Ultrasonography was performed at the end of the experimental period (2 days before slaughter) to assess longissimus muscle height (cm) and width (cm), as well as the rump muscle length (cm) and rump fat thickness (mm). Animals were scanned by a trained professional on the left side for ultrasonography using an Aloka 183 500 V machine (Corometrics Medical Systems. Wallingford. CT). The ultrasound was equipped with a 184 MHz 17.2 cm linear array transducer. The *Longissimus thoracis* muscle images (which corresponds to the commercial striploin cut) were taken in the intercostal space between the 12^{th} and 13^{th} ribs. To measure the rump muscle length and rump fat thickness, an imaginary line was considered between the animal's ischium and ileum, parallel to the vertebral column, comprising the anatomical region where cuts such as rump-steak and rump cap are obtained. Subsequently, the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, IA, USA) was used for image analysis.

2.6. Apparent total tract digestibility, nitrogen balance and microbial crude protein synthesis

2.6.1. Sampling

Beef steers were submitted to a digestibility trial from day 62 to 65 of experimental period (5 consecutive days). Throughout the digestibility trial, feedstuffs (corn silage and concentrate) and orts samples were daily collected. During the trial, fecal samples from all steers were collected by the hand grab technique directly from the rectum. Samples were obtained four times a day (0600 a.m., 0900 a.m., 1200 a.m., and 1600 p.m.). The fresh samples from each animal were proportionally used to build a composite sample per pen for each sampling period within a day. Thus, a 12.5 g aliquot of fresh fecal material from each animal was used to form a composite sample (50 grams), which was immediately used for pH determination. To determine fecal pH, feces were diluted in 100 mL of water and homogenized using a stick. Lastly, fecal pH was measured using a previously calibrated portable pH meter (model HI 208; SP Labor, São Paulo, Brazil). The remaining fecal content was stored at -20º C for further chemical analysis.

Two urine samples were collected by the spot technique from the heaviest animal of each pen in the morning (0800 a.m.) on the first and last day of the digestibility trial. The urine content obtained was homogenized and filtered with a double layer of gauze. A 10 mL aliquot of urine was immediately acidified [40 mL H_2SO_4 (0.036N)] to avoid N loss. In addition, to obtain a concentrated urine sample, 5 drops of sulfuric acid P.A. were added to a 30 mL aliquot of urine. All samples were subsequently stored at -20º C until chemical analysis.

2.6.2. Chemical analysis and calculations

All chemical analysis was performed at the Animal Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Lavras (UFLA). The feedstuffs, orts and fecal samples were previously dried in a forced-air oven at 65º C until

achievement of a constant weight. After drying, samples were ground in 1 and 2 mm sieves using a Wiley mill (Wiley® TE-680, Philadelphia, PA, USA), being subsequently stored at room temperature. All samples were chemical analyzed for moisture (method No G-003/1), ash (M-001/1), nitrogen (N-001/1), ether extract (EE; G-004/1), ash- and protein- free neutral detergent fiber (NDFap; N-002/1) and NDF*i* (F-009/2) considering the analytical guidelines of the Brazilian National Institute of Science and Technology (INCT-CA) (Detmann et al., 2012). The starch analysis was also performed according to the INCT-CA recommendations (method No G-007/1). Readings were performed using a UV/visible spectrophotometer at 630 nm.

The total digestible nutrients (TDN) values were estimated according to Detmann et al. (2010), as: $TDN = CP_{ad} + NFC_{ad} + NDF_{d} + 2.25 \times EE_{ad}$ (being: CP_{ad} = crude protein apparently digestible, NFC_{ad} = non-fiber carbohydrate apparently digestible, NDF_{d} = digestible fraction of neutral detergent fiber, and EE_{ad} = apparently digestible etheric extract). The non-fiber carbohydrate was obtained as: NFC = 100 - (%NDF + %CP + %EE + %Ash) (Detmann et al., 2010).

The apparent total tract digestibility coefficients were estimated as the difference between intake and the fecal content divided by the intake. The indigestible detergent fiber (NDF*i*) was used as an internal indicator for fecal production mensuration (Equation 2). The NDFi was determined using the 2 mm grounding samples in an autoclave, after *in situ* incubation (288 h) using rumen cannulated beef animals (Detmann et al., 2012).

$$Fecal\ production\ (kg/day) = \frac{dietary\ NDFi\ (kg/day)}{fecal\ NDFi\ (kg/kg)} \quad \text{Eq. (2)}$$

Moreover, for N balance purposes, the diet components, fecal and urine samples were chemical analyzed for N concentration. The total N content in the urine was determined by the Kjeldahl method (AOAC, 1990). Creatinine concentration was used

for urinary volume determination. Creatinine determination was done through colorimetric reaction using a commercial kit (K067, Bioclin, Belo Horizonte, MG, Brazil). The N retained was considered as the difference between the total N intake and the N losses in the feces and urine.

The microbial crude protein, as well as its efficiency (expressed in g of microbial protein per kg of TDN), were estimated using the urine purine derivatives technique. Allantoin analysis was done by colorimetric method, as described by Chen & Gomes (1992). The values were transformed to grams per day through the product between allantoin concentration (AL) and the urinary volume. Then, the values were transformed to mmol/day considering the product between allantoin concentration and its molecular weight. The daily excretion of uric acid (UA) (mmol / day) were estimated according to equation 3 described below (Valadares Filho et al., 2016):

$$UA_{Excretion}\ (mmol/day) = 0.1104\ \times AL \qquad \text{Eq. (3)}$$

The purine derivatives (PD) excretion were obtained as the sum of allantoin and uric acid (mmol/d). Purine absorption (PA) and ruminal synthesis of microbial crude protein were calculated using Equation 4 and 5, respectively (Prates et al., 2012). Microbial crude protein synthesis was estimated considering the product between the ruminal synthesis of nitrogenous compounds by 6.25.

$$Purine\ absortion\ (mmol/day) = \frac{PD\ -\ (0.405\ mmol/kg^{0.75})}{0.99} \qquad \text{Eq. (4)}$$

Where: PD = purine derivatives, [- 0. 405 mmol/ $kg^{0.75}$] = endogenous excretion of purine derivatives, 0.99 = recovered absorbed purines.

$$Ruminal\ MCP\ (g/day) = \frac{70\ \times PA}{0.93\ \times 0.11 \times 1000} \quad \text{Eq. (5)}$$

Where: 70 = purine N content (mg N/mol), 0.93 = purine digestibility, and 0.11 = ratio between purine N content: total N of microorganism.

2.7. Fecal score

The fecal score was performed by a trained person three times a week in the morning (0900 a.m.) and afternoon (300 p.m.). The score was assigned considering a gradual scale (1 to 5). Briefly, each point on the scale was represented as follows: (1) watery and diarrheal feces. Evacuation of fecal material in jets. Feces with a strong odor, and brownish gray color. Animals were commonly presenting feces in the rear portion of the body. Possible presence of mucus and gas bubbles in the feces; (2) Pasty feces, smeared on the ground. Gray coloring. Presence of mucus. Fecal material without the presence of concentric circles. Presence of long fiber and whole grains in the fecal material; (3) Feces containing concentric circles (not too pronounced), with a mild odor and a humid appearance; (4) Feces with a firm appearance and conical shape. Unlike score 3, feces with score 4 do not present depression in the center of the fecal mass. Presence of bumps resembling rings. The occasional presence of a shiny layer over such bumps; (5) Hard feces with very pronounced interconnected concentric rings. Dry feces that do not stick to the touch. Dark on the outside and lighter on the inside.

2.8. Slaughter, carcass assessment and liver abscesses

Steers were slaughtered in a commercial slaughterhouse (Piracicaba, São Paulo, Brazil). All slaughtered procedures were performed in accordance with the standard protocols proposed by the Sanitary and Industrial Inspection Regulation for Animal Products of Origin (Brazil, 1997). The animals were stunned by the captive's bold

technique and subsequently subjected to the bleeding and dressing process. After the hot carcass weight (HCW) was recorded, the carcasses were stored in a cold room (4º C for 24 h).

Total carcass gain was considered as: Total carcass gain = Slaughter packing carcass weight - (initial BW × 50%). It was assumed that all animals had the same initial carcass yield. The daily carcass gain was obtained considering the product between the total carcass gain and the period (expressed in days). The biological efficiency was calculated as the ratio between the average DMI and the carcass yield.

The rib eye area and subcutaneous fat thickness (SFT) was measured in the left half carcass, in the Longissimus thoracis muscle section. To rib eye area determination was used an acetate-based paper. The rib eye area was designed by a trained professional on the transparent surface, being the images subsequently digitized and analyzed using the ImageJ® software (National Institutes of Health, Bethesda, Maryland, USA). Moreover, the SFT was measured with a precision caliper with a millimeter-scale positioned at ¾ of the medial border of the Longissimus thoracis muscle.

2.9. Meat quality and composition

At slaughter, meat samples were collected from the heaviest animal of each pen for the qualitative parameters analysis. Samples were vacuum-packed, frozen (-20ºC) and sent to the Meat Laboratory located at the Animal Science Department of UFLA. Initially, the frozen meat samples were cut in beef of 2.54-cm-thick each. Each beef was re-identified, vacuum repackaged and frozen. Subsequently, one beef per animal was thawed overnight at -4º C (~16 hours) and weighed. For color analysis, the beef samples were removed from the packages and exposed to atmospheric air for 30 minutes (to allow myoglobin oxygenation). The color analysis was performed using the Minolta Chroma Meter colorimeter (CM-700, Kônica Minolta Sensing Inc, Osaka, Japan), calibrated to a

soft tile pattern. The evaluation was performed considering the CIE L*a*b* system, in which L* = the index associated with brightness; b* = intensity of red; and a* = intensity of yellow. Six color readings were performed per beef. Concomitant with the color analysis, beefs were evaluated for pH, using a previously calibrated portable pH meter (Model HI 99,163; Hanna, Woonsocket, RI, USA). Three pH readings were performed per beef. The same unaged beef was used for the cooking weight loss determination. This parameter was calculated as the weight difference prior and post beef cooking. Beef was cooked using a grill and a thermometer (positioned inside the beef). After the achievement of 71ºC, beef was placed at room temperature, and weighted to determine the cooking loss (after temperature stabilization). Lastly, the same unaged beef was used for Warner-Bratzler Square Shear Force (WBSF) analysis. Six rectangular samples (1 × 1 × 3 cm) per beef were manually obtained. Samples were completely sheared perpendicularly to the fibers direction, by a Warner-Bratzler blade coupled to a TA.XTplus texturometer (Stable Micro System Ltd., Godalming, Surrey, United Kingdom). Other beef from the same animal were exposed to maturation under (1º C) during 14 days' *post mortem.* After this period, the samples were analyzed for color, pH, cooking losses and Warner-Bratzler Square Shear Force parameters.

Other beef from each animal was used in the proximate composition analysis. The chemical analysis were performed as described by Ramírez-Zamudio et al. (2022). Briefly, to chemical composition determination, the beef was cleaned (i.e., subcutaneous fat was removed) and grounded in a meat processor. A 100 g sample per animal was positioned on a plate and subsequently analyzed in the FoodScan™ (AOAC method No 2007-04; FOSS, Hillerod, Denmark).

2.10. Statistical analysis

The outcomes were analyzed in a completely randomized design with a factorial arrangement 2 × 2, using the MIXED procedure of SAS. The pen was considered as the experimental unit. The statistical model considered the effects of the MON, GRN, and their interaction. The following statistical model was used:

$$Yij = \mu + Mi + Tj + (MT)ij + \varepsilon ij$$

Where: Y_{ij} is the observed measure; μ is the general average; M_i is the fixed effect of monensin; T_j is the fixed effect of the GRN; MT_{ij} is the interaction between M and T and ε_{ij} is the random error associated with Y_{ij}. with $e_{ij} \sim N (0.\sigma_e^2)$.

When relevant ($P < 0.05$), the initial shrunk body weight was used as a covariate for statistical analysis for the corresponding parameters. When not relevant ($P > 0.05$), this information was removed from the model. All data were checked for normality (Shapiro-Wilk Test) using the UNIVARIATE procedure of SAS 9.2 (SAS Inst. Inc.. Cary. NC). For all variables and their interactions, data was presented as treatment means ± standard error of the mean. Differences were declared at $P < 0.05$ and trends was discussed when $P > 0.05$ and $P \leq 0.10$.

3. RESULTS

3.1. Performance, voluntary feed intake and feed efficiency

At the beginning of the experimental period, the shrunk body weight was similar between all experimental treatments ($P \geq 0.69$; Table 2). At the end of the experimental period, animals fed MON + GRN tended to have lower final SBW (MON × GRN: $P = 0.08$) than the other treatments. During the adaptation phase, there were no differences toward ADG as a function of the feeding regimens used ($P \geq 0.58$). However, the total

ADG (0 to 102 days) was reduced by the MON use in combination with GRN in the diet (MON × GRN: $P = 0.02$) compared to the single GRN use in the diet (Table 2).

The DMI during the finishing phase and the DMI considering the entire experimental period were affected by the MON + GRN use in the diet (MON × GRN: $P \leq 0.03$). Steers fed MON had greater DMI during the finishing phase and during the total experimental period compared to other treatments (Table 2). The day-by-day variation in dry matter intake during the adaptation, final and total periods were affected by the associated use of MON plus GRN in the diet ($P \leq 0.01$). During the adaptation period, steers fed diets without additives demonstrated a greater day-by-day DMI variation compared to steers fed MON, GRN or GRN + MON. During the finishing and total experimental period, steers fed control diets exhibited the greater day-by-day DMI variation, while steers fed MON had the lowest day-by-day DMI variation (Table 2).

The feed efficiency (G: F) was ~5.7 % higher for steers fed GRN in the diet compared to steers fed diets without GRN inclusion ($P = 0.04$; with 0.167 *vs.* 0.158 kg of BW/Kg of DMI, for steers fed with and without GRN).

3.2. Blood parameters and Nutrient accretion

Any MON × GRN interaction was detected for urea or glucose blood concentrations ($P \geq 0.25$, Table 3). Nevertheless, there was a tendency ($P = 0.06$) toward ~7.8% additional urea concentration for steers fed without GRN compared to steers fed with GRN.

Regarding the ultrasound measurements, the *Longissumus* muscle width was improved by the isolated use of GRN in detriment of other three treatments (MON × GRN: $P = 0.02$; Table 3). On the other hand, the Longissimus muscle height, the ratio LM width: LM height, the rump muscle length, and the rump fat thickness measurements were not affected by the monensin or GRN use, or by its interaction ($P \geq 0.05$).

3.3. Nutrient intake and apparent total tract digestibility

Steers fed MON had greater DM intake during digestibility trial than the remaining treatments (MON × GRN: $P = 0.04$; Table 4). Steers fed GRN diets had lower CP and starch intake ($P < 0.01$) than those fed diets with GRN inclusion during the digestibility period. The NDF intake was greater for steers fed MON than for the other treatments (MON × GRN: $P = 0.03$).

Any MON × GRN interactions were detected for DM and diet components digestibility ($P \geq 0.43$, Table 4). Steers fed diets with GRN inclusion tended to have ~5.6% additional DM digestibility ($P = 0.09$) than steers fed diets without this additive.

3.4. Nitrogen balance and microbial protein synthesis

Any MON × GRN interaction or MON effects were detected for N balance outcomes ($P \geq 0.29$, Table 4). Steers fed diets without GRN had ~18.5% greater N intake relative to animals fed diets with GRN ($P < 0.01$; 204.5 vs. 172.5 g/day for diets with or without GRN, respectively). Fecal nitrogen excretion was lower for steers fed GRN diets compared to those fed without GRN inclusion in the diet ($P = 0.02$). The urinary N, total N excretion and the N balance were similar between steers fed with or without GRN ($P \geq 0.14$).

The microbial crude protein synthesis (g/day) was reduced by the associated used of MON + GRN (MON × GRN*:* $P = 0.02$). The microbial protein synthesis per kg of CP intake tended to be lower for steers fed CON and MON + GRN diets compared to the GRN treatment (MON × GRN*:* $P = 0.08$). The microbial protein synthesis per kg of TDN intake tended (MON × GRN*:* $P = 0.10$) to be lower for MON + GRN compared to other treatments.

3.5. Fecal pH and score

The fecal pH was not affected by any dietary additive studied or by their interactions ($P \geq 0.43$). There was an associative effect of MON plus GRN additives used on the fecal score during the finishing period ($P = 0.02$) and on the frequency of animals with adequate fecal score during the finishing period ($P = 0.05$). Steers fed control diets had lower fecal score compared to those fed MON. Moreover, at the finishing period, the CON treatments were those that presented the lower frequency of animals with adequate fecal score compared to other treatments.

3.6. Carcass measurements

Animals from the GRN treatment had a greater hot carcass weight compared to the other three treatments (MON × GRN: $P = 0.04$). Animals fed diets with GRN had ~8% additional total carcass gain and daily carcass gain ($P = 0.04$; Table 6). They also had ~3.1% greater carcass yield compared to those fed without the GRN inclusion ($P = 0.02$). Moreover, the GRN inclusion in the diet demonstrated a beneficial effect on the steers biological efficiency (P = 0.01, 0.086 vs. 0.100 kg/kg for diets without and with GRN, respectively). At slaughter, GRN group tended to have the greatest ribeye area (MON × GRN: $P = 0.08$; Table 6). The backfat thickness were similar between treatments ($P \geq 0.59$).

3.7. Meat quality parameters

The color parameters (L*, a*, b*), Chrome, Hue, myoglobin pigments, cooking losses and beef pH in the unaged and in meat exposed to maturation during 14 days were similar between the treatments ($P \geq 0.05$, Table 6). The WBSF at time zero and time 14 of maturation was lower for steers fed MON than those fed without MON ($P \leq 0.03$, Table 7a).

Regarding the centesimal composition (Table 7b), steers fed diets with monensin inclusion ($P < 0.01$) had ~2.7% more protein content than animals fed without this ionophores (22.1 *vs*. 22.7% for diets with and without MON, respectively).

4. Discussion

Consistent with our hypothesis, there were differences toward the associative use of MON + GRN for steers exposed to a dietary challenge. The monensin addition to finishing diets containing GRN promoted a negative effect on the steers performance. Such response, may be associated to the negative effect of MON + GRN on the microbial protein synthesis and efficiency. Monensin is a carboxylic ionophore well-knower by disrupts transmembrane movement and the ions intracellular balance of ruminal microorganisms (Rezaei Ahvanooei et al., 2023), specially of gram-positive bacteria. As consequence, its use impair the growth of cellulolytic, proteolytic and lactate producing bacteria, as well as methanogenic archeas (Marques and Cooke, 2021). Therefore, for instance, MON reduce microbial populations, and the microbial protein synthesis due to its selective response on certain microorganism types. Additionally, MON reduce the ammonia ruminal pool, presenting a "protein-sparing' effect, because its use reduce deamination and proteolysis (Chen and Russell, 1991). By the other hand, once Timafeed release ammonia intermittently occur a lower ruminal ammonia availability as a function of its use. Thus, collectively these effects justified the MON + GRN inhibitory effect on the rumen microbiota, suggesting impaired ruminal N metabolism due its associate use.

The GRN technology showed be a promising technology to improve beef cattle anabolism. As described above, steers fed GRN had greater *Longissumus* muscle weight, hot carcass weight, total and daily carcass gain, carcass yield, and rib eye area. Such response might be explained by the greater synchronism between the non-protein nitrogen

and the carbohydrates sources degradation in the ruminal environment. The ruminal velocity of ammonia release is a crucial factor for dietary transformation into microbial protein (Melo et al., 2021). Compared to the conventional urea sources commonly used in feedlot diets, gradual release urea avoids a faster ammonia release in the rumen, which in turn is associated with an inefficient N use by microorganism, and to a possible toxicity (Benedeti et al., 2014). Thus, the intermittent ammonia supply by Timafeed might optimized the ruminal fermentation pattern. In other words, as consequence of GRN technology use, the ammonia assimilation demonstrated be improved, leading to a greater microbial protein in the small intestine. Therefore, there was a greater AA availability in the bloodstream available for productive purposes, especially for anabolism. Lastly, the lack of significate MON × GRN interactions indicate the independence of dietary additives on these outcomes.

For each animal, in your respectively physiological state, there is an optimum nutrient level that need to be supplied through the feed intake. The feed intake is driven by a feedback cascade, involving several signals from the diet, but also from the gastrointestinal tract, liver and body reserves to the central nervous system (Forbes, 1999). Considering the day-by-day DMI variation, steers fed diet without additives demonstrated a greater DMI fluctuation compared to steers fed MON, GRN or GRN + MON during the adaptation period. Additionally, during the finishing and total experimental period, steers fed CON diet exhibited the greater day-by-day DMI variation, while steers fed MON had the lowest variation. Highly fermentable diets result in a quickly organic acids production in the ruminal environment, which may reduce the ruminal pH and lead to different acidogenic thresholds (subacute and acute acidosis) (Aschenbach et al., 2011). Considering that the ruminal pH is determined by dynamic between the acids production and its removal from rumen (by absorption through the

rumen wall and passage), as well as by the ruminal neutralization capacity (Penner et al., 2009), we postulated that animals fed CON diet had prolonged periods within a day with lower rumen pH than other treatments (Dijkstra et al., 2012). As consequence, these animals probably presented greater activation of chemoreceptors present in the rumen wall (sensible to ruminal pH and to VFA concentration), which led to reduced ruminal motility and greater DMI fluctuations (Furlan et al., 2006).

Conversely, in the present study, steers fed MON-enriched diet presented lower DMI fluctuation and greater daily feed intake during the finishing and total experimental periods than the CON, GRN or GRN+MON groups. For ruminants fed feedlot diets, the feed intake is mainly controlled by the energy level of the diet. According to the hepatic oxidation theory [For review see: Allen et al. (2009)], the propionate act as the primary satiety signal for ruminants fed diets rich in ruminal degraded starch. The greater propionate availability after the meals in such scenario, improve gluconeogenesis but also stimulates the acetil-CoA oxidation, increasing the hepatocytes energy status, which in turn, intensify the signals carried from the liver to the brain (through the vagus nerve), promoting satiety. Follow this reasoning, might be expected that higher monensin inclusion level may suppressed the feed intake (Allen et al., 2009), once this additive is well known to promote substantial increases in propionate production (Gupta et al., 2019). Nevertheless, in the present study, MON was the treatment with best response on the feed intake. This pattern differ from those reported by a meta-analysis using 40 peer-reviewed articles focusing on growing and finishing beef cattle (Duffield et al., 2012) in which the MON use reduced the DMI. In contrast to this previous results reported in the literature, the greater DMI can be due to the lower DMI variation and due to greater NDF intake for steers fed MON. Herein, the greater fiber intake might be stimulated rumination, salivation, and ruminal motility (Carvalho et al., 2016), avoiding ruminal pH

peaks and favoring the DM in animals fed MON. Lastly, the smaller day-by-day DMI variation during the adaptation period for steers fed GRN compared to those without this product, can be associated to the yeast presence in Timafeed composition. Consistently, Arambel and Kent (1990) suggested that products containing yeasts may be more effective under stress conditions (such as adaptation period) rather than under normal dietary conditions.

The use of GRN in the diet tended to improve the total tract DM digestibility of the diet. It should be noted that steers fed GRN had lower DMI during the finishing and total periods compared with steers fed without this supplement. Thus, improved DM digestibility of DM was probably not a direct effect of Timafeed use, but a response to the reduced passage rate due to the less voluntary feed intake (Ngidi et al., 1990). Other interesting response verified in the present study was the favorable responses verified on the biological efficiency, feed efficiency and carcass deposition efficiency. Together these findings suggest that steers fed GRN need less inputs to achieve the same productive level than their contemporaneous, contributing to overcome the challenge of higher production costs in the livestock.

The present results suggested that using slow-release nitrogen is possible to maintain the D-lactate and glucose concentrations in similar levels achieved by the use of diets containing monensin for beef cattle. The blood urea levels were lower for steers fed GRN than for those fed diets without GRN inclusion. Urea is built in the liver in amounts proportional to ruminal ammonia levels. Herein, such response may be sustained by a reduced ammonia absorption from portal-drained viscera for GRN group as a consequence of the intermittent urea liberation from Timafeed. Consistently, previously studies performed with beef cattle using urea-calcium as a slowly source of nitrogen

liberation (Melo et al., 2021) demonstrated that such technology resulted in a reduced ammonia absorption.

Highly fermentable diets are also closely associated to hindgut pH drop in cattle. As consequence of ruminal disturbance, a greater amounts of indigestible substrates might achieve the hindgut, causing an additional perturbation, including bacterial community shifts, increased short-chain fatty acids production, and fecal pH depression, which in turn lead to deleterious effects on the intestinal permeability and mucosal integrity (Neubauer et al., 2020). Nevertheless, in the current study, any effects on fecal pH were detected as a function of feeding regimens evaluated. Despite the lack of difference on this parameter, steers fed MON presented improved fecal score compared to steers fed CON diet. This response might be sustained by the DMI data. The smaller variation in the DMI for steers fed MON in the total experimental period compared the control treatment may explain the improvements in the frequency of animals with appropriate fecal scores, once the fecal score is an acidosis metric.

The WBSF is a parameters related to the meat tenderness (Destefanis et al., 2008), which reflect the force required to cut the cooked meat (Girard et al., 2012). The MON use reduced the meat Warner-Bratzler Square Shear Force, indicating a softer meat as a function to the MON use. The WBSF is mainly affected by myofibres and connective tissue (Girard et al., 2012). Considering that collagen content in the centesimal analysis were similar between treatments, the lower WBSF may be related to the myofibrilar components. Thus, we postulated that steers fed MON had a greater protein turnover and thus a greater breakdown of myofibrillar proteins. However, these findings need further investigation.

5. Conclusion

The use of a progressive and controlled N release additive showed great potential for improving the performance and efficiency of N synchronization. Thus, in summary the GRN technology showed be a promising technology to be used to boost muscle hypertrophy and carcass production of beef cattle. However, our findings suggest that its use may be avoid when this additive is used in combination with monensin, due to its potential negative effects on the ruminal ammonia pool.

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7. Tables

Table 1. Ingredients and chemical composition of experimental diets.

Item	CON	MON	GRN	MONGRN
		Ingredients, *g/kg of DM*		
Corn silage	290	290	290	290
Ground corn	649.9	649.9	649.9	649.9
Soybean meal	28.4	28.4	11.4	11.4
Urea	11.6	11.6	4.60	4.60
Mineral premix[1]	20.0	20.0	20.0	20.0
Kaolin	0.10	-	0.10	-
Rumensin 200	-	0.10	-	0.10
Timafeed Boost[2]	-	-	24.0	24.0
		Chemical composition, *g/kg of DM*		
Dry matter	71.6	71.6	71.7	71.7
Crude protein	12.5	12.5	12.5	12.5
Neutral detergent fiber	23.8	23.8	23.9	23.9
Ether extract	3.48	3.48	3.47	3.47
Ca	0.50	0.50	0.50	0.50
P	0.25	0.25	0.25	0.25
Mg	0.21	0.21	0.21	0.21
Na	0.22	0.21	0.22	0.21
TDN	79.5	79.5	78.8	78.8

[1] Mineral Premix (PECPRO Animal Nutrition, Alfenas, Minas Gerais, Brazil)
[2] Timafeed Bost: *Analytical constituintes*: Crude ash - 40%, Insoluble ash in hydrochloric acid - 25%, Crude protein - 116%, Crude fibre - 2%, Crude oil and fats - 2%, Sodium - 3%, Magnesium - 1.5%. *Additives*: Urea and its derivatives - 3d1 Urea 380000 mg per kg - Binder- 1g568 Clinoptilolite of sedimentary origin 110000 mg per kg, E562 Sepiolite 63000 mg per kg - Mycotoxin Reducers- 1m558 Bentonite 95000 mg per kg. *Composition*: Distillers' dried grains and solubles, Concentrated distillers solubles, Sodium sulphate, Yeasts, and Ammonium sulphate.

Table 2. Performance, voluntary feed intake and feed efficiency of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

	Feeding regimen					*P*-value		
	Control	MON	GRN	MONGRN	SEM	MON	GRN	MON × GRN
				Performance data				
Initial SBW, *kg*	385	381	381	382	6.28	0.80	0.79	0.69
Final SBW, *kg*	529	530	533	522	3.08	0.09	0.58	0.08
ADG adaptation, *kg/d*	0.942	1.110	0.950	1.052	0.24	0.58	0.92	0.89
ADG total, kg/d	1.728^{ab}	1.743^{ab}	1.776^{a}	1.666^{b}	0.27	0.08	0.58	0.02
				Intake measurements				
DMI adaptation, *kg/d*	8.18	8.29	8.35	8.29	0.05	0.68	0.10	0.11
DMI finishing, *kg/d*	11.1^{b}	11.9^{a}	10.9^{b}	10.5^{b}	0.26	0.33	< 0.01	0.03
DMI total, *kg/d*	10.6^{b}	11.3^{a}	10.4^{b}	10.1^{b}	0.21	0.32	< 0.01	0.02
DMI adaptation, *g/kg BW*	21.0	21.2	21.4	21.3	0.16	0.85	0.19	0.20
DMI total, *g/kg BW*	24.3^{b}	25.7^{a}	23.6^{b}	23.3^{b}	0.46	0.19	< 0.01	0.05
DMIvar adaptation, *%*	9.81^{a}	8.54^{b}	7.65^{b}	8.03^{b}	0.50	0.39	0.01	0.01
DMIvar finishing, *%*	4.51^{a}	1.83^{c}	3.31^{b}	3.51^{ab}	0.43	< 0.01	0.59	< 0.01
DMIvar total, *%*	5.38^{a}	2.89^{c}	3.82^{b}	4.22^{b}	0.35	< 0.01	0.78	< 0.01
G:F, *kg of BW/kg of DMI*	0.162	0.154	0.170	0.164	< 0.01	0.11	0.04	0.84

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release, SBW = shrunk body weight.

Table 3. Blood parameters and nutrient accretion of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON × GRN
				Blood Parameters				
D –Lactato, *mmol/L*	1.8	2.1	1.9	1.6	0.24	0.94	0.33	0.21
Urea, *mg/dL*	34.3	31.8	29.0	28.0	2.30	0.46	0.06	0.73
Glucose, *mg/dL*	85.1	79.8	81.2	87.4	5.28	0.93	0.71	0.25
				Nutrient accretion				
LM height, *cm*	6.75	6.82	7.33	7.18	0.49	0.94	0.30	0.80
LM width, cm	15.0[b]	15.1[b]	16.1[a]	14.7[b]	0.35	0.04	0.27	0.02
LM width : LM height	2.27	2.25	2.24	2.07	0.15	0.53	0.46	0.59
Rump muscle length, *cm*	9.03	8.79	8.52	8.99	0.37	0.74	0.68	0.35
Rump fat thickness, *mm*	5.32	5.88	4.33	4.20	1.01	0.83	0.19	0.73

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release

Table 4. Nutrients intake, apparent total tract digestibility, nitrogen balance and microbial crude protein of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON×GRN
				Nutrients intake				
DM, *kg/day*	10.63[b]	11.31[a]	10.42[b]	10.14[b]	0.22	0.37	< 0.01	0.04
CP, *kg/day*	1.18	1.26	1.13	1.10	0.03	0.46	< 0.01	0.12
NDF, *kg/day*	3.53[b]	3.73[a]	3.51[b]	3.40[b]	0.06	0.47	0.01	0.03
Starch, *kg/day*	4.99	5.27	4.85	4.73	0.10	0.38	< 0.01	0.06
TDN, *kg/day*	7.58	7.99	7.96	7.68	0.23	0.76	0.87	0.15
				Apparent total tract digestibility				
DM, *g/kg of nutrient*	691	688	722	734	22.17	0.83	0.09	0.72
CP, *g/kg of nutrient*	704	691	713	726	27.09	0.99	0.43	0.64
NDF, *g/kg of nutrient*	484	470	502	510	24.08	0.88	0.23	0.65
Starch, *g/kg of nutrient*	867	871	871	896	12.91	0.27	0.27	0.43
				Nitrogen balance and microbial crude protein				
N intake, *g/day*	196	213	174	171	5.97	0.29	< 0.01	0.42
Fecal N, *g/day*	57.75	66.40	48.17	44.78	2.71	0.49	0.02	0.45
Fecal N, *% N intake*	29.55	30.85	28.68	27.40	14.58	0.99	0.43	0.64
Urinary N, *g/day*	87.11	106.31	85.77	87.24	8.09	0.48	0.49	0.55
Urinary N, *% N intake*	45.12	51.03	49.85	51.52	16.19	0.64	0.75	0.79
Total N excretion, *g/day*	145	172	133	135	8.89	0.38	0.14	0.42
Total N excretion, *% N intake*	74.68	81.88	78.53	78.92	17.82	0.67	0.96	0.70
N balance, *g/day*	51.06	40.77	37.44	38.87	0.22	0.81	0.67	0.75
N balance, *% N intake*	10.63	11.31	10.43	10.15	0.22	0.67	0.96	0.70
MCP, *g/day*	907[ab]	1042[a]	1073[a]	830[b]	73.7	0.47	0.76	0.02
MCP, *g/kg of CP intake*	771	830	952	757	136	0.33	0.43	0.08
MCP, *g/kg of TDN intake*	120	130	134	109	20.1	0.45	0.77	0.10

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release, DM = Dry matter, OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fiber, TDN = Total digestible nutrients, MCP = Microbial crude protein.

Table 5. Fecal pH and score of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON×GRN
Fecal pH	5.27	5.34	5.22	5.21	0.11	0.76	0.43	0.74
Fecal score adaptation, *arbitrary units*	3.31	3.40	3.34	3.38	0.05	0.24	0,96	0.68
Fecal score finishing, *arbitrary units*	2.89[b]	3.06[a]	3.00[ab]	2.98[ab]	0.04	0.07	0.71	0.02
Adequate fecal score adaptation[1], *%*	98.6	100	97.3	99.9	1.08	0.07	0.50	0.56
Adequate fecal score finishing[1], *%*	94.3[b]	99.9[a]	99.4[a]	99.6[a]	1.32	0.04	0.08	0.05

[1]Adequate fecal score = frequency of animals with adequate feces score (score 3). Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release.

Table 6. Carcass measurements and liver abscess at slaughter of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON×GRN
Hot carcass weight, *kg*	291[b]	294[b]	306[a]	294[b]	3.24	0.16	0.03	0.04
Total carcass gain, *kg*	96	99	111	100	3.67	0.23	0.04	0.06
Daily carcass gain, *kg/d*	0.940	0.966	1.087	0.973	0.04	0.24	0.04	0.06
Carcass yield, *%*	55.2	55.6	57.7	56.6	0.65	0.61	0.02	0.25
Biological efficiency, *kg/kg*	0.087	0.086	0.105	0.095	0.005	0.28	0.01	0.45
Carcass deposition efficiency, *kg of DM/ @*	174	177	146	158	8.91	0.38	0.02	0.63
Ribeye area, *cm^2*	79.6	81.5	85.1	78.9	2.23	0.35	0.52	0.08
Ribeye area, *cm^2/100 kg carcass*	15.3	15.4	15.9	14.9	0.46	0.30	0.88	0.24
Backfat thickness, *mm*	2.81	2.83	2.46	2.97	0.49	0.59	0.84	0.63

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release.

Table 7a. Meat quality parameters of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON×GRN
				Unaged meat				
Color L*	40.0	39.2	40.4	39.3	1.34	0.48	0.86	0.92
Color a*	17.0	16.4	17.2	17.0	1.11	0.71	0.72	0.81
Color b*	9.52	9.44	10.2	9.49	0.98	0.69	0.72	0.76
Chrome	19.5	18.9	19.9	19.5	1.43	0.71	0.71	0.95
Hue	28.9	29.6	30.4	28.3	1.07	0.52	0.95	0.21
Deoxymyoglobin, %	21.8	19.6	19.0	21.3	1.21	0.94	0.67	0.07
Oxymyoglobin, %	50.4	51.8	52.9	50.6	1.21	0.72	0.56	0.14
Metamyoglobin, %	27.8	28.6	28.1	28.0	0.50	0.48	0.69	0.41
Cooking losses, %	26.6	26.6	19.3	28.9	4.29	0.28	0.57	0.27
WBSF, N	83.8	81.3	85.8	63.7	5.22	0.03	0.14	0.07
pH	5.80	5.84	5.62	5.85	0.14	0.36	0.59	0.51
				Meat aged for 14 days				
Color L*	39.3	37.2	38.4	38.1	1.89	0.52	0.99	0.62
Color a*	20.2	19.1	20.8	19.2	1.50	0.38	0.79	0.85
Color b*	12.6	11.5	13.2	11.7	1.51	0.41	0.79	0.89
Chrome	23.8	22.4	24.7	22.6	2.06	0.40	0.79	0.87
Hue	31.5	30.0	32.0	30.3	1.47	0.28	0.80	0.95
Deoxymyoglobin, %	13.6	13.9	11.0	13.3	2.20	0.56	0.47	0.66
Oxymyoglobin, %	59.3	58.3	62.1	59.4	2.79	0.52	0.49	0.75
Metamyoglobin, %	27.1	27.8	26.8	27.3	0.69	0.43	0.64	0.89
Cooking losses, %	265	21.1	24.7	18.9	3.39	0.11	0.58	0.96
WBSF, N	55.1	51.9	53.5	39.4	3.58	0.02	0.06	0.14
pH	5.68	5.86	5.67	5.82	0.12	0.19	0.87	0.92

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release.

Table 7b. Meat quality parameters of steers fed monensin, gradual-N-realease or monensin plus gradual-N-release enriched-diets.

Item	Feeding Regimen				SEM	*P-value*		
	Control	MON	GRN	MONGRN		MON	GRN	MON×GRN
			Centesimal composition					
Protein, %	22.7	22.3	22.7	22.0	0.25	< 0.01	0.53	0.47
Collagen, %	1.7	1.5	1.7	1.6	0.09	0.21	0.81	0.44
Fat, %	1.9	1.8	1.7	2.1	0.32	0.61	0.88	0.43
Moisture, %	74.1	74.6	74.2	74.1	0.48	0.65	0.76	0.56
Mineral matter, %	1.3	1.3	1.4	1.7	0.22	0.45	0.22	0.39

Abbreviations: CON = Control (finishing diet without ionophore or gradual-N-release based product), MON = Monensin-enriched diet (Rumensin®, Elanco Animal Health, Greenfield, IN), GRN = Gradual-N-release enriched diet (Timafeed Boost®, Roullier Group, Saint-Malo, FR), MONGRN = monensin associated to gradual-N-release.