FEDERAL UNIVERSITY OF LAVRAS
DEPARTMENT OF ANIMAL SCIENCE

MASTER'S DISSERTATION

INTERACTION OF FLINT CORN GRAIN PROCESSING AND UREA SOURCE, AND THE INCLUSION OF CALCAREOUS MARINE ALGAE IN FINISHING DIETS FOR NELLORE CATTLE: EFFECTS ON GROWTH PERFORMANCE, CARCASS TRAITS, AND NUTRITIONAL CHARACTERISTICS

Master's student: Igor Gomes Fávero
Advisor: Dr. Erick Darlisson Batista
Co-advisor: Dr. Daniel Rume Casagrande

2025

IGOR GOMES FÁVERO

INTERACTION OF FLINT CORN GRAIN PROCESSING AND UREA SOURCE, AND THE INCLUSION OF CALCAREOUS MARINE ALGAE IN FINISHING DIETS FOR NELLORE CATTLE: EFFECTS ON GROWTH PERFORMANCE, CARCASS TRAITS, AND NUTRITIONAL CHARACTERISTICS

Dissertation presented to the Federal University of Lavras, as part of the Animal Science Graduate Program requirements, in Ruminant Nutrition and Production, to obtain the Master's tittle in Animal Science.

Advisor: Dr. Erick Darlisson Batista
Co-advisor: Dr. Daniel Rume Casagrande

LAVRAS – MINAS GERAIS
2025

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Favero, Igor Gomes.
Interaction of flint corn grain processing and urea source, and the inclusion of calcareous marine algae in finishing diets for Nellore cattle: effects on growth performance, carcass traits, and nutritional characteristics / Igor Gomes Favero. - 2025.
94 p. : il.

Orientador: Erick Darlisson Batista
Coorientador: Daniel Rume Casagrande

Dissertação (Mestrado Acadêmico) - Universidade Federal de Lavras, 2025.
Bibliografia.

1. Ruminant. 2. Beef Cattle. 3. Feedlot. 4. Entheric Methane Emission. 5. Sustainability. I. Darlisson Batista, Erick . II. Rume Casagrande, Daniel . III. Universidade Federal de Lavras. IV. Título.

IGOR GOMES FÁVERO

INTERACTION OF FLINT CORN GRAIN PROCESSING AND UREA SOURCE, AND THE INCLUSION OF CALCAREOUS MARINE ALGAE IN FINISHING DIETS FOR NELLORE CATTLE: EFFECTS ON GROWTH PERFORMANCE, CARCASS TRAITS, AND NUTRITIONAL CHARACTERISTICS

Dissertation presented to the Federal University of Lavras, as part of the Animal Science Graduate Program requirements, in Ruminant Nutrition and Production, to obtain the Master's tittle in Animal Science.

APPROVED on July 9, 2025

Dr. Erick Darlisson Batista, Federal University of Lavras
Dr. Daniel Rume Casagrande, Federal University of Lavras
Dr. Diogo Fleury Azevedo Costa, University of São Paulo

LAVRAS – MINAS GERAIS

2025

For God and Miraculous Medal about all for the blessings bestowed…

For my dad Nadir Fávero and mom Maria Otília Gomes Fávero for my creation and love in my whole life…

For aunt Teresinha about all affection and love…

I dedicate

ACKNOWLEDGMENT

Firstly, to God and Our Lady of Aparecida for giving me strength and the will to be better at every moment of my undergraduate life.

To my mother Maria Otília, to my father Nadir. To my grandparents Maria and Orlando Borges, Maria and Jovino Fávero. To my aunts Teresinha and Vera Luce. To my uncle Geraldo and Olair.

To the Federal University of Lavras and all the professors who were part of my academic formation.

To my girlfriend Ana Claudia Degli Exposti for all the good times and companionship.

To all my friends I made in graduate school.

To the Brazilian Beef Cattle Team – NEPEC for all the professional and personal growth it made me acquire.

To the Beef Cattle Sector and its professors for their guidance and encouragement.

I will be forever grateful to everyone who was part of every moment of my graduation. Thank y'all!

I thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (Finance Code 001) for funding my scholarship.

I would also like to acknowledge Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), grant number APQ-02327-21, for partially funding the first trial.

I sincerely thank Celtic Sea Minerals and Trouw Nutrition and for their financial support. Their investment and partnership were essential to the successful development of this research and to the quality of the results presented in this dissertation.

SUMMARY

CHAPTER 2 - INTERACTION OF FLINT CORN GRAIN PROCESSING AND UREA SOURCE IN DIETS FOR FINISHING CATTLE: IMPACT ON PERFORMANCE, CARCASS TRAITS, NUTRIENT UTILIZATION, AND ENZYME ACTIVITIES INVOLVED IN NITROGEN AND ENERGY METABOLISM

CHAPTER 1

EFFECTS OF CALCAREOUS MARINE ALGAE ON FEEDLOT PERFORMANCE, CARCASS TRAITS, NUTRIENT DIGESTION AND ENTERIC METHANE EMISSIONS OF FEEDLOT-FINISHED NELLORE HEIFERS

ABSTRACT

Global population growth has intensified the demand for productive and environmentally sustainable livestock systems. In this context, *Lithothamnium calcareum*, a calcareous marine algae, has emerged as a natural buffering agent to improve ruminal fermentation and reduce methane emissions. Thus, the objective of this study was to evaluate the effects of *L. calcareum* supplementation on feedlot performance, carcass traits, nutrient digestibility, nitrogen metabolism, urinary and fecal pH, and enteric methane emissions in Nellore heifers during the finishing phase. Thirty-six heifers with an initial body weight (BW) of 270 ± 7 kg were allocated in individual pens and distributed in a randomized complete block design. The experimental diets were formulated with a 25:75 forage-to-concentrate ratio (DM basis) and provided *ad libitum*, with daily adjustments to control feed refusals. Two treatments were evaluated: 1) Sodium bicarbonate (1.8% DM in the diet, providing 110 g/heifer/day), and 2) Calcareous marine algae (supplemented with 60 g of *L. calcareum* /heifer/day). The experiment lasted 96 days, including 12 days of dietary adaptation followed by 84 days on the finishing diet. Refusals were collected and quantified daily to estimate dry matter intake (DMI), by calculating the difference between the amount offered and refused. BW was measured on days 0, 13, 41, 69 and 96, before the morning feeding. Enteric methane (CH_4) emissions were measured using the sulfur hexafluoride (SF_6) tracer gas technique during three distinct periods of the experiment: days 21 to 25, 49 to 53, and 77 to 81. Fecal and urine samples were also collected during three periods (days 27–32, 55–60, and 83–88). Data was analyzed using the MIXED procedure of SAS 9.4. There were no significant treatment effects on feedlot performance ($P \geq 0.560$), carcass traits ($P \geq 0.681$) and apparent total tract digestibility ($P \geq 0.212$). Urine pH was significantly lower for heifers consuming calcareous marine algae ($P \leq 0.001$) compared to those receiving sodium bicarbonate. Fecal N excretion (53.82 vs. 47.87 g/day; $P = 0.079$) tended to be lower in heifers fed with calcareous marine algae. Heifers fed diets supplemented with calcareous marine algae showed a tendency for an 8.2% reduction ($P = 0.075$) in enteric methane emissions compared to those receiving sodium bicarbonate. In summary, *L. calcareum* proved to be a viable natural alternative to sodium bicarbonate in finishing diets for Nellore heifers, maintaining performance and carcass traits, with potential to enhance ruminal microbial activity and reduce enteric CH_4 emissions, although further studies are needed.

Keywords: clean production, finishing phase, ruminants, sustainability.

SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

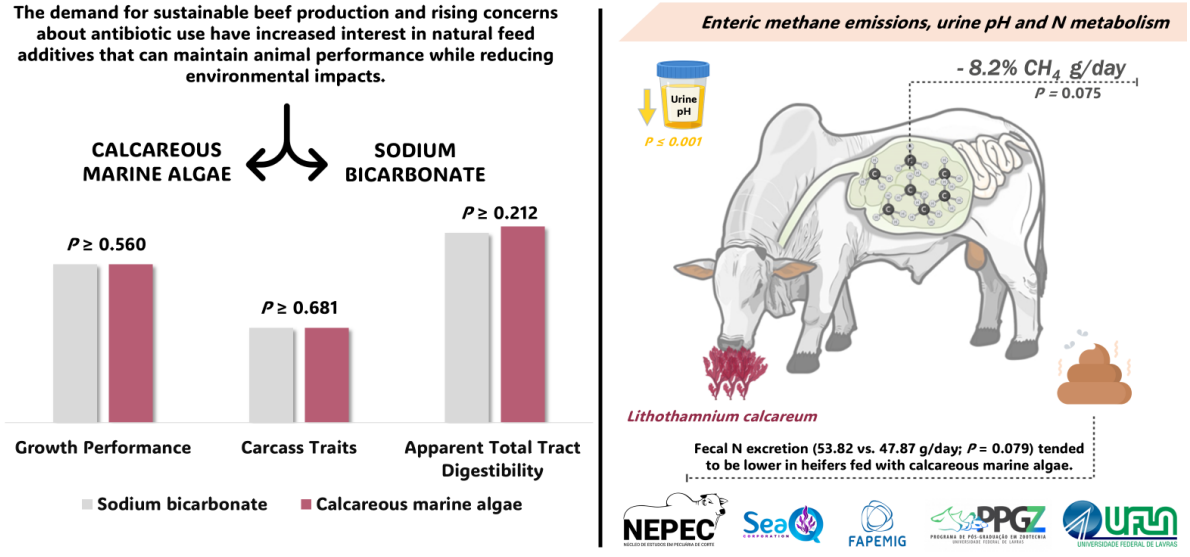
The first study, presented in Chapter 1, evaluated the impacts of using *Lithothamnium calcareum* as an alternative buffering agent to sodium bicarbonate in finishing diets for Nellore heifers, considering growth performance, nutrient digestibility, nitrogen utilization, and greenhouse gas emissions. The results generated relevant scientific, technological, environmental, and economic impacts for intensive ruminant production systems within the context of tropical livestock production. Technologically, the inclusion of *L. calcareum* maintained parameters related to ruminal environment stability and nutrient utilization efficiency at levels comparable to those obtained with a conventional buffering agent, demonstrating its technical viability as a nutritional alternative and expanding the range of functional feed additives available for feedlot finishing diets. Environmentally, the results indicate a potential mitigation of enteric methane emissions, contributing to reductions in the environmental footprint of beef production, aligning with emerging sustainability demands and global commitments related to greenhouse gas mitigation in agriculture. From an economic perspective, the use of *L. calcareum* presents potential impacts on the diversification of nutritional inputs, reduction in dependence on conventional feed additives and ionophores, and increased flexibility in feed formulation, which may positively influence the economic sustainability of tropical production systems. Social and extension-related impacts arise from the dissemination of scientific and technical knowledge with direct application in the beef cattle production chain, benefiting rural producers, consultants, and students of agricultural sciences, and promoting the adoption of emerging nutritional technologies at the commercial level.

INFOGRAPHIC

SUSTAINABLE BEEF PRODUCTION: EVALUATING CALCAREOUS MARINE ALGAE AS A NATURAL ADDITIVE IN BEEF HEIFERS DURING THE FINISHING PHASE

The growing demand for sustainable beef production calls for alternative strategies that can maintain animal performance while reducing the environmental impacts of livestock systems. At the same time, public concerns regarding antibiotic use in animal nutrition, driven by the precautionary principle, have increased the interest in natural feed additives. This study evaluated *Lithothamnium calcareum*, a natural calcareous marine algae, as a replacement for the conventional buffer, sodium bicarbonate, in finishing diets for feedlot Nellore heifers. Over a 96-day feeding period, the heifers received high-concentrate diets with either sodium bicarbonate or *L. calcareum* as a buffering agent. The results showed that the inclusion of *L. calcareum* maintained feedlot performance, carcass traits and nutrient digestibility comparable to those observed with sodium bicarbonate. Importantly, the use of the algae tended to reduce enteric methane emissions by approximately 8.2% and slightly lowered fecal nitrogen losses, which is relevant since manure nitrogen is a major source of ammonia (NH_3) and nitrous oxide (N_2O) emissions with significant environmental impact. These findings indicate that *L. calcareum* could be a promising natural additive to help stabilize rumen pH, support healthy fermentation, and contribute to the mitigation of greenhouse gas emissions. Overall, adopting natural additives such as calcareous marine algae may represent an important step towards more sustainable and environmentally responsible beef production without compromising animal growth performance.

EFFECTS OF CALCAREOUS MARINE ALGAE ON FEEDLOT PERFORMANCE, CARCASS TRAITS, NUTRIENT DIGESTION AND ENTERIC METHANE EMISSIONS OF FEEDLOT-FINISHED NELLORE HEIFERS



1. INTRODUCTION

Currently, there is a significant challenge in meeting the growing global demand for ruminant products due to the continuous expansion of the world population (Geber et al., 2013). With Brazil holding a substantial share of global meat exports and approximately 12% of the world's cattle herd (ABIEC, 2024), there is an increasing pressure from consumers and governments to further reduce the environmental footprint from red meat production (Mayberry et al., 2019).

Most greenhouse gas (GHG) emissions from livestock production occur in the form of methane (CH_4), which is primarily produced through enteric fermentation of feed substrates in the rumen (Roque et al., 2021). The concern over CH_4 emissions is significant due to its average atmospheric lifetime retention of approximately 12.4 years (Myhre et al., 2014) and its high global warming potential, as it is 28 times more impactful than carbon dioxide (CO_2) (U.S. EPA, 2021).

In addition to environmental concerns, methane production in ruminants can be significant as it represents up to 12% of gross energy (GE) intake that could otherwise be utilized for physiological processes but is instead released into the atmosphere through eructation (Beauchemin et al., 2009a). Reducing CH_4 emissions in beef cattle could potentially increase productivity by conserving metabolizable energy (ME) that would, otherwise, be lost. Studies, including those by Beauchemin et al. (2008) and Knapp et al. (2014), indicate that dietary strategies targeting CH_4 reduction can improve ME availability, resulting in increased body weight gain (BWG) and improving feed efficiency. These findings highlight the potential of CH_4 mitigation to enhance cattle productivity by reallocating this excess conserved energy toward growth.

Some nutritional strategies, such as the use of ionophore additives, have been employed to reduce methanogenesis and thereby increase energy efficiency (Hristov et al., 2013). Despite their effectiveness, the use of antibiotics as growth promoters may be limited in the future due to social and governmental pressures (Callaway et al., 2003) associated with the potential development of antibiotic-resistant pathogens (Russell & Houlihan, 2003). For example, the European Union and Denmark banned the use of ionophores as growth promoters in animal production in 2006 and 1999, respectively (European Union, 2006).

As an alternative to ionophores, some non-antibiotic and natural additives, including a range of algae spp, such as *Lithothamnium calcareum*, are being investigated to determine their potential to manipulate rumen fermentation and reduce CH_4 production. *L. calcareum* has a honeycomb structure that results in the slow release of minerals in an acidic environment (Cruywagen et al., 2015). These characteristics may be beneficial to rumen fermentation and CH_4 abatement where the release of a

bicarbonate ion can react with one proton, while the release of a carbonate ion can neutralize two protons (Cruywagen et al., 2015), providing twice the buffering capacity of sodium bicarbonate.

Guerreiro (2025) conducted a study with *L. calcareum*, in association with monensin, demonstrating its potential to reduce CH_4 production when compared to essential oils. However, no studies have been conducted on the effect of *L. calcareum*, on CH_4 production when used in the diet without another additive.

Thus, we hypothesized that calcareous seaweeds have the potential to reduce methane emissions, increase energy efficiency, and improve the productive performance of Nellore heifers. The objective of this study was to evaluate the effect of *L. calcareum* on the mitigation of GHG production, digestibility, and growth performance of Nellore heifers during the finishing phase.

2. LITERATURE REVIEW

2.1 Diets for feedlot-finishing cattle in Brazil

Approximately 12% of the global cattle herd is in Brazil, which produced 11.8 million tons of carcass weight equivalent (CWE) in 2023 (ABIEC, 2025), representing 15% of the world's total beef production. In terms of exports, Brazil is the largest exporter of beef, accounting for 21% of all beef traded internationally (ABIEC, 2025). Furthermore, the percentage of feedlot-finished animals slaughtered, compared to the total number of cattle slaughtered, has increased from 12.6% to 19.9% over the past five years (ABIEC, 2019; 2025).

Feeding represents the most significant component of production costs in feedlot systems, accounting for up to 90% of daily expenditures per animal (DSM, 2024). Consequently, nutritional planning plays a crucial role in influencing both productivity and economic returns (Valadares Filho et al., 2023). To intensify production, feedlot strategies have increasingly focused on enhancing nutrient use efficiency through dietary adjustments, particularly the adoption of high-concentrate diets rich in non-structural carbohydrates. According to a nationwide survey of Brazilian feedlot nutritionists by Monsalve & Millen et al. (2025), finishing diets typically contain an average of 84.3% concentrate on a dry matter (DM) basis. These high-concentrate formulations are largely based on cereal grains, which contribute substantial amounts of rapidly fermentable non-structural carbohydrates. Unlike structural carbohydrates, which are slowly degraded in the rumen, starches, sugars, and pectins undergo rapid fermentation (González et al., 2012). As a result, animals consuming these energy-dense diets experience intensified ruminal fermentation and increased production of volatile fatty acids (VFAs) (Millen et al., 2022).

Corn, containing high energy density with approximately 72% starch on a DM basis, is the predominant grain used in cattle feedlots in Brazil, and is present in 97.2% of finishing diets (Silvestre & Millen, 2021; Huntington, 1997). However, flint corn is the predominant type used in these rations (Bernardes & Castro, 2019; Oliveira & Millen, 2014; Pinto & Millen, 2018), despite it having a higher proportion of vitreous endosperm and lower starch availability compared with dent corn (Corona et al., 2006; Correa et al., 2002).

The availability and digestibility of the starch in corn kernels can vary based on the type and extent of kernel processing. Such processing can enhance the action of proteolytic bacteria and protease enzymes, which break down the protein matrix surrounding starch granules (Junges et al., 2017), thereby increasing starch availability (Hoffman et al., 2011).

Although fine grinding remains the primary corn processing method in most Brazilian feedlots (Millen et al., 2009; Oliveira & Millen, 2014; Pinto & Millen, 2018), there is a growing interest in ensiling techniques to further optimize starch digestibility (Bernardes & Castro, 2019). In corn, the starch–protein matrix is mainly composed of hydrophobic zein proteins, which are classified as prolamins (Zinn et al., 2002). Consequently, even after milling, this matrix may act as a physicochemical barrier to starch digestion by rumen microorganisms (Owens et al., 1986; Hoffman et al., 2011; McAllister et al., 1990).

Efficient feedlot practices are crucial for maintaining and potentially enhancing productivity. The use of high-energy diets, particularly with flint corn as the primary energy source, highlights the need for specific processing techniques to increase starch availability and digestibility in the gastro-intestinal tract of feedlot cattle. Such improvements can increase the nutritional value of feed and may contribute to better feed conversion efficiency (Owens et al., 1986; Hoffman et al., 2011; Bernardes & Castro, 2019).

2.2 Enteric methane emissions

The sustainability of the beef industry has become a widely discussed topic in the news and on social media, with attention often focusing on the potential negative impacts on the environment and climate (Dressler et al., 2024). The primary GHG emissions from livestock include CO_2, CH_4, and N_2O. Methane, a significant GHG, was first detected in the atmosphere in the 1940s (Migeotte, 1948). Since then, atmospheric CH_4 concentration has been correlated with population growth (Moss et al., 2000), with anthropogenic activities contributing approximately 60% of global CH_4 emissions (Intergovernmental Panel on Climate Change - IPCC, 2022).

There are three main sources of CH_4 emissions globally: natural (such as swamps, oceans, and termite populations), those associated with energy generation and waste management (including gas

burning, coal, waste ponds, and landfills) and emissions associated with agricultural activities (such as rice cultivation and livestock) (Millen et al., 2016). Ruminant livestock species, such as cattle, buffalo, sheep, and goats, emit CH_4 from the fermentation of feedstuffs during digestion. In cattle, CH_4 emissions arise from enteric fermentation (85-90%) and, to a lesser extent, from fecal excretion (Pereira et al., 2015).

The primary products of fermentation in the rumen are VFAs, including acetate, propionate, and butyrate. However, fermentation also generates byproducts such as hydrogen (H_2), ammonia, and CO_2 (Janssen, 2010). During glycolysis, when NAD+ is reduced to NADH, the released H_2 must be re-oxidized to regenerate NAD+ for subsequent phosphorylation. Methanogenic archaea in the rumen utilize these byproducts, particularly H_2, to produce CH_4 (McGovern et al., 2020). These organisms act as terminal electron acceptors (Pereira et al., 2015) and are considered the primary sink for H_2 in the rumen (Ellis et al., 2008).

Methanogens play a crucial digestive role in the rumen by removing H_2, which could otherwise accumulate and inhibit fermentation rates and microbial function (Ellis et al., 2008; Van Kessel & Russell, 1996; McAllister & Newbold, 2008). Consequently, the metabolic pathways involved in H_2 production and utilization are critical factors to consider when developing strategies to reduce CH_4 emissions (Martin et al., 2010).

Economically, CH_4 production from enteric fermentation in beef cattle represents a decreased efficiency in cattle production. According to Johnson & Johnson (1995) these emissions result in a loss of 2% to 12% of GE intake, thereby reducing the potential for conversion of GE into ME. The proportion of GE lost to CH_4 production is highly dependent on the acetic to propionic acid ratio (A:P) in the rumen, which is primarily influenced by the type of feed; high forage/roughage diets are typically associated with a higher A:P, potentially leading to greater CH_4 associated production losses (Johnson & Johnson, 1995).

High-energy diets show increased DM digestibility and greater ruminal propionate proportions, reflecting their higher starch and lower fiber content. This composition contributed to reduction in calculated CH_4 loss and an increase in ME content in high-energy diets. Therefore, high-energy diets are likely optimal for precision feeding strategies, especially when aiming to reduce the environmental footprint of cattle production (Galyean et al., 2023).

Reducing CH_4 production from beef cattle theoretically may increase production by converting more digestible energy (DE) into ME that would otherwise be lost to gas production. For instance, Beauchemin et al. (2008) observed that dietary interventions targeting methanogenesis can increase ME, supporting higher body weight gain rates in beef cattle. Similarly, Knapp et al. (2014) emphasized that reducing CH_4 emissions can free up energy, directly supporting productivity and demonstrating the

potential of mitigation strategies to increase cattle production. Minimizing enteric CH_4 emissions, while increasing biological efficiency and the utilization of dietary nutrients is essential for achieving sustainable livestock production (Min et al., 2020).

2.3 Additives

Roughage inclusion levels in finishing diets offered to cattle are reportedly to be 16.8% of the diet (on a DM basis) in finishing diets (Silvestre & Millen, 2021), which represents approximately a 42% reduction compared to the initial Brazilian feedlot survey conducted in 2009 (Millen et al., 2009). This reduction aligns with an increasing trend toward incorporating high-starch content into feedlot diets, a strategy often adopted to maximize growth performance and feed efficiency (Crawford et al., 2022).

Nonetheless, energy-dense diets containing high levels of rapidly fermenting carbohydrates can lead to organic acid accumulation (Boerman et al., 2015). Thus, resulting in a drop in ruminal pH due to the production rate of the acids being higher than the absorption rate, which consequently elevates the risk of metabolic disorders, such as sub-acute ($5.6 > pH < 5.8$) and acute ($pH < 5.6$) ruminal acidosis, bloating, and potentially other downstream issues like ruminitis and liver abscesses. These disorders can amplify inflammatory responses due to damage to the rumen epithelium and cecum, ultimately impacting animal productivity (Owens et al., 1998; Nagaraja & Lechtenberg, 2007).

Manipulating ruminal fermentation is a crucial strategy for enhancing production efficiency, a primary objective in ruminant nutrition research. Furthermore, the use of feed additives is widely recognized as a direct approach to influence ruminal fermentation (Stivari et al., 2014; Clemmons et al., 2019). According to Silvestre & Millen (2021), 99.8% of surveyed nutritionists reported that their clients utilized some form of additive in finishing diets, with antibiotic ionophores, such as monensin, being the most used (86.1% of the participants recommended its use). However, public concerns regarding antibiotic use in animal nutrition, driven by the precautionary principle, have increased. The emergence of antibiotic-resistant bacterial strains in animal-derived products has prompted some countries to implement regulatory measures to address this concern (Russell & Houlihan, 2003; Cameron & McAllister, 2016).

As a result, various alternatives to ionophores have been investigated to provide safe and effective solutions for ruminant nutrition. These include calcareous marine sources (Cruywagen et al., 2015; Neville et al., 2019) as well as sodium bicarbonate (Rauch et al., 2012).

2.3.1 Calcareous marine algae

Calcareous seaweed (*L. calcareum*) is a renewable mineral resource primarily composed of calcium and magnesium carbonate ($CaCO_3$ and $MgCO_3$, respectively), along with variety of

micronutrients, including Fe, Mn, B, Ni, Cu, Zn, Mo, Se, and Sr (Dias, 2000). The unique honeycomb structure of this seaweed allows for a gradual mineral release in the rumen, extending its buffering action compared to sodium bicarbonate (Cruywagen et al., 2015). This structural feature may explain the algae's mode of action and their potential for mitigating CH_4 emissions, by absorbing CH_4 molecules.

Calcareous marine algae, which contain over than 30% calcium predominantly as calcium carbonate, act as an effective buffer: bicarbonate ions (HCO_3^-) neutralize one proton, whereas carbonate ions (CO_3^{2-}) can neutralize two protons (Rossi et al., 2019). Supporting this, Rossi (2019) observed that calcareous marine algae reduced the incidence of acidosis-related disorders and improved average daily gain (ADG), feed conversion, and both fiber and DM digestibility compared to sodium bicarbonate. These benefits can be attributed to the superior buffering capacity of the algae, as indicated by a higher ruminal pH (6.25 [calcareous marine algae] vs 5.62 [sodium bicarbonate]) and reduced pH fluctuations. Specifically, animals receiving sodium bicarbonate treatment spent an average of 4:09 h/day with ruminal pH < 5.6, whereas those supplemented with calcareous marine algae only spent 00:12 h/day below this threshold during the transition from adaptation to finishing diets. Additionally, this high proton-neutralizing capacity of these algae may also explain their role in reducing CH_4 emissions.

Previous studies (Calitz, 2009; Mesgaran et al., 2013) indicated that calcareous marine algae could act as a buffer in lactating dairy cow diets. When included at 0.3% of dietary DM, calcareous marine algae resulted in higher milk yield compared to diets containing 0.125% (Cruywagen et al., 2004). The optimal dose to maximize milk production was determined to be 80 g calcareous marine algae DM/head/day. No additional gains were observed, with buffer levels at 0.6% or 0.9%. In comparison to sodium bicarbonate (180 g/head/day), *L. calcareum* offered at 90 g DM/head/day increased rumen pH and supported higher milk yields of dairy cows on a high-concentrate diets, when compared to the control group (Cruywagen et al., 2007).

In a study by Comparin et al. (2013), heifers on pasture consuming 1 kg protein-energy supplement DM/head/day were compared to those consuming the same supplement at 0.99 kg DM/animal/day plus 0.01 kg of *L. calcareum*. No statistical differences in DM intake, ADG, dressing percentage, or rumen pH were observed. However, supplementation with calcareous algae reduced ADG by 8.7% numerically, the authors suggested that the amount of *L. calcareum* used may have been insufficient to induce significant changes in animal production and rumen fermentation parameters.

Makkar et al. (2016) reviewed the application of various seaweeds in animal nutrition, noting that data on their use with ruminants remains limited. This underscores the need for further research on calcareous seaweed, particularly its buffering effects on pH control and animal production. Additionally, studies investigating the influence of *L. calcareum* alone on GHG production are yet to be conducted.

2.3.2 Sodium bicarbonate

Sodium bicarbonate, a fast-acting source of bicarbonate (HCO_3^-), is commonly used as a buffer in cattle diets to neutralize rumen acids, which in turn raises rumen pH. This makes sodium bicarbonate supplementation a cost-effective and efficient strategy for preventing rumen acidosis, particularly in high-energy diets that promote increased ruminal fermentation and the production of short-chain fatty acid (SCFA; Van Soest, 1994). However, due to its high solubility and short duration in the rumen, sodium bicarbonate alone may not sustain prolonged pH stability over time, and thus, longer-lasting buffering strategies may be warranted.

Erdman (1988) reviewed 83 studies and observed that sodium bicarbonate supplementation increased DM intake (DMI) and fat-corrected milk production in corn silage-based diets but had no effect in alfalfa haylage or other hay crop silages. The findings suggest that the high fiber content in alfalfa haylage may inherently stimulate greater saliva production, which itself serves as a natural buffer by promoting chewing activity and, consequently, promoting bicarbonate and phosphate ion release.

The buffering action of bicarbonate ions is essential for maintaining an optimal ruminal pH for microbial function, typically in the range of 5.8 to 6.8 (Cunningham & Klein, 2008). In addition to dietary supplementation, endogenous bicarbonate enters the rumen via saliva, which contributes approximately 250 g of Na_2HPO_4 and 1 to 2 kg of $NaHCO_3$ daily (Cunningham & Klein 2008). This salivary buffering is complemented by bicarbonate influx through the rumen epithelium, which releases HCO_3^- during SCFA absorption. These combined mechanisms help regulate rumen pH and mitigate acid accumulation, particularly in diets with high fermentation potential.

Buffering in the rumen involves the removal of hydrogen ions (H^+) via both bicarbonate and phosphate systems. In the bicarbonate system, H^+ combine with bicarbonate (HCO_3^-) to form carbonic acid (H_2CO_3), which then decomposes into CO_2 and water (H_2O).

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

CO_2 is expelled through eructation, while the remaining water and bicarbonate ions sustain rumen pH (Van Soest, 1994). Phosphate, which has a pKa of 7.2, also contributes under near-neutral pH. However, bicarbonate (pKa of 6.1) is responsible for 82% of the total buffering capacity of bovine saliva (Millen, 2016), varying with the ruminal content's pH.

Daily dosing recommendations for sodium bicarbonate typically range from 110 to 225 g/cow but may be adjusted based on diet composition and rumen pH requirements. Calculations for sodium bicarbonate inclusion rate can also be expressed as a percentage of the diet to optimize rumen buffering according to specific dietary conditions (Erdman, 1988).

3. OBJECTIVE

The overall objective was to evaluate the effect of a calcareous marine algae (*L. calcareum*) on feedlot performance, carcass traits, nutrient digestibility, nitrogen metabolism, urinary and fecal pH, enteric methane emissions of Nellore heifers during the finishing phase.

4. MATERIAL AND METHODS

The experiment was conducted at the Research Feedlot of the Department of Animal Science (DZO), College of Animal Science and Veterinary Medicine (FZMV), Federal University of Lavras (UFLA), in Lavras, State of Minas Gerais, Brazil. All experimental protocols and activities in this trial were approved by the Ethics Committee on Animal Use of UFLA (protocol number 019/21).

4.1 Experimental design, animals, and housing

Nellore heifers (n = 36; 15 months of age, 270 ± 7 kg initial body weight [BW], mean ± SD) were allocated to 36 individual pens in a randomized complete block design experiment. Heifers were randomly assigned to concrete and semi-covered pens (18 pens/treatment; 20 m²), each equipped with an individual feed bunk and an automatic water trough. Upon arrival at the feedlot, before the step-up diets, the heifers were offered a receiving diet containing 80% corn silage, 17% ground corn, 1.6% soybean meal, 0.4% urea, and 1.0% mineral mixture (DM basis) to allow for adaptation to the new environment. Prior to the beginning of the experiment, heifers were processed using the following protocol: dewormed (Treo® Ace; Zoetis, Brazil) and vaccinated against rabies (Rai-Vet; Laboratório Bio-Vet S/A, Vargem Grande Paulista, São Paulo, Brazil), respiratory diseases, and leptospirosis (CattleMaster® 4+L5; Zoetis), as well as received prophylaxis for botulism and other ruminant diseases (StarVac®; Labovet Produtos Veterinários Ltda, Feira de Santana, Bahia, Brazil). After a 21-day adaptation period to the facilities, the heifers were gradually adapted to experimental diets over 12 days using three step-up diets (four days for each step), with an incremental increase of 5% in concentrate (from 60% to 65% to 70%, respectively), until reaching the finisher diet containing 75% concentrate and 25% corn silage inclusion (DM basis). The experimental period lasted 96 days, consisting of 12 days for diet adaptation and 84 days for the finishing phase, which was divided into three periods (days 13–40, 41–68, and 69–97).

4.2 Dietary treatments

The experimental treatment diets involved the inclusion of two different feed additives (i.e., calcareous marine algae [*L. calcareum*] and sodium bicarbonate) in the otherwise identical finishing ration fed to all heifers. The inclusion of corn silage was 25.0% (DM basis), and the remainder of the

diets consisted of 63.3% ground corn, 5.8% soybean meal from a single lot, 1.2% feed-grade urea, and 2.0% of a feedlot premix (without any additives). A commercial seaweed product (Beecomb®, SeaQ Corporation, Sant Cugat del Vallès, Spain) was supplemented at 0.9% DM in the diet, allowing the heifers to consume approximately 60 g *L. calcareum*/heifer/day. The sodium bicarbonate treatment (1.8% DM in the diet, providing 110 g/heifer/day) was used as a positive control. All the diets were formulated to provide sufficient energy, protein, minerals, and vitamins to meet BR-CORTE (2023) requirements for Nellore heifers in the finishing phase, targeting an estimated ADG of 1.3 kg/day.

Table 1. Ingredients and nutritional composition of the experimental diets

Item	Sodium Bicarbonate	Calcareous marine algae
Ingredient, % of DM		
Corn Silage	25.0	25.0
Corn, dry ground	63.3	63.3
Soybean meal	5.8	5.8
Feed-grade urea	1.2	1.2
Feedlot premix	2.0	2.0
Sodium Bicarbonate	1.8	-
Limestone	0.9	-
Seaweed (*Lithothamnium*)	-	0.9
Kaolin	-	1.8
Estimated chemical composition, % of DM		
DM, % as-fed	70.67	70.74
Crude Protein	12.66	12.76
Starch	47.50	47.50
NDF	23.17	23.44
Ether extract	3.30	3.30

4.3 Feeding management and measurements

Corn was ground to a mean particle size of 1.42 mm, and particle size distribution was described according to Yu et al. (1998), using sieves with square pores of 6.0, 3.5, 2.0, and 1.25 mm (Produtest T Model; Telastem Peneiras para Análises Ltda, São Paulo, SP, Brazil). The particle size of corn silage was determined according to the Penn State Particle Separator method (Lammers et al., 1996), using sieves of 19, 8, and 4 mm. All ingredients were weighed on a fixed scale for each animal. Total mixed rations (TMR) were manually mixed and offered *ad libitum* twice daily at 0700 and 1600 h with daily allowances based on previous days intake ± 5%. Samples of all ingredients in each concentrate batch and corn silage were collected daily throughout the study, while one sample of the leftovers from each pen was collected twice a week. A weekly representative diet composite sample was obtained and

partially dried in a forced-air oven (55°C for 72 h). The weekly DM content of corn silage was used to adjust the ingredient proportions in the diets if DM deviated by more than 3% from the average. These samples were ground through a 1 mm screen using a Willye Super-type mill (STAR FT-80; Fortinox; Marconi Ltda, Piracicaba, SP, Brazil), and the laboratory DM content of the samples was determined by drying at 105°C for 16 h. Dry matter intake for each pen was calculated as the difference between the amount of feed offered each day and the total amount of feed refused at the end of each week, after correction for DM content of both feed offered and feed refused.

4.4 Animal production and carcass traits

Once the heifers were assigned to their respective treatment groups, they were reweighed individually on days 0 and 97 of the experimental period, after a 16-hour feed curfew). Body weight was also measured on days 13, 41, and 69 of the experimental periods, and shrunk body weight (SBW) was estimated using a non-linear equation developed for Zebu cattle to account for gut fill ($0.8915 \times BW^{0151}$; BR CORTE - 2023). The ADG was calculated as the difference between the initial, intermediate, and final shrunk BW, divided by the number of days of each period or overall. The gain-to-feed (G:F) ratio was calculated as ADG divided by DM intake for each period and over the entire experimental period. At the end of the experiment, the heifers were transported to a commercial abattoir (Frigorífico Iper Ltda., Divinópolis, MG, Brazil) for carcass measurements. Pre-slaughter handling was conducted following good animal welfare practices, and slaughtering procedures followed strict guidelines established and regulated by the Sanitary and Industrial Inspection Regulation for Animal Origin Products in Brazil (Brazil, 1997). Dressing percentage was calculated based on the final hot carcass weight (HCW) and BW after fasting.

4.5 Urine and fecal sampling

Fecal grab samples were collected from the rectum of individual heifers each day within the three separate sample collection periods throughout the experimental period (i.e., days 27 to 32, 55 to 60, and 83 to 88) To ensure sample representation and homogeneous sampling, samples were collected at 0600 h, 0900 h, 1200 h, 1500 h and 1800 h on days 1, 2, 3, 4 and 5 of each collection period respectively. A sub-sample (10 g) of each sample was homogenized with an equal weight of deionized water for subsequent analyses. Another sub-sample of feces was stored to prepare a composite sample representative of the entire collection period, which was later used for additional laboratory analyses.

Urine spot samples were collected concurrently, and urine pH was immediately measured using a portable pH meter (TEC-7p MPp, MS Tecnopon Equipamentos Especiais LTDA, Piracicaba, SP). Daily aliquots of urine (2 mL) were acidified in 0.036 N sulfuric acid (8 mL) and stored at -20°C. After each sampling period (described above), the frozen subsamples were pooled to form a composite sample. This composite sample was used to determine the concentration of purine derivatives for each heifer,

which are considered indirect indicators of microbial protein synthesis in the rumen (Chen & Gomes, 1995; Valadares et al., 1999).

4.6 Apparent total tract digestibility

The apparent total tract nutrient digestibility was assessed using samples of feed offered and refused and feces collected during the three five-day sample collection described in section 4.5. Feed offered and residues (section 4.3), and fecal samples (section 4.5) were collected on each day of the collection period and oven-dried (55°C), ground to pass through a 2 mm screen, and a subsample of approximately 50% of the ground material was further ground to pass through a 1 mm screen for subsequent chemical analysis. After conducting the studies, digestibility was calculated as follows:

Fecal Excretion (gDM/day) = iNDF intake (g/day) / [iNDF] fecal (g/gDM)

Apparent total tract digestibility = [(Intake – Fecal Excretion) / Intake] × 100

4.7 Enteric methane emission

Enteric CH_4 emission was measured from individual heifers using the sulfur hexafluoride (SF_6) tracer gas technique (Johnson & Johnson, 1995) in each of three separate collection periods which were immediately prior to the fecal and urine sample collection periods (section 4.5). One month prior to the first collection period, a small brass permeation tube was placed in the rumen to allow the tracer gas to equilibrate in the rumen. Five days prior to the first gas collection period, the heifers were fitted with gas collection halters to acclimate to the collection apparatus prior to sample collection. The setup included a 0.127 mm stainless steel capillary tube halter, and a 15 μm in-line filter was placed on the heifer's head and connected to an evacuated sampling vessel (Johnson et al., 2007). Before the experiment, collected canisters made of polyvinyl chloride were attached to a vacuum pump in the laboratory to create negative pressure (approximately −13.15 psi). As the vacuum in the sampling vessel slowly dissipated, the negative pressure continuously drew in the air sample around the animals' mouth and nose. The gas samples were obtained continuously through a capillary tube connected to a collecting container placed on the neck of each heifer. Canisters were removed from heifers at 0600 h each day with the sampled gas representative of gases present in breath samples collected over the previous 24 h period. Additional PVC canisters were placed near the experimental feedlot to monitor the ambient daily concentration ("basal concentration") of CH_4 and SF_6 during each sampling period.

After collection of gas samples from canisters, pure nitrogen was added (approximate pressure of 1.5 psi above ambient) to the canisters, and then CH_4 and SF_6 concentrations were analyzed using gas chromatography (Agilent HP-6890, Wilmington, DE, USA; or Shimadzu® GC-2014, Kyoto, Japan). Both chromatographs were equipped with a flame ionization detector and a megabore column (0.53 mm, 30 m) Plot HP-Al/M ([for CH_4]) and an electron capture detector (μ-ECD) and megabore column HP-

MolSiv ([for SF_6]), with two 0.5 cm^3 loops coupled to two six-way valves. The calibration curves were established using gas standards certified by White Martins (Praxair), with concentrations in ppt (54 ± 9, 97 ± 9, and 954 ± 98 ppt) for SF_6 and in ppm (0.996; 4.98; 10.36, and 51.57 ppm) for CH_4, according to Westberg et al. (1998). The CH_4 flux released by the animal was calculated from the SF_6 flux, correlating the results to the known rate of tracer release in the rumen (Westberg et al., 1998), following the equation:

$$CH_4\ emission = QSF_6 \times \left\{\frac{\left[(CH_4)_y - (CH_4)_b\right]}{\left[(SF_6)_y - (SF_6)_b\right]}\right\}$$

where CH_4 emission = CH_4 emission rate per animal; QSF_6 = known SF_6 emission rate from the capsule in the rumen; $(CH_4)_y$ = CH_4 concentrations in the collection device; $(CH_4)_b$ = basal concentration of CH_4; $(SF_6)_y$ = SF_6 concentration in the collection device; and $(SF_6)_b$ = basal SF_6 concentration.

The CH_4 emissions were expressed as CH_4 production (g/day), CH_4 yield (g/kg DM intake) and CH_4 intensity (g/kg ADG).

4.8 Sample analysis

Samples of feed offered, including individual ingredients, feed residues and feces were ground in a knife mill to pass through a 2 mm screen with approximately 50% then subsequently ground through a 1 mm screen. Samples of each material ground through 1 mm sieves (feed offered, feed residues and feces) were analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012) for DM content (dried overnight at 105°C; method INCT-CA no. G-003/1), ash content (complete combustion in a muffle furnace at 600°C for 4 h; method INCT-CA no. M-001/1), with organic matter (OM) calculated by difference, neutral detergent fiber (NDF) corrected for ash and protein content (NDFap; using heat-stable α-amylase, omitting sodium sulfite, and correcting for residual ash and protein; method INCT-CA no. F-002/1), and nitrogen (N) content (Leco FP-528; Leco Corp., St Joseph, MI, USA). The crude protein (CP) content was calculated by multiplying the nitrogen content by 6.25 (Van Soest, 1994). From samples processed through a 2 mm sieve, an internal marker (indigestible NDF [iNDF]) was used to estimate total fecal output. The iNDF content was determined as the residual NDF remaining after 288 h of ruminal *in situ* incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY), according to Valente et al. (2011). Starch analysis followed the recommended INCT-CA procedure (method No G-007/1). Measurements were taken using a UV/visible spectrophotometer set to 630 nm.

The concentration of creatinine, allantoin, uric acid, and urea in urine were determined by spectrophotometry. Creatinine and allantoin were analyzed using the colorimetric methods of Bartels et al. (1972) and Chen & Gomes (1995), respectively. Uric acid and urea were measured with enzymatic

kits. Excretion of purine derivatives was calculated from the sum of the amounts of allantoin and uric acid excreted in the urine. From this finding, the absorbed purines were calculated by the following equation (Verbic et al., 1990):

$$AP = \frac{PD - 0.385 \times BW^{0.75}}{0.85}$$

where AP is the number of absorbed purines (mmol/d), PD is the amount of excreted purine derivatives (mmol/d), 0.85 is the recovery of absorbed purines as purine derivatives in the urine (mmol/mmol), and 0.385 is the excretion of endogenous purine derivatives in the urine per unit of metabolic size (mmol).

The microbial synthesis of nitrogenous compounds in the rumen (Nmic, g/d) was estimated according to Chen & Gomes (1992) as follows:

$$Nmic = \frac{70 \times AP}{0.83 \times R \times 1{,}000}$$

where R is the N_{RNA}:N_{TOTAL} ratio in the microorganisms (mg/mg), 70 is the nitrogen content in purines (mg/mol), and 0.83 is the intestinal digestibility of the microbial purines (mg/mg). It was adopted R = 0.176 (Valadares Filho, 1995).

4.9 Statistical analysis

All statistical analyses were conducted using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with animals as the experimental unit. Treatments and feedlot periods were treated as fixed effects, and pens were treated as random effects. The statistical model for data analysis was as follows:

$$Y_{ijm} = \mu + T_i + P_j + A_m + \varepsilon_{ijm}$$

where: Y_{ijm} = variable from treatment i, feedlot period j, and animal m; μ = average; T_i = fixed effect of treatment I; P_j = fixed effect of period j; A_m = random effect of animal m; ε_{ijm} = error.

Statistical analyses were conducted to compare treatment effects using Fisher's protected least significant difference (LSD) test. A similar model was applied to data on urine and fecal pH, CH_4 production, CH_4 yield and CH_4 intensity, incorporating time as a repeated measure. For these repeated measures, either compound symmetry or heterogeneous compound symmetry was used as the covariance structure, with the optimal choice determined by the lowest Akaike information criterion (AIC). When a significant treatment × time interaction was observed, simple effects were analyzed using the SLICE option within the LSMEANS statement. In the absence of interaction, and when the main effects of diet and time were significant, comparisons among diets and time points were also conducted

using the LSD test. Statistical significance was defined as $P \leq 0.05$, and results with $0.05 < P < 0.10$ were interpreted as indicative of trends.

5. RESULTS

5.1 Growth performance

No period × treatment interactions were observed for feedlot performance or carcass characteristics measured at the slaughterhouse ($P \geq 0.560$).

By design, initial BW did not differ between treatments ($P = 0.923$; Table 2). Heifers fed diets containing calcareous marine algae had similar final BW ($P = 0.991$), ADG ($P = 0.991$), DM intake ($P = 0.692$), and G:F ($P = 0.560$) compared to those receiving sodium bicarbonate. Additionally, carcass traits measured at slaughter, including HCW ($P = 0.695$) and dressing percentage ($P = 0.682$), were not affected by treatment.

The DM intake ($P < 0.001$) of heifers was lowest in the first period (6.48 kg/heifer/day), followed by the second period (7.02 kg/heifer/day), and highest during the third period (7.33 kg/heifer/day). The ADG of heifers was significantly higher ($P < 0.001$) during the third period of the experiment (1.31 kg/heifer/day) compared to the first and second (0.849 kg/day) which were not significantly different from each other. Consequently, G:F was influenced by period ($P < 0.001$), with the third period (0.18) having a more efficient feed conversion compared to the first and second periods (0.13).

Table 2. Effects of sodium bicarbonate and calcareous marine algae on performance and carcass traits of Nellore heifers during the finishing phase

Item	Sodium bicarbonate	Calcareous marine algae	SEM	*P*-value
Feedlot production				
Initial BW, kg	268	269	7.3	0.923
Final BW, kg	368	368	4.1	0.991
Average daily gain, kg	0.902	0.902	0.037	0.991
DM intake, kg	7.19	7.08	0.28	0.692
G:F	0.127	0.130	0.005	0.560
Carcass traits				
HCW, kg	203	202	2.26	0.695
Dressing, %	55.6	55.5	0.42	0.681

SEM = standard error of the mean; BW = body weight; HCW = hot carcass weight; DM – dry matter; G:F = gain:feed

5.2 Urine and fecal pH

There was a tendency to a significant treatment x period interaction for urine pH (Table 3; P = 0.055). Urine pH was significantly lower ($P < 0.001$) for heifers consuming the ration containing the calcareous marine algae on the first (7.88 *vs.* 7.26), second (8.18 *vs.* 7.83), and third (8.26 *vs.* 7.91) collection periods compared to those consuming the ration containing sodium bicarbonate. Urine pH was also significantly lower ($P < 0.001$) during the first collection period (7.57) compared to the second (8.00) and third (8.09) collection periods, which did not differ.

No period × treatment interaction was detected for fecal pH (P = 0.529). Fecal pH was unaffected by dietary treatment at each of the collection periods and when averaged across the entire experimental period (Table 3; P = 0.144). However, fecal pH was significantly lower in the second collection period (5.93) compared to samples collected during the first (6.14) and third (6.14) collection periods.

Table 3. Effects of sodium bicarbonate and calcareous marine algae on the pH of urine and faeces collected from Nellore heifers during the finishing phase

Item	Treatment		Period	SEM	*P*-value		
	Sodium bicarbonate	Calcareous marine algae			Treatment	Period	Treatment*Period
Urine pH							
Day 35	7.88	7.26	7.57[a]	0.07	<0.001	<0.001	0.055
Day 63	8.18	7.83	8.00[b]				
Day 91	8.26	7.91	8.09[b]				
Overall	8.11	7.67	7.89				
Fecal pH							
Day 35	6.16	6.12	6.14[a]	0.06	0.144	<0.001	0.529
Day 63	5.94	5.93	5.93[b]				
Day 91	6.22	6.07	6.14[a]				
Overall	6.11	6.03	6.07				

5.3 Apparent total tract nutrient digestibility

Total tract nutrient digestibility of DM (P = 0.434), OM (P = 0.212), starch (P=0.691), NDF (P = 0.315) and CP (P = 0.902) were unaffected by the inclusion of calcareous marine algae in the ration (Table 4).

Table 4. Effects of sodium bicarbonate and calcareous marine algae on the apparent total tract digestibility of nutrients by Nellore heifers during the finishing phase

Item	Sodium bicarbonate	Calcareous marine algae	SEM	*P*-value
Apparent total tract digestibility, %				
Dry matter	60.85	62.27	1.63	0.434
Organic matter	62.18	64.42	1.61	0.212
Starch	86.25	85.50	1.61	0.691
Neutral detergent fiber	44.33	47.13	2.69	0.315
Crude protein	61.74	61.98	1.37	0.902

5.4 Nitrogen utilization

Nitrogen (N) intake was similar between heifers fed sodium bicarbonate and those fed calcareous marine algae (145.7 g/d vs. 143.2 g/d; $P = 0.668$). Fecal N excretion tended to be lower in the calcareous marine algae treatment compared to sodium bicarbonate (53.82 vs. 47.87 g/d; $P = 0.079$). Urinary N excretion (65.80 vs. 69.96 g/d; $P = 0.507$) and total N excretion (116.54 vs. 109.01 g/d; $P = 0.174$) were not affected by treatments.

Urinary excretion of purine derivatives did not differ significantly between the treatments. Specifically, allantoin (92.19 vs. 82.23 mmol/d; $P = 0.270$), uric acid (10.51 vs. 9.26 mmol/d; $P = 0.195$), and total purine derivative excretion (102.7 vs. 91.48 mmol/d; $P = 0.262$) showed no significant differences. Similarly, estimated microbial protein synthesis did not differ between treatments ($P = 0.262$), with values of 41.50 and 35.18 g/d for sodium bicarbonate and calcareous marine algae, respectively.

The efficiency of microbial protein synthesis was not influenced by dietary treatment, whether expressed as grams per kilogram of digestible organic matter (5.87 vs. 5.00 g/kg DOM; $P = 0.355$) or crude protein intake (44.28 vs. 37.18 g/kg CP; $P = 0.316$), for sodium bicarbonate and calcareous marine algae, respectively.

Table 5. Effects of sodium bicarbonate and calcareous marine algae on nitrogen metabolism and estimated microbial protein supply by Nellore heifers during the finishing phase

Item	Sodium bicarbonate	Calcareous marine algae	SEM	*P*-value
N intake, g/day	145.7	143.2	5.81	0.668
N excretion				
Fecal, g/day	53.82	47.87	3.03	0.079
Urinary, g/day	65.80	69.96	4.38	0.507
Total N excretion, g/day	116.54	109.01	4.95	0.174
Purine derivarites excretion				
Allantoin, mmol/day	92.19	82.23	9.81	0.270
Uric acid, mmol/day	10.51	9.26	1.04	0.195
Total purine derivatives, mmol/day	102.7	91.48	10.85	0.262
Microbial protein				
Nmic, g/day	41.50	35.18	6.12	0.262
Efficiency of microbial protein synthesis				
g/kg DOM	5.87	5.00	1.04	0.355
g/kg CP	44.28	37.18	7.81	0.316

5.5 Enteric methane emissions

No period × treatment interactions were observed for CH_4 production (g/day; $P = 0.859$), CH_4 yield (g/kg DMI; $P = 0.772$), or intensity (g/kg ADG; $P = 0.737$).

Methane production tended to be reduced ($P = 0.075$) by 8.2% in heifers fed rations containing calcareous marine algae compared to heifers consuming rations containing sodium bicarbonate. No differences were observed between the three sampling periods ($P = 0.929$).

Calculated CH_4 yield ($P = 0.204$) and CH_4 intensity ($P = 0.315$) did not differ between heifers fed rations containing the calcareous marine algae or sodium bicarbonate. Additionally, calculated CH_4 yield did not differ across the three sampling periods ($P = 0.142$). However, calculated CH_4 intensity was significantly lower ($P < 0.001$) in the third sampling period compared to the first two sampling periods, which did not differ from each other.

Table 6. Effects of sodium bicarbonate and calcareous marine algae on enteric methane emissions from Nellore heifers during the finishing phase

Item	Sodium bicarbonate	Calcareous marine algae	SEM	*P*-value		
				Treatment	Period	Treatment*Pediod
Methane emissions						
Production, g/day						
d 27	145	134				
d 55	150	132	8.32	0.075	0.929	0.859
d 83	147	138				
Overall	147	135				
Yield (g/kg DM intake)						
d 27	22.1	21.6				
d 55	21.8	19.3	1.31	0.204	0.142	0.772
d 83	20.3	19.1				
Overall	21.4	20.0				
Intensity (g/kg ADG)						
d 27	169.2	168.8				
d 55	170.3	166.0	10.73	0.315	<0.0001	0.737
d 83	111.6	102.4				
Overall	155.0	145.7				

6. DISCUSSION

L. calcareum, a red calcareous marine algae harvested from northern Europe and South America, is valued for its high concentrations of bioavailable calcium and magnesium. Calsamiglia et al. (2011) suggest that its buffering capacity may help stabilize ruminal pH, reduce the risk of subacute acidosis, and improve feed efficiency. This action may explain the lack of differences observed in DM intake in the present study, suggesting that *L. calcareum* maintained rumen pH at levels comparable to sodium bicarbonate, a conventional buffering agent widely used in livestock diets. Moreover, the similarity in DMI between treatments suggests that both additives promoted similar effects on feed acceptability. These findings align with Rossi et al. (2019), Cruywagen et al. (2015) and Wu et al. (2015) who also reported no significant effects on DM intake when replacing sodium bicarbonate with *L. calcareum* in cattle diets. Similarly, Guerreiro et al. (2025) reported no significant differences in ADG, DMI, or G:F when comparing the supplementation of calcareous marine algae in combination with monensin to monensin alone in beef cattle. These findings are consistent with the present study, in which performance parameters such as ADG and G:F ratio were also not affected by the inclusion of calcareous marine algae. Given DM intake is a major determinant of cattle productivity, the absence of any differences in DM intake in the current experiment likely explains the lack of significant differences in ADG and carcass traits (NASEM, 2021). The results of the current experiment demonstrate that there

are no negative effects of including *L. calcareum* in the ratio of finishing beef heifers. Given the growing demand for sustainable and antibiotic-free livestock production, *L. calcareum* emerges as a promising additive for feedlot operations seeking to balance productivity with environmental responsibility.

The inclusion of *L. calcareum* did not significantly affect the apparent total tract digestibility of DM, OM, NDF, or CP when compared to sodium bicarbonate. These results suggest that the use of *L. calcareum* as a buffering agent does not impair nutrient utilization and maintains digestive efficiency comparable to conventional buffers. Although statistical differences were not observed, numerical improvements in fiber digestibility were noted in heifers fed rations containing the calcareous marine algae. This numerical difference may be associated with higher ruminal pH conditions, particularly during the finishing phase, promoted by the gradual and prolonged release of minerals from *L. calcareum* due to its porous structure (Cruywagen et al., 2015). Stabilization of ruminal pH is particularly relevant for maintaining fibrolytic microbial populations, the effect of pH on fiber digestion in the rumen has been extensively documented (Erdman, 1988; Russell & Wilson, 1996). Previous studies have shown that the activity of cellulolytic bacteria declines when pH drops below 5.7 (Dijkstra et al., 1992; Lescoat & Sauvant, 1995; Imamidoost & Cant, 2005). While this mechanism remains hypothetical in the context of our study, it highlights the importance of rumen pH stability for optimal fiber utilization. Our findings are consistent with those of Montanez-Valdez et al. (2016), who observed that calcified seaweed in an *in-situ* study in high-concentrate diets for ruminants didn't compromise NDF digestibility. Collectively, these results support the potential use of *L. calcareum* as a functional feed additive capable of promoting digestive stability and efficiency under intensive feeding conditions.

Regarding the pH, these contrasting responses in urinary and fecal pH highlight that systemic and local buffering mechanisms may be independently modulated by the physicochemical properties of dietary additives. This difference may be partially explained by the distinct solubility of the compounds: sodium bicarbonate dissolves readily in water (approximately 9.6 g/100 mL at 20 °C; Lide, 1995), while calcareous marine algae exhibit limited solubility. *L. calcareum* contains various crystalline forms of calcium carbonate ($CaCO_3$), including calcite, aragonite, and vaterite, with decreasing solubility in that order (Railsback, 2006). Additionally, *L. calcareum* is a porous mineral compound with a slower dissolution rate, which may lead to a more gradual and less pronounced systemic buffering response (Cruywagen et al., 2015). The urine pH is generally used as an indicator of metabolic acid or alkali load (Sanchez, 2003). The observed reduction in urinary pH may have reflected alteration in blood pH. The kidneys play a vital role in minimizing this change making the urine pH alkaline, by excreting more H^+ and conserving more HCO_3^- (Roche et al. 2003a). Under alkaline conditions, the kidney increases alkali excretion and suppresses H^+ excretion to maintain normal blood pH so urine pH increases (Cohen & Kassirer, 1982). Increased urine pH indicates that buffers may be useful to alleviate systemic acidosis. Thus, its buffering effect is slower and more prolonged compared to sodium bicarbonate, which is highly

soluble and rapidly dissociates, providing immediate buffering action in both the rumen and systemically. Conversely, no treatment effect was observed on fecal pH, indicating that both additives effectively stabilized ruminal pH and prevented excessive acid flow to the hindgut. However, a period effect was detected, with a reduction in fecal pH during the mid-feedlot phase, reflecting temporal changes in fermentative activity and microbial adaptation to high-concentrate diets.

The similar nitrogen intake observed between heifers fed sodium bicarbonate and those receiving *L. calcareum* confirms the DMI and crude protein digestibility data. Heifers supplemented with *L. calcareum* tended to exhibit lower fecal nitrogen losses compared to those fed sodium bicarbonate, suggesting a slight improvement in nitrogen digestibility, possibly related to the buffering properties of *L. calcareum*, which help maintain a stable ruminal environment favorable for microbial proteolysis. However, when total nitrogen excretion through both urine and feces is considered, a shift in the excretion pathway can be noted. It is important to highlight that urinary nitrogen, although not statistically affected, represents the main route for volatile nitrogen losses to the environment and poses a greater risk of ammonia emissions compared to fecal nitrogen (Castillo et al., 2000). The excretion of purine derivatives, including allantoin, uric acid, and total purines, did not differ significantly between treatments. Since these metabolites are recognized as indirect indicators of microbial protein synthesis (Chen & Gomes, 1995), the absence of significant variation suggests that *L. calcareum* maintained microbial activity at levels comparable to sodium bicarbonate. This finding is consistent with the similar estimates of total microbial protein synthesis and its efficiency, expressed per unit of crude protein or digestible organic matter intake. Nevertheless, such differences may not have been detected due to the absence of direct ruminal sampling.

Although heifers supplemented with *Lithothamnium calcareum* exhibited an 8.2% reduction in daily CH_4 production compared to those receiving sodium bicarbonate, this difference represented a statistical tendency. Previous findings by Guerreiro et al. (2025) demonstrated that the combination of *L. calcareum* with monensin significantly reduced enteric CH_4 emissions compared to a blend of essentials oils. In a recent meta-analysis, Cooke et al. (2024) reported that monensin, when used alone, reduced CH_4 production by approximately 15% across various dietary contexts, confirming its efficacy as a CH_4 mitigation strategy in ruminants. These results suggest that while monensin independently reduces CH_4 emissions, its association with *L. calcareum* may enhance this effect synergistically. Although the present study evaluated the impact of algae alone, the directional response observed suggests a potential role for *L. calcareum* in mitigating CH_4. One possible mode of action may involve its adsorptive capacity, similar to that described for biochar in ruminant diets. The porous, honeycomb-like structure (Cruywagen et al., 2015) of calcified marine algae may promote physical entrapment or adsorption of CH_4 molecules within its matrix. This structural feature, coupled with its slow-release mineral profile, may help stabilize the ruminal environment and facilitate the removal of CH_4 or its

precursors (e.g., H_2) from the rumen ecosystem. Biochar studies have shown that its high surface area and microporosity can function as a sink for gases such as CH_4 and NH_3, thereby modulating microbial fermentation dynamics (Leng et al., 2012a; Saleem et al., 2018). While direct evidence for similar mechanisms in marine algae is limited, the comparable physicochemical characteristics of *L. calcareum* suggest that such adsorptive processes may play a role in reducing enteric CH_4 emissions. In this study, CH_4 yield (g/kg DM intake) and intensity (g/kg ADG) were not significantly affected by treatment. However, a decline in CH_4 intensity was observed over time, particularly during the final feedlot phase, likely due to improved feed efficiency and higher ADG, which are known to reduce methane output per unit of animal productivity (Moss et al., 2000). In summary, although statistical significance was not achieved, the observed trends support the hypothesis that *L. calcareum* may contribute to CH_4 mitigation in finishing cattle. Given the increasing interest in natural feed additives that enhance environmental sustainability without impairing animal performance, it represents a promising strategy that warrants further validation in large-scale, long-duration studies.

7. CONCLUSIONS

In conclusion, the results of this study suggest that *L. calcareum* can serve as a viable natural alternative to sodium bicarbonate in the finishing diets of finishing Nellore heifers, maintaining expected body weight gain, nutrient digestibility, and carcass traits. Furthermore, the additive showed potential to enhance ruminal microbial activity and reduce enteric CH_4 emissions, although further research is required to confirm its efficacy with respect to these variables. Overall, *L. calcareum* demonstrated potential as a sustainable additive for inclusion of rations of beef cattle in finishing systems.

8. REFERENCES

ABIEC – ASSOCIAÇÃO BRASILEIRA DAS INDÚSTRIAS EXPORTADORAS DE CARNE. Perfil da pecuária no Brasil – *Relatório Anual 2019 e 2025. ABIEC*, 2019, 2025.

BARTELS, H.; BÖHMER, M.; HEIERLI, C. Serum creatinine determination without protein precipitation. *Clinica Chimica Acta*, v. 37, p. 193-197, 1972.

BEAUCHEMIN, K. A.; KREUZER, M.; O'MARA, F.; McALLISTER, T. A. Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture*, v. 48, n. 2, p. 21-27, 2008.

BEAUCHEMIN, K. A.; McALLISTER, T. A.; McGINN, S. M. Dietary mitigation of enteric methane from cattle. *CABI Reviews*, 2009, p. 1-18.

BERNARDES, T.; CASTRO, T. PSXII-12 Silages and roughage sources in the Brazilian beef feedlots. *Journal of Animal Science*, v. 97, Suppl. 3, p. 411-411, 2019.

BOERMAN, J. P.; POTTS, S. B.; VANDEHAAR, M. J.; LOCK, A. L. Effects of partly replacing dietary starch with fiber and fat on milk production and energy partitioning. *Journal of Dairy Science*, v. 98, n. 10, p. 7264-7276, 2015.

BR CORTE – COMITÊ DE NUTRIÇÃO DE BOVINOS DE CORTE. BR Corte 4.0 – Tabelas de exigências nutricionais de bovinos de corte (4ª ed.). Pedro Del Bianco Benedeti, Flávia Adriane de Sales Silva, Douglas Teixeira Saraiva, Sidnei Antônio Lopes, Luiz Fernando Costa e Silva, Diego Zanetti e Sebastião de Campos Valadares Filho (Orgs.). *Produção Independente*, 2023.

CALITZ, T. The effect of acid buf and combinations of acid buf and sodium bicarbonate in dairy cow diets on production response and rumen parameters (Doctoral dissertation). *University of Stellenbosch*, Stellenbosch, 2009.

CALLAWAY, T. R.; EDRINGTON, T. S.; RYCHLIK, J. L.; GENOVESE, K. J.; POOLE, T. L.; JUNG, Y. S.; NISBET, D. J. Ionophores: their use as ruminant growth promotants and impact on food safety. *Current Issues in Intestinal Microbiology*, v. 4, n. 2, p. 43-51, 2003.

CAMERON, A.; McALLISTER, T. A. Antimicrobial usage and resistance in beef production. *Journal of Animal Science and Biotechnology*, v. 7, p. 1-22, 2016.

CALSAMIGLIA, S.; BLANCH, M.; FERRET, A.; MOYA, D. Is subacute ruminal acidosis a pH related problem? Causes and tools for its control. *Animal Feed Science and Technology*, v. 172, n. 1-2, p. 42-50, 2012.

CASTILLO, A. R.; KEBREAB, E.; BEEVER, D. E.; FRANCE, J. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. *Journal of Animal and Feed Sciences*, v. 9, n. 1, p. 1-32, 2000.

CHEN, X. B.; GOMES, M. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of the technical details. *International Feed Resources Unit*, Rowett Research Institute, Aberdeen, 1992.

CLEMMONS, B. A.; VOY, B. H.; MYER, P. R. Altering the gut microbiome of cattle: considerations of host-microbiome interactions for persistent microbiome manipulation. *Microbial Ecology*, v. 77, p. 523-536, 2019.

COHEN, J. J.; KASSIRER, J. P. Acid-base. 1st ed. *Little, Brown and Co.,* Boston, 1982.

COMPARIN, M. A. S.; MORAIS, M. da G.; ALVES, F. V.; COUTINHO, M. A. da S.; FERNANDES, H. J.; FEIJÓ, G. L. D.; OLIVEIRA, L. O. F. de; COELHO, R. G. Desempenho, características

qualitativas da carcaça e da carne de novilhas Brangus suplementadas em pastagem recebendo diferentes aditivos nutricionais. *Revista Brasileira de Saúde e Produção Animal*, v. 14, n. 3, p. 574-586, 2013. Disponível em: http://www.rbspa.ufba.br

COOKE, R. F.; ELOY, L. R.; BOSCO, S. C.; LASMAR, P. V.; DE SIMAS, J. M.; LEIVA, T.; DE MEDEIROS, S. R. An updated meta-analysis of the anti-methanogenic effects of monensin in beef cattle. *Translational Animal Science*, v. 8, txae032, 2024.

CORONA, L.; OWENS, F. N.; ZINN, R. A. Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. *Journal of Animal Science*, v. 84, n. 11, p. 3020-3031, 2006.

CORREA, C. E. S.; SHAVER, R. D.; PEREIRA, M. N.; LAUER, J. G.; KOHN, K. Relationship between corn vitreousness and ruminal in situ starch degradability. *Journal of Dairy Science*, v. 85, n. 11, p. 3008-3012, 2002.

CRAWFORD, D. M.; RICHESON, J. T.; PERKINS, T. L.; SAMUELSON, K. L. Feeding a high-energy finishing diet upon arrival to high-risk feedlot calves: Effects on health, performance, ruminal pH, rumination, serum metabolites, and carcass traits. *Journal of Animal Science*, v. 100, n. 9, skac194, 2022.

CRUYWAGEN, C. W.; SWIEGERS, J. P.; TAYLOR, S. J.; COETZEE, E. The effect of Acid Buf in dairy cow diets on production response and rumen parameters. In: *Journal of Dairy Science*, v. 87, p. 46, 2004. American Dairy Science Association, Savoy, IL, USA.

CRUYWAGEN, C. W.; TAYLOR, S. J.; BEYA, M. M. The effect of buffering dairy cow diets with limestone, Acid Buf or sodium bicarbonate plus limestone on production response and rumen parameters. *Journal of Dairy Science,* v. 90, p. 561, 2007.

CRUYWAGEN, C. W.; TAYLOR, S.; BEYA, M. M.; CALITZ, T. The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. *Journal of Dairy Science*, v. 98, n. 8, p. 5506-5514, 2015.

CUNNINGHAM, J. G.; KLEIN, B. G. *Textbook of Veterinary Physiology (4th ed.).* Rio de Janeiro: Elsevier, 2008.

DETMANN, E.; SOUZA, M.; VALADARES FILHO, S. D. C.; QUEIROZ, A. C.; BERCHIELLI, T. T.; SALIBA, E. O. S.; AZEVEDO, J. A. G. *Métodos para análise de alimentos-INCT*. Viçosa, MG, Brazil: Suprema Gráfica, 2012.

DIAS, G. Granulados bioclásticos: algas calcárias. *Revista Brasileira de Geofísica*, v. 18, p. 307-318, 2000.

DIJKSTRA, J.; NEAL, H. D. S. C.; BEEVER, D. E.; FRANCE, J. Simulation of nutrient digestion, absorption and outflow in the rumen: model description. *The Journal of Nutrition*, v. 122, n. 11, p. 2239-2256, 1992.

DRESSLER, E. A.; BORMANN, J. M.; WEABER, R. L.; ROLF, M. M. Use of methane production data for genetic prediction in beef cattle: A review. *Translacional Animal Science*, v. 8, txae014, 2024.

DSM. Censo Confinamento Brasil 2024. Disponível em: https://www.dsm.com/tortuga/pt_BR/home.html. Acesso em: 2025.

ELLIS, J. L.; DIJKSTRA, J.; KEBREAB, E.; BANNINK, A.; ODONGO, N. E.; MCBRIDE, B. W.; FRANCE, J. Aspects of rumen microbiology central to mechanistic modelling of methane production in cattle. *The Journal of Agricultural Science*, v. 146, n. 2, p. 213-233, 2008.

ERDMAN, R. A. Dietary buffering requirements of the lactating dairy cow: A review. *Journal of Dairy Science*, v. 71, p. 3246-3266, 1988.

EUROPEAN UNION. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency. *Official Journal of the European Union,* L 396/1, 2006. Disponível em: https://eur-lex.europa.eu

GALYEAN, M. L.; HALES, K. E. Feeding management strategies to mitigate methane and improve production efficiency in feedlot cattle. *Animals*, v. 13, n. 4, 2023.

GERBER, P. J.; STEINFELD, H.; HENDERSON, B.; MOTTET, A.; OPIO, C.; DIJKMAN, J.; et al. Tackling Climate Change through Livestock: A Global Assessment of Emissions and Mitigation Opportunities. *FAO*, Rome, 2013.

GUERREIRO, P.; COSTA, D. F. A.; LIMEDE, A. C.; CONGIO, G. F. S.; MESCHIATTI, M. A. P.; BERNARDES, P. A.; SANTOS, F. A. P. Effects of monensin, calcareous algae, and essential oils on performance, carcass traits, and methane emissions across different breeds of feedlot-finished beef cattle. *Ruminants*, v. 5, n. 1, p. 2, 2025.

GONZÁLEZ, L. A.; MANTECA, X.; CALSAMIGLIA, S.; SCHWARTZKOPF-GENSWEIN, K. S.; FERRET, A. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Animal Feed Science and Technology*, v. 172, p. 66-79, 2012.

HALL, M. B.; HUNTINGTON, G. B. Nutrient synchrony: Sound in theory, elusive in practice. *Journal of Animal Science*, v. 86, Suppl. 14, p. E287-E292, 2008.

HOFFMAN, P.; et al. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *Journal of Dairy Science*, v. 94, n. 5, p. 2465-2474, 2011.

HUNTINGTON, G. B. Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science*, v. 75, n. 3, p. 852-867, 1997.

IMAMIDOOST, R.; CANT, J. P. Non-steady-state modeling of effects of timing and level of concentrate supplementation on ruminal pH and forage intake in high-producing, grazing ewes. *Journal of Animal Science*, v. 83, n. 5, p. 1102-1115, 2005.

INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE (*IPCC*). Climate Change 2022: Impacts, Adaptation and Vulnerability. Available at: https://www.ipcc.ch/report/ar6/wg2/.

JANSSEN, P. H. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology*, v. 160, p. 1-22, 2010.

JOHNSON, K.; WESTBERG, H.; MICHAL, J.; COSSALMAN, M. The SF_6 tracer technique: methane measurement from ruminants. In: MAKKAR, H. P.; VERCOE, P. E. (Eds.). *Measuring Methane Production from Ruminants*, p. 33-67. Springer, Dordrecht, The Netherlands, 2007.

JOHNSON, K. A.; JOHNSON, D. E. Methane emissions from cattle. *Journal of Animal Science*, v. 73, p. 2483-2492, 1995.

JUNGES, D.; et al. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. *Journal of Dairy Science,* v. 100, n. 11, p. 9048-9051, 2017.

KNAPP, J. R.; LAUR, G. L.; VADAS, P. A.; WEISS, W. P.; TRICARICO, J. M. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science*, v. 97, p. 3231-3261, 2014.

LAMMERS, B. P.; BUCKMASTER, D. R.; HEINRICHS, A. J. A simple method for the analysis of particle sizes of forage and total mixed rations. *Journal of Dairy Science*, v. 79, n. 5, p. 922-928, 1996.

LENG, R. A.; INTHAPANYA, S.; PRESTON, T. R. Biochar lowers net methane production from rumen fluid in vitro. *Livestock Research for Rural Development*, v. 24, n. 6, p. 103, 2012.

LESCOAT, P.; SAUVANT, D. Development of a mechanistic model for rumen digestion validated using the duodenal flux of amino acids. *Reproduction Nutrition Development*, v. 35, n. 1, p. 45-70, 1995.

LIDE, D. R. CRC handbook of chemistry and physics: A ready-reference book of chemical and physical data. *Boca Raton: CRC Press*, 1995.

MAKKAR, H. P. S.; TRAN, G.; HEUZÉ, V.; GIGER-REVERDIN, S.; LESSIRE, M.; LEBAS, F.; ANKERS, P. Seaweeds for livestock diets: A review. In: *Animal Feed Science and Technology*, v. 212, p. 1-17, 2016. Elsevier.

MARQUES, R. D. S.; COOKE, R. F. Effects of ionophores on ruminal function of beef cattle. *Animals*, v. 11, n. 10, p. 2871, 2021.

MARTIN, C.; MORGAVI, D. P.; DOREAU, M. Methane mitigation in ruminants: From microbe to the farm scale. *Animal*, v. 4, p. 351-365, 2010.

MAYBERRY, D.; BARTLETT, H.; MOSS, J.; DAVISON, T.; HERRERO, M. Pathways to carbon-neutrality for the Australian red meat sector. *Agricultural Systems*, v. 175, p. 13-21, 2019.

MCALLISTER, T. A.; NEWBOLD, C. J. Redirecting rumen fermentation to reduce methanogenesis. *Australian Journal of Experimental Agriculture*, v. 48, p. 7-13, 2008.

MCALLISTER, T. A.; et al. Effect of ruminal microbial colonization on cereal grain digestion. *Canadian Journal of Animal Science*, v. 70, n. 2, p. 571-579, 1990.

MCGOVERN, E.; MCGEE, M.; BYRNE, C. J.; KENNY, D. A.; KELLY, A. K.; WATERS, S. M. Investigation into the effect of divergent feed efficiency phenotype on the bovine rumen microbiota across diet and breed. *Scientific Reports*, v. 10, p. 15317, 2020.

MESGARAN, D. In vitro usage of various non-organic compounds to subdue acidogenic value and enhance the fermentation of alfalfa hay based diets by mixed rumen microbiota. *International Journal of Livestock Production,* v. 4, n. 10, p. 165-171, 2013.

MIGEOTTE, M. V. Spectroscopic evidence of methane in the Earth's atmosphere. *Physical Review*, v. 73, p. 519-520, 1948.

MILLEN, D. D.; PACHECO, R. D. L.; ARRIGONI, M. D. B.; GALYEAN, M. L.; VASCONCELOS, J. T. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. *Journal of Animal Science*, v. 87, n. 10, p. 3427-3439, 2009.

MILLEN, D. D.; PACHECO, R. D. L.; CABRAL, S. L.; CURSINO, L. L.; WATANABE, D. H. M.; RIGUEIRO, A. L. N. Ruminal acidosis. In: *Rumenology* (1st ed., p. 127-156). Cham, Switzerland: Springer, 2016.

MIN, B. R.; SOLAIMAN, S.; WALDRIP, H. M.; PARKER, D.; TODD, R. W.; BRAUER, D. Dietary mitigation of enteric methane emissions from ruminants: A review of plant tannin mitigation options. *Animal Nutrition*, v. 6, n. 3, p. 231-246, 2020.

MONSALVE, J. G.; MILLEN, D. D. A snapshot of nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil in 2023. *Frontiers in Veterinary Science*, v. 12, p. 1518571, 2025.

MONTAÑEZ-VALDEZ, O. D.; PINOS-RODRÍGUEZ, J. M.; ROJO-RUBIO, R. O. L. A. N. D. O.; SALINAS-CHAVIRA, J.; MARTÍNEZTINAJERO, J. J.; SALEM, A. Z. M.; AVELLANEDA-

CEVALLOS, J. H. Effect of a calcified-seaweed extract as rumen buffer on ruminal disappearance and fermentation in steers. *Indian Journal of Animal Sciences*, v. 82, p. 430-432, 2012.

MOSS, A. R.; JOUANY, J.-P.; NEWBOLD, J. Methane production by ruminants: Its contribution to global warming. *Annales de Zootechnie, EDP Sciences*, p. 231-253, 2000.

MYHRE, G.; SHINDELL, D.; BRÉON, F. M.; COLLINS, W.; FUGLESTVEDT, J.; HUANG, J.; ZHANG, H.; et al. Anthropogenic and natural radiative forcing. In: *Climate Change 2013 - The Physical Science Basis*, p. 659-740, 2014.

NAGARAJA, T. G.; LECHTENBERG, K. F. Acidosis in feedlot cattle. Veterinary Clinics of North America: *Food Animal Practice,* v. 23, n. 2, p. 333-350, 2007.

NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE. Nutrient requirements of beef cattle. 8th rev. ed. Washington, DC: *The National Academies Press*, 2021.

NEVILLE, E. W.; FAHEY, A. G.; GATH, V. P.; MOLLOY, B. P.; TAYLOR, S. J.; MULLIGAN, F. J. The effect of calcareous marine algae, with or without marine magnesium oxide, and sodium bicarbonate on rumen pH and milk production in mid-lactation dairy cows. *Journal of Dairy Science*, v. 102, p. 8027-8039, 2019.

OLIVEIRA, C.; MILLEN, D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. *Animal Feed Science and Technology*, v. 197, p. 64-75, 2014.

OWENS, F. N.; HILL, W. J.; SECRIST, D. S.; GILL, D. R. Acidosis in cattle: A review. *Journal of Animal Science*, v. 76, p. 275-286, 1998.

OWENS, F. N.; ZINN, R.; KIM, Y. Limits to starch digestion in the ruminant small intestine. *Journal of Animal Science*, v. 63, n. 5, p. 1634-1648, 1986.

PEREIRA, L. G. R.; VET, M.; MACHADO, F. S.; CAMPOS, M. M.; GUIMARAES JUNIOR, R.; TOMICH, T. R.; REIS, L. G.; COOMBS, C.; VET ST, M. Enteric methane mitigation strategies in ruminants: A review. *Revista Colombiana de Ciencias Pecuarias,* v. 28, 2015.

PINTO, A. C.; MILLEN, D. D. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: The 2016 Brazilian survey. *Canadian Journal of Animal Science*, v. 99, n. 2, p. 392-407, 2018.

RAILSBACK, L. B. Some Fundamentals of Carbonate Geology. *University of Georgia*, 2006. Disponível em: https://railsback.org/Fundamentals/FundamentalsCarbs.html

RAUCH, R. E.; ROBINSON, P. H.; ERASMUS, L. J. Effects of sodium bicarbonate and calcium magnesium carbonate supplementation on performance of high producing dairy cows. *Animal Feed Science and Technology*, v. 177, n. 3-4, p. 180-193, 2012.

ROCHE, J. R.; DALLEY, D.; MOATE, P.; GRAINGER, C.; RATH, M.; O'MARA, F. Dietary cation-anion difference and the health and production of pasture-fed dairy cows 2. Nonlactating periparturient cows. *Journal of Dairy Science*, v. 86, n. 3, p. 979-987, 2003.

ROQUE, B. M.; VENEGAS, M.; KINLEY, R. D.; DE NYS, R.; DUARTE, T. L.; YANG, X.; KEBREAB, E. Red seaweed (Asparagopsis taxiformis) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS ONE*, v. 16, n. 3, 2021.

ROSSI, C. A. S.; COMPIANI, R.; BALDI, G.; TAYLOR, S. J.; RIGHI, F.; SIMONI, M.; QUARANTELLI, A. Replacing sodium bicarbonate with half amount of calcareous marine algae in the diet of beef cattle. *Revista Brasileira de Zootecnia*, v. 48, e20180129, 2019.

RUSSELL, J. B.; HOULIHAN, A. J. Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiology Reviews*, v. 27, n. 1, p. 65-74, 2003.

RUSSELL, J. B.; WILSON, D. B. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of Dairy Science*, v. 79, n. 8, p. 1503-1509, 1996.

SALEEM, A. M.; RIBEIRO JR, G. O.; YANG, W. Z.; RAN, T.; BEAUCHEMIN, K. A.; McGEOUGH, E. J.; McALLISTER, T. A. Effect of engineered biocarbon on rumen fermentation, microbial protein synthesis, and methane production in an artificial rumen (RUSITEC) fed a high forage diet. *Journal of Animal Science*, v. 96, n. 8, p. 3121-3130, 2018.

SANCHEZ, W. K. The latest in dietary cation-anion difference (DCAD) Nutrition. *Proceeding of 43rd Annual Dairy Cattle Day*, 26 March 2003.

SILVESTRE, A. M.; MILLEN, D. D. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia*, v. 50, p. 1-25, 2021.

STIVARI, T. S. S.; CHEN, R. F. F.; GAMEIRO, A. H.; MONTEIRO, A. L. G.; RAINERI, C.; SILVA, J. B. A. Feasibility of grazing sheep production systems using long-term economic indicators and the methodology of the soil expectation value. *Brazilian Journal of Veterinary Research and Animal Science,* v. 51, n. 2, p. 149-157, 2014.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (U.S. EPA). *Inventory of U.S. greenhouse gas emissions and sinks*. 2021. Disponível em: https://www.epa.gov/sites/production/files/2021-04/documents/us-ghg-inventory-2021-main-text.pdf

VALADARES FILHO, S. C. Microbial protein synthesis, crude protein ruminal degradation and intestinal digestibility in cattle. Proceedings of International Symposium on the Nutritional Requirements of Ruminants. Viçosa, MG: *JARD*, p. 201-234, 1995.

VALADARES, R. F. D.; BRODERICK, G. A.; VALADARES FILHO, S. D. C.; CLAYTON, M. K. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *Journal of Dairy Science*, v. 82, n. 12, p. 2686-2696, 1999.

VALADARES FILHO, S. C.; COSTA E SILVA, L. F.; ROTTA, P. P.; GIONBELLI, M. P.; CHIZZOTTI, M. L.; MARCONDES, M. I.; PRADOS, L. F. Exigências nutricionais de zebuínos puros e cruzados – BR-CORTE 2023. Viçosa, MG: *Universidade Federal de Viçosa*, 2023.

VALENTE, T. N. P.; DETMANN, E.; QUEIROZ, A. C.; VALADARES FILHO, S. C.; GOMES, D. I.; FIGUEIREDO, D. M. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. *Revista Brasileira de Zootecnia*, v. 40, p. 2565-2573, 2011.

VAN KESSEL, J. S.; RUSSELL, J. B. The effect of pH on ruminal methanogenesis. *FEMS Microbiology Ecology*, v. 20, p. 205-210, 1996.

VAN SOEST, P. J. Nutritional ecology of the ruminant. *Cornell University Press*, 1994.

VERBIC, J.; MACLEOD, N. A.; ØRSKOV, E. R. Excretion of purine derivatives by ruminants: Effect of microbial nucleic acid infusion on purine derivative excretion by steers. *Journal of Agricultural Science,* v. 114, p. 243-248, 1990.

WESTBERG, H.; JOHNSON, K.; COSSALMAN, M.; MICHAL, J. A SF6 tracer technique: methane measurement from ruminants. *Washington State University,* Pullman, WA, USA, 1998.

WU, Z.; BERNARD, J. K.; TAYLOR, S. J. Effect of feeding calcareous marine algae to Holstein cows prepartum or postpartum on serum metabolites and performance. *Journal of Dairy Science*, v. 98, p. 4629-4639, 2015.

YU, P.; HUBER, J. T.; SANTOS, F. A. P.; SIMAS, J. M.; THEURER, C. B. Effects of ground, steam-flaked, and steam-rolled corn grains on performance of lactating cows. *Journal of Dairy Science*, v. 81, n. 3, p. 777-783, 1998.

ZINN, R. A.; OWENS, F. N.; WARE, R. A. Flaking corn: Processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *Journal of Animal Science*, v. 80, n. 5, p. 1145-1156, 2002.

CHAPTER 2

INTERACTION OF FLINT CORN GRAIN PROCESSING AND UREA SOURCE IN DIETS FOR FINISHING CATTLE: IMPACT ON PERFORMANCE, CARCASS TRAITS, NUTRIENT UTILIZATION, AND ENZYME ACTIVITIES INVOLVED IN NITROGEN AND ENERGY METABOLISM

ABSTRACT

Improving starch and nitrogen utilization efficiency is crucial for enhancing growth performance and sustainability in beef feedlot systems that rely heavily on flint corn, which has lower starch digestibility than dent corn. Ensiling flint corn can increase starch availability, but its interaction with different non-protein nitrogen (NPN) sources, such as coated urea, remains unclear. Coated urea may better synchronize nitrogen release with rumen fermentation, improving microbial protein synthesis and nitrogen retention. We hypothesized that the inclusion of coated urea, from a product designed to partially deliver urea to the post-ruminal gastrointestinal tract, in corn-based diets would impact performance, metabolism, carcass and meat characteristics of Nellore bulls. Hence, the objective was to evaluate the effects of flint corn grain processing methods [CPM; dry ground corn (DGC) or rehydrated and ensiled corn grain (REC)] and urea source [US; feed-grade urea (U) and coated urea (CU)] on intake, growth performance, carcass traits, nutrient digestibility, diet energy content, nitrogen metabolism, and blood parameters in finishing Nellore bulls. Eighty Nellore bulls (26-mo age; initial body weight [BW] = 388 ± 45.7 kg) were allocated into 28 pens (7 pens/treatment; 6 pens with 3 bulls and 1 pen with 2 bulls). Treatments were arranged in a 2 × 2 factorial arrangement: 1) DGC + U; 2) DGC + CU; 3) REC + U; and 4) RSC+CU. Bulls were adapted to finishing diets over a period of 14 days and fed a total of 86 days, totaling 100 days. There were no significant interactions between corn processing and urea source for growth performance ($P \geq 0.29$), carcass characteristics ($P \geq 0.27$), dietary net energy and fecal characteristics ($P \geq 0.14$). However, bulls fed REC showed greater growth performance (final BW, ADG, feed efficiency; $P \leq 0.02$), carcass traits (HCW, carcass ADG, carcass gain:feed; $P \leq 0.01$), improved dietary net energy ($P < 0.01$) and apparent total tract digestibility of DM, OM, CP, and starch ($P \leq 0.01$) compared to DGC. Fecal starch was reduced by 53% with REC (5.5% vs. 11.6%; $P < 0.01$), and fecal nitrogen also decreased ($P \leq 0.01$). Similarly, coated urea improved the same parameters in growth performance ($P \leq 0.01$), carcass traits ($P \leq 0.04$), dietary net energy ($P \leq 0.04$), and nitrogen retention (45 vs. 42 g/d; $P \leq 0.01$) compared with feed-grade urea, and slightly improved starch digestibility ($P = 0.05$) relative to feed-grade urea. Blood metabolites and meat quality traits were unaffected ($P > 0.10$). In summary, reconstituted corn grain silage markedly improved flint corn energy value on digestibility, growth performance, and feed efficiency compared with fine grinding corn, independently of the urea source. When cattle are finished on diets based on flint corn like the ones used in this study, independent of the processing method coated urea seems to be the optimal source of supplemental NPN.

Keywords: beef cattle, feedlot, non-protein nitrogen, starch.

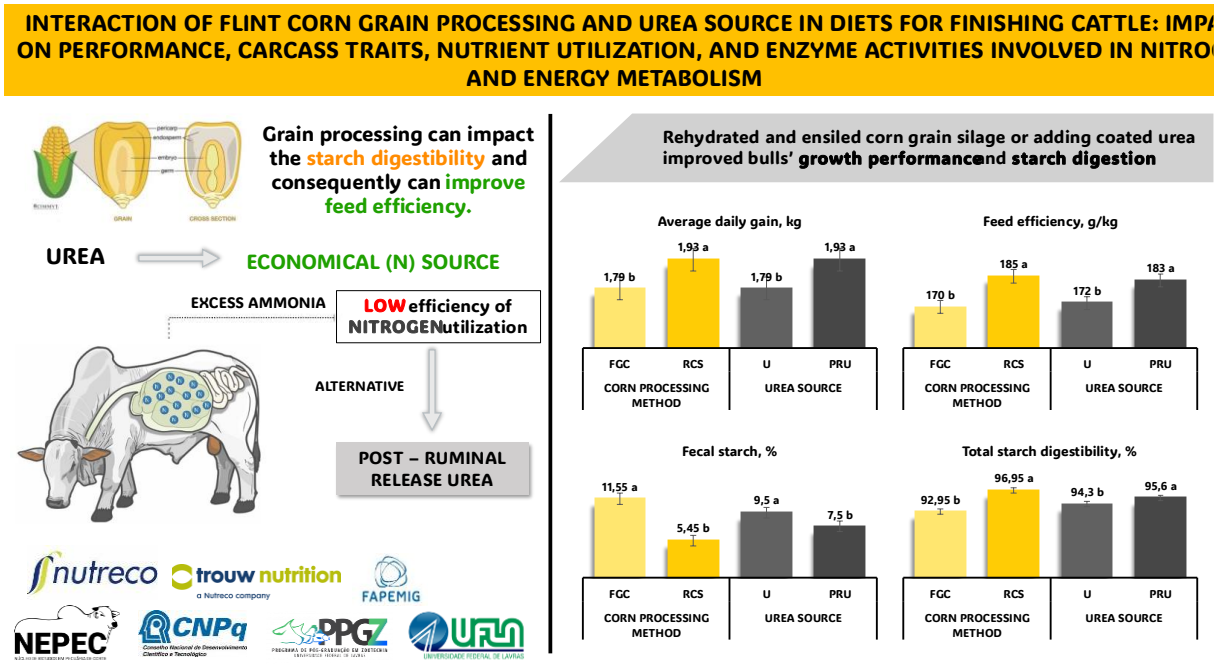
SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS

The study presented in Chapter 2 evaluated the impacts of flint corn grain processing associated with different non-protein nitrogen (NPN) sources on productive performance, nutrient digestibility, carcass traits, dietary energy content, and nitrogen metabolism in finishing Nellore bulls under commercial feedlot conditions. The results generated relevant scientific, technological, environmental, and economic implications for intensive beef cattle production in tropical regions, particularly in Brazil, where flint corn and NPN supplementation remain central components of finishing diets. From a technological standpoint, the findings demonstrated that rehydration and ensiling of flint corn markedly enhanced starch and crude protein digestibility, dietary net energy, feed efficiency, and carcass performance, while coated urea further improved nitrogen retention and carcass outcomes relative to conventional feed-grade urea, highlighting the importance of synchronizing starch and nitrogen release for optimal rumen fermentation and nutrient use efficiency. Environmentally, reductions in fecal starch and fecal nitrogen excretion suggest improvements in nutrient utilization efficiency and decreased nutrient losses to the environment, contributing to strategies for sustainable intensification of beef cattle production and improved nitrogen use efficiency. From an economic perspective, improvements in feed efficiency, dietary energy value, and carcass performance associated with the use of rehydrated and ensiled flint corn and coated urea indicate potential gains in feedlot profitability through enhanced nutrient conversion and reduced dependence on more costly protein sources. Social impacts arise from the generation of technical information with direct applicability to commercial cattle production, supporting decision-making by producers, consultants, nutritionists, and industry stakeholders, and contributing to the advancement of tropical feedlot nutrition through improved understanding of flint corn utilization and nitrogen management.

INFOGRAPHIC

CORN PROCESSING AND UREA TECHNOLOGIES TO OPTIMIZE PERFORMANCE AND SUSTAINABILITY IN BEEF FEEDLOTS

Flint corn is the predominant grain used in Brazilian feedlots; however, its starch digestibility is lower than that of dent corn, mainly due to its starch-protein matrix and vitreous endosperm. To enhance starch digestion, ensiling methods have been increasingly adopted in Brazilian feedlots, surpassing dry grinding as the preferred processing method in recent years. Furthermore, nitrogen availability from urea sources may interact with starch availability in the rumen, depending on the corn processing method, thereby influencing rumen fermentation and microbial protein synthesis. To investigate this interaction, we evaluated two flint corn grain processing methods (dry grounding and rehydration and ensiling) and two urea sources (feed-grade and a coated product) to assess their effects on feedlot growth performance, carcass traits, diet energy content, nutrient digestibility, blood parameters, and nitrogen metabolism of finishing Nellore bulls. Overall, our findings that feeding diets with rehydrated and ensiled corn grain silage or adding coated urea improved bulls' growth performance and starch digestion, thereby increasing energy availability for growth without affecting metabolic parameters. Additionally, incorporating rehydrated and ensiled corn grain or coated urea in finishing bull diets has the potential to improve nitrogen utilization efficiency, thereby enhancing overall sustainability of finishing phase by reducing nitrogen excretion.



1 INTRODUCTION

Flint corn is the predominant grain in Brazilian feedlots (Bernardes & Castro, 2019; Monsalve & Millen, 2025), though it has lower digestibility than dent corn (Correa et al., 2002; Corona et al., 2006). Starch availability depends on kernel processing, which influences proteolytic bacterial activity and protein matrix breakdown, ultimately enhancing starch digestion (Junges et al., 2017; Hoffman et al., 2011). Most Brazilian feedlots historically rely on fine grinding corn (DGC) as the primary processing method (Millen et al., 2009; Silvestre & Millen, 2021), but there is growing interest in ensiling methods [reconstituted corn grain silage (REC) and high-moisture corn (HMC)] due to their potential to improve starch utilization (Owens et al., 1986; Benton et al., 2005). Together, HMC and REC account for 36% of primary and 55% of secondary grain processing methods actually used in Brazilian feedlots (Monsalve & Millen, 2025). Evidence for dietary sorting has been provided for cattle fed finishing diets as cattle fed high-grain diets sort the total mixed ration (TMR) for long particles as a strategy to alleviate a low ruminal pH (Yang & Beauchemin 2006; DeVries et al. 2014).

Beyond starch digestibility, nitrogen (N) availability and its interaction with starch impact rumen fermentation and microbial protein synthesis (MPS; Casper et al., 1999). Since the 1950s, urea has been widely used as an economical N source, replacing true protein sources like soybean meal while maintaining animal performance (Sinclair et al., 2012). Ruminants have an adaptive advantage in recycling urea, supplying N for MPS (Reynolds & Kristensen, 2008). However, rapid urea hydrolysis in the rumen can lead to excess ammonia, surpassing microbial utilization capacity and resulting in inefficient N utilization, increased excretion, and potential environmental concerns (Huber & Kung, 1981; Huntington, 1986). Strategies to improve N efficiency include slow-release non-protein N (NPN) sources such as biuret, starea, linseed oil-coated urea, and formaldehyde-treated urea, though these have shown limited advantages over conventional urea (Mudd, 1977; Tamminga & Van Hellemond, 1977; Miller, 1979; Owens et al., 1980). Another approach is to provide postruminal N sources, which can lower ammonia peaks and enhance MPS by optimizing N retention and recycling (Atkinson et al., 2007; Wickersham et al., 2008). Studies comparing ruminally degradable and postruminally delivered urea indicate that the latter provides a more stable N supply for microbial growth, improving urea-N recycling (Carvalho et al., 2020; Nichols et al., 2022).

A deeper understanding of urea-N recycling could lead to improved N utilization in ruminants (Calsamiglia et al., 2010). Diet manipulation, such as increasing fermentable carbohydrates or modifying grain processing methods (Kennedy & Milligan, 1980; Theurer et al., 2002), may shift starch digestion from the small intestine to the rumen, potentially enhancing N recycling and MPS (Hailemariam et al., 2021). However, research on replacing conventional urea with coated urea (CU) to increase postruminally urea in diets with different corn processing methods and its effects on N metabolism and cattle performance remains limited.

This study hypothesizes that corn grain processing methods interact with the urea source, with REC-based diets combined with CU potentially improving nitrogen recycling, increasing MPS, reducing urinary nitrogen excretion, and enhancing overall nitrogen utilization efficiency. To test this, the effects of flint corn processing methods – dry ground corn (DGC; 1.77 mm) and reconstituted corn grain silage (REC; 2.15 mm) – were evaluated in combination with feed-grade urea (U) and CU. The study aimed to assess impacts on feedlot growth performance, carcass traits, diet energy content, nutrient digestibility, blood parameters, and nitrogen metabolism in finishing Nellore bulls.

2 LITERATURE REVIEW

2.1 Corn processing methods

Corn is widely utilized in finishing cattle diets as the primary energy source, accounting for 91.67% of the main grains used in Brazilian feedlot systems (Monsalve & Millen, 2025), primarily due to its high starch concentration, which can reach up to 72% of the grain's dry matter (Huntington, 1997). In Brazil, flint-type hybrids (*Zea mays* ssp. *indurata*) are predominantly cultivated. These hybrids are characterized by a highly vitreous endosperm, contrasting with the dent-type corn (*Zea mays* ssp. *indentata*) commonly used in other countries (Correa et al., 2002; Silvestre & Millen, 2021). According to Correa et al. (2002), Silvestre & Millen (2021), and Monsalve & Millen (2025), flint corn represents approximately 97.06% of the corn grain used in Brazilian feedlots, primarily due to its greater resistance to pests, diseases, and adverse environmental conditions.

The main differences between corn types are attributed to the proportion and structural characteristics of the endosperm. Flint corn exhibits a predominance of vitreous endosperm, where starch granules are densely embedded within a protein matrix, limiting enzymatic hydrolysis in both the rumen and small intestine (Rooney & Pflugfelder, 1986; Taylor & Allen, 2005). In contrast, dent corn contains a higher proportion of floury endosperm, in which starch granules are more loosely arranged and more susceptible to enzymatic digestion.

Starch is primarily composed of two glucose polymers: amylose and amylopectin. Amylose is a linear polymer consisting of α-1,4 glycosidic linkages, while amylopectin is a highly branched polymer composed of α-1,4 linear chains with α-1,6 branching points every 20–25 glucose units. Due to its branched structure, amylopectin exposes more non-reducing ends, making it more susceptible to enzymatic hydrolysis than amylose. Consequently, amylopectin is more rapidly and extensively fermented (Millen et al., 2016).

In addition to starch structure, flint corn contains higher concentrations of prolamins, particularly zein, an alcohol-soluble protein that accumulates around starch granules, forming a

hydrophobic matrix that impairs water penetration and enzymatic degradation (Taylor & Emmambux, 2010). This dense protein network is closely associated with the reduced starch digestibility observed in highly vitreous corn hybrids (McAllister et al., 1993). Therefore, the combination of compacted amylopectin and elevated zein content highlights the need for processing strategies aimed at disrupting this matrix to improve starch utilization throughout the gastrointestinal tract.

The primary objective of grain processing is to enhance the nutritive value of corn by improving starch digestibility and nutrient availability, thereby optimizing animal performance (Theurer et al., 1986). For flint corn, processing is particularly important due to its intrinsic resistance to digestion. Although it has lower baseline digestibility, flint corn often responds more positively to processing, as mechanical or biochemical disruption of its structure significantly increases starch solubilization and degradation (Correa et al., 2002; Giuberti et al., 2014).

Historically, dry grinding has been the most common grain processing method in Brazilian feedlot systems. However, the most recent national survey revealed that HMC silage has surpassed dry grinding for the first time, accounting for 36.1% of reported practices (Monsalve & Millen, 2025), representing a 22.21% increase compared to the previous survey (Silvestre & Millen, 2021). Mechanical dry grinding disrupts the pericarp and reduces particle size, thereby increasing the surface area for microbial colonization and enzymatic activity in the rumen and intestine (Santos et al., 2016). Nevertheless, this method may not be sufficient to disrupt the protein matrix surrounding starch granules, especially in high-zein flint corn (Hoffman et al., 2011).

Alternatively, rehydration followed by anaerobic ensiling enhances the proteolysis of the protein matrix, thereby improving starch accessibility for microbial and enzymatic digestion (Ferraretto et al., 2013). This process involves adding water to dry ground corn achieve a moisture level of 30–35%, followed by anaerobic storage (Pereira et al., 2013). Notably, starch digestibility exhibits a quadratic response to fermentation time, with maximum digestibility typically observed around 330 days of storage. However, inadequate fermentation periods or poor storage conditions may compromise feed hygiene and limit digestibility (Ferraretto et al., 2013).

During ensiling, microbial and endogenous plant enzymes partially degrade the prolamin matrix, particularly zein, improving starch exposure to ruminal and post-ruminal enzymatic activity (Ferraretto et al., 2013; Hoffman et al., 2011). Compared to dry ground corn, HMC generally shows superior starch digestibility due to its softer endosperm and increased porosity induced by fermentation (Owens et al., 1986; Zinn et al., 2002).

Total starch digestibility in ruminants is strongly influenced by both the type and intensity of grain processing (Owens et al., 1986; McAllister et al., 1993). While both dry grinding and HMC processing improve starch accessibility, the extent of this improvement depends on hybrid

characteristics, processing conditions, and the animal's physiological response (Correa et al., 2002; Taylor & Allen, 2005). In general, dry grinding promotes mechanical disruption (Santos et al., 2016), whereas rehydration and fermentation induce biochemical and structural modifications through proteolysis of the prolamin matrix (Hoffman et al., 2011; Ferraretto et al., 2013).

Studies using flint corn in finishing cattle diets have reported total starch digestibility values ranging from 81% to 86% for dry ground corn, while values exceeding 95% have been observed with rehydrated and ensiled corn (Gervásio et al., 2021; Godoi et al., 2021; Jacovaci et al., 2021). Nonetheless, these results may vary depending on factors such as grain moisture, ensiling duration, dietary composition, and overall management practices (Owens et al., 2005; Zinn et al., 2002). Therefore, rehydration followed by ensiling represents a promising strategy to enhance starch utilization in flint corn–based diets, although its implementation should be carefully evaluated under diverse production conditions.

2.2 Nitrogen in ruminant nutrition

Ruminants evolved approximately 50 million years ago, developing a highly specialized digestive system that enabled them to thrive on fibrous diets and efficiently utilize simple nitrogen compounds (Van Soest, 1994). One of the most critical evolutionary adaptations was their capacity to recycle nitrogen effectively, particularly via urea nitrogen recycling. This mechanism allows ruminants to conserve nitrogen by redirecting hepatic urea into the gastrointestinal tract, primarily to the rumen, through saliva or passive diffusion across the ruminal wall. In the rumen, ureolytic microorganisms hydrolyze urea into ammonia (NH_3), which is subsequently used for microbial protein synthesis of high biological value (Lapierre & Lobley, 2001; NASEM, 2016).

This nitrogen-conserving strategy enabled ruminants to adapt to and persist in nutrient-poor environments, such as grasslands and arid regions, where dietary protein availability is limited or highly variable. Thus, urea recycling was essential for the ecological success and global dispersion of ruminant species. This recycling mechanism becomes particularly relevant under low-protein dietary conditions, enhancing nitrogen retention and reducing nitrogen losses to the environment. The extent of urea recycling is influenced by dietary protein content, fermentable energy availability, ruminal pH, and the physiological state of the animal (Van Soest, 1994).

In ruminant diets, urea is the primary source of NPN, characterized by high solubility, rapid ruminal availability, and low cost compared to true protein sources. Despite the strategic role of NPN in ruminant nutrition, conventional protein sources continue to be essential. Proteins are composed of amino acid chains linked by peptide bonds and exhibit variable ruminal degradability depending on their source, processing methods, and solubility (Van Soest, 1994; Valadares Filho et al., 2023). After ingestion, dietary protein is classified as either rumen degradable protein (RDP), which is rapidly

utilized by rumen microbes, or rumen undegradable protein (RUP), which bypasses ruminal fermentation and is digested in the small intestine (NASEM, 2016).

To meet the protein requirements of ruminants, the metabolizable protein (MP) supply is considered. MP refers to the fraction of protein absorbed in the small intestine as amino acids and small peptides. It comprises three main components: microbial protein synthesized in the rumen, digestible RUP, and a minor fraction of endogenous protein from digestive secretions and sloughed epithelial cells (NASEM, 2016).

The metabolic fate of nitrogen begins in the rumen, where dietary protein and NPN are subjected to extensive fermentation and used for MPS. Rumen bacteria secrete extracellular enzymes to hydrolyze proteins into peptides and amino acids. These amino acids are either incorporated into microbial biomass or deaminated to yield carbon skeletons and ammonia (McDonald et al., 2011). When urea is the nitrogen source, the enzyme urease, produced by several rumen bacterial species, catalyzes its hydrolysis into ammonia and carbon dioxide (Abdoun et al., 2006). The resulting ammonia may either be absorbed across the rumen wall or incorporated into microbial amino acid synthesis, provided that sufficient fermentable energy is available (Calsamiglia et al., 2010; Hristov et al., 2019).

Following absorption, ammonia enters the portal bloodstream and is transported to the liver, where it is promptly converted into urea as a detoxification mechanism. This conversion is essential for preventing systemic toxicity, as elevated blood ammonia concentrations can disrupt cellular acid–base balance, limit the availability of Krebs cycle intermediates, and impair neurotransmitter synthesis (Kozloski, 2017). Once synthesized in the liver, urea follows two main routes: urinary excretion or recycling. Urea excretion via urine is the predominant nitrogen loss pathway and is proportional to protein intake, fermentable energy supply, and the efficiency of microbial metabolism (Lapierre & Lobley, 2001; Hristov et al., 2019). A proportion of urea, however, is recycled to the gastrointestinal tract, particularly to the rumen, either through saliva or by diffusion across the ruminal epithelium, where microbial urease reconverts it into ammonia for microbial use (Abdoun et al., 2006; Lapierre & Lobley, 2001).

Nutritional strategies that aim to improve nitrogen retention by stimulating urea recycling are crucial for enhancing nitrogen utilization efficiency, reducing reliance on costly protein supplements, and mitigating environmental nitrogen losses. These strategies shift the focus from the ruminal environment alone to a whole-animal perspective. To this end, technologies have been developed to reduce ruminal solubility of urea and extend nitrogen release along the gastrointestinal tract.

Beyond slow-release systems, recent research has explored targeted nitrogen delivery to post-ruminal compartments. Continuous abomasal infusion of urea has been shown to offer benefits, including improved ruminal pH stability, reduced ruminal ammonia (NH_3) concentrations, and enhanced

neutral detergent fiber (NDF) digestibility (Carvalho et al., 2020). Similarly, Oliveira et al. (2020) reported reduced ruminal NH_3 concentrations and pH, increased microbial crude protein yield per unit of digestible organic matter, and a greater contribution of recycled nitrogen to MPS.

Based on these findings, a prototype of rumen-protected urea (coated urea, CU) was developed to deliver nitrogen gradually along the digestive tract. This non-commercial prototype, produced by Trouw Nutrition R&D (Amersfoort, the Netherlands), consists of feed-grade urea (Yara, Brunsbüttel, Germany) encapsulated with layers of hydrogenated palm oil (predominantly C16:0 and C18:0). The fat matrix reduces ruminal solubilization while maintaining total tract digestibility (Häussner et al., 2020; European Patent Office, Application No. PCT/EP2018/076234). The product provides a crude protein equivalent of 225%, though it contains approximately 18% less nitrogen than uncoated urea due to the lipid coating.

Technologies that modulate the site and rate of nitrogen release, such as coated urea and post-ruminal delivery systems, represent promising approaches to improve nutrient utilization and animal performance. By enhancing nitrogen retention and digestibility, these innovations contribute to more efficient and environmentally sustainable feedlot systems. A holistic understanding of nutrient form, release kinetics, and site-specific absorption is essential for designing feeding strategies tailored to the demands of high-performance ruminant production.

2.3 Interaction between rumen fermentable energy and protein

The interaction between fermentable energy and N sources in the rumen plays a central role in microbial protein synthesis and the overall efficiency of nitrogen utilization in ruminants. Several factors influence the supply of MP, including the availability of energy and degradable nitrogen, dietary composition, feed management, and the presence of essential nutrients such as minerals and vitamins (Owens & Sapienza, 2014). Energy and protein are frequently the most limiting nutrients in ruminant diets, warranting significant attention in nutritional evaluation systems (Mapato et al., 2010).

This interaction is based on the premise that optimal rumen fermentation requires synchrony between the availability of fermentable energy and degradable nitrogen. When both substrates are concurrently available, ruminal microorganisms can effectively incorporate NH_3 and amino acids into microbial biomass, thereby increasing the yield of MPS and enhancing nitrogen retention (Bach et al., 2005; Calsamiglia et al., 2010).

Maintaining a balanced energy-to-protein ratio is crucial for preventing metabolic disorders and promoting optimal animal performance. In situations where N is supplied more than available energy, NH_3 accumulates in the rumen and bloodstream, increasing the risk of toxicity and impairing feed intake and animal welfare (Detmann et al., 2007; Camargo et al., 2022). Conversely, excessive energy supply

relative to N may decrease microbial growth efficiency and increase heat increment, leading to reduced intake (Illius & Jessop, 1996). Therefore, properly formulated diets must ensure adequate synchrony between energy and nitrogen supply to support microbial activity and maximize voluntary feed intake (Cherdthong & Wanapat, 2010).

Achieving perfect synchrony under practical feeding conditions, however, remains a challenge due to the inherent variability in feeding ingredient characteristics. Structural carbohydrates such as cellulose and hemicellulose are slowly fermented, while non-structural carbohydrates like starch and soluble sugars are rapidly degraded in the rumen (Hall et al., 1999). Protein degradability also varies depending on the source, heat treatment, and solubility (Van Soest, 1994). As a result, the notion of achieving perfect synchrony between energy and nitrogen may be biologically unrealistic. Hall & Huntington (2008) demonstrated that asynchronous nutrient supply did not impair the performance of beef and dairy cattle, indicating that total nutrient balance over time may be more important than moment-to-moment synchrony.

Diet composition is a primary determinant of rumen fermentation patterns, influencing substrate availability and microbial efficiency (Hall & Huntington, 2008). Factors such as ingredient type, chemical composition, and grain processing have a significant impact on nutrient release dynamics (Dewhurst et al., 2000). While the concept of synchrony has been widely discussed, other researchers argue that adequate energy availability within an acceptable range of N levels may be sufficient for optimal MPS (Keim et al., 2011). In this context, urea recycling emerges as an important adaptive mechanism that enables ruminants to compensate for transient nutrient imbalances, thereby making precise synchrony less critical (Valkeners et al., 2004; Cabrita et al., 2006).

Recent advances in nutritional strategies have focused on modulating the site and rate of nitrogen release along the gastrointestinal tract. Protected or coated urea products have been developed to reduce rapid ruminal solubility and extend N availability beyond the rumen, facilitating better alignment with slow fermentable carbohydrate sources (Carvalho et al., 2020;). Abomasal infusion of urea has been shown to stabilize ruminal pH, reduce NH_3 concentrations, and improve fiber digestibility, highlighting the benefits of post-ruminal N delivery (Oliveira et al., 2020).

In a classical review, Johnson (1976) categorized carbohydrates into three groups according to their ruminal degradation rate: (1) structural carbohydrates (e.g., cellulose and hemicellulose), which ferment slowly; (2) non-structural carbohydrates (e.g., starch), with intermediate degradation rates; and (3) simple sugars (e.g., molasses), which are rapidly fermented. According to Thomas (1973), the ideal scenario for optimizing NH_3 incorporation into microbial protein synthesis would involve a nitrogen release pattern that mirrors carbohydrate fermentation kinetics. Johnson (1976) proposed theoretical ruminal available nitrogen (RAN) release curves to simulate this synchrony, but empirical evidence

suggests that even coated urea products seldom match the nitrogen demands imposed by various carbohydrate types.

Thus, achieving precise synchrony between energy and nitrogen release remains a theoretical goal rather than a practical standard. As Hall & Huntington (2008) emphasized, in complex biological systems, strategies that prioritize the overall balance and sustained nutrient availability, through both dietary and endogenous sources, may offer greater support for microbial growth and overall animal performance.

Comparative studies confirm that diets not specifically designed to synchronize energy and nitrogen can yield similar or even superior outcomes. For instance, Archibeque et al. (2007) showed that oscillating crude protein (CP) concentrations over time in finishing diets did not impair animal performance. Likewise, Menezes et al. (2019) reported no negative effects on intake, digestibility, or nitrogen balance in cattle receiving diets with fluctuating CP levels, and noted improvements in N use efficiency. These findings support the idea that urea recycling provides a physiological buffer that compensates for temporal imbalances in nutrient supply.

Moreover, evidence shows that diets rich in rapidly fermentable carbohydrates enhance urea transport into the rumen. Huntington (1989) observed increased urea entry in steers fed a starch-based diet compared to those receiving alfalfa-based diets. Similarly, Scott et al. (2020) found that sheep fed high-concentrate diets exhibited increased hepatic urea synthesis and greater return of urea to the gastrointestinal tract, along with lower ruminal ammonia concentrations. This is consistent with earlier findings by Seal et al. (1992), who demonstrated that rapidly fermentable carbohydrates stimulate microbial incorporation of RAN when energy availability is sufficient (Bach et al., 2005). Conversely, elevated ruminal ammonia can inhibit epithelial urea transport, as shown by Abdoun et al. (2006), underscoring the importance of balancing N supply with fermentable energy.

In summary, the interaction between fermentable energy and protein in the rumen is a key determinant of nitrogen utilization efficiency. Diets formulated with highly fermentable carbohydrates can improve microbial N capture and stimulate urea recycling, thereby promoting gastrointestinal N retention and reducing urinary N excretion. Understanding the regulatory factors involved, including carbohydrate type, CP content, protein degradability, and epithelial nitrogen transport, provides a foundation for developing more efficient and environmentally sustainable feeding strategies.

3. OBJECTIVE

The overall objective was to evaluate the effects of flint corn processing methods (dry ground corn and reconstituted corn grain silage) in combination with feed-grade urea and coated urea, on growth

performance, carcass characteristics, dietary energy value, nutrient digestibility, blood metabolites, and nitrogen metabolism in finishing Nellore bulls.

4. MATERIAL AND METHODS

4.1 Location

The trial was conducted at the Experimental Feedlot of the Department of Animal Science (DZO), College of Animal Science and Veterinary Medicine (FZMV), Federal University of Lavras in Lavras, Minas Gerais, Brazil. All experimental protocols and activities in this trial were approved by the Ethics Committee on Animal Use of the Federal University of Lavras (protocol number 038/20).

4.2 Preparation of whole-plant corn Shredlage® and reconstituted and ensiled corn

A corn hybrid (Pioneer® 30S40) was planted in a field and harvested in February 2020 with a DM content of 38% as whole plant corn Shredlage®. The Shredlage was harvested using a self-propelled forage harvester equipped with a silage harvester head set for a 22-mm theoretical length. The Shredlage was stored in a bunker silo (length × width × height of 42 × 5 × 1.5 m) allowed to ferment for about 12 weeks to use. Flint corn was acquired from the same source (Lag Lavras Armazens Gerais, Lavras, Minas Gerais, Brazil), at an approximately 13% moisture content (87% DM) and stored in metal bins until their use. Prior to the beginning of the experiment, a total of 40,000 kg of corn grain were ground using a stationary mill Nogueira TN-8 (Nogueira Máquinas Agrícolas, São João da Boa Vista, SP). Water was added with 2 kg/ton of a blend of organic acids on a fresh weight basis until the DM decreased to approximately 65%. The blend of organic acids (Fylax® Forte HC, Selko, Trouw Nutrition Brazil) was diluted with 1.6 kg of tap water and applied by spraying onto the ground kernels during mixing. After about 10 min of mixing, reconstituted grains were ensiled in bunker reinforced concrete silos (1.0 m inside diameter × 1.0 m long × 8.0 cm wall thickness) with a mean density of 1,000 kg of fresh material/m3.

4.3 Experimental design, animals, and housing

Eighty Nellore bulls (26-mo age, 388 ± 45.7 kg initial body weigh [BW], mean ± SD) were weighed and blocked by initial BW from light to heavy, and allocated into 1 of 28 pens (24 pens of 3 bulls and 4 pens of 2 bulls). Cattle within BW blocks were randomly allocated into pens (7 pens/treatment; 6 pens with 3 bulls + 1 pen with 2 bulls) with 4 m wide × 10 m deep; 4 m of linear concrete bunk space) and a common automatic water fountain was shared between 2 adjacent pens. At the arrival to the feedlot, before step-up diets, bulls were fed a diet containing (DM basis) 80% corn silage, 17% ground corn, 1.6% soybean meal, 0.4% urea, and 1.0% mineral mixture. Prior to the onset of the experiment, bulls were dewormed (Treo® Ace; Zoetis, Brazil) and vaccinated against rabies (Rai-

Vet; Laboratório Bio-Vet S/A, Vargem Grande Paulista, São Paulo, Brazil), respiratory diseases and Leptospirosis (CattleMaster® 4+L5; Zoetis), and prophylaxis of botulism and other diseases of ruminants (StarVac®; Labovet Produtos Veterinários Ltda, Feira de Santana, Bahia, Brazil).

After a period of 21 d for adaptation to the facilities, bulls were then gradually adapted to the experimental diets over 14-d using 3 step-up diets (6-d for step 1 and 4-d for step 2 and 3), each with an incremental increase in 22.3% of the final diet (from 33 to 55.3 to 77.6, respectively) until reaching the proportion of 72% of concentrate and 28% of corn silage per dietary DM. The experimental period lasted 100 d, 14 d for diet adaptation period (referred as adaptation period) and 86 d of finishing diet feeding period (referred as feedlot period).

4.4 Dietary treatments

Experimental treatment diets (Table 1) consisted of 1) fine ground corn (DGC) with feed-grade urea (U) diet (DGC+U); 2) rehydrated and ensiled corn (REC) with U (REC+U); 3) DGC with coated urea (CU; DGC+CU), and 4) REC with CU (REC+CU). The corn Shredlage® inclusion was 28.0% (DM basis) and remainder of the diets consisted of 63% corn (ground or rehydrated and ensiled), 6.3% soybean meal from a single lot, 0.1% ammonium sulphate and 3.0% of a mineral, vitamin, and monensin supplement (Table 1). The same flint corn was used in both diets, differing due to the processing (ground or reconstituted and ensiling). A non-commercial CU product (N4C4®, SIPENA SAS, St Malo, France) was supplemented at 1.35% DM in the diets, for bulls to consume approximately 140 g daily of N4C4®, a blended, controlled release urea product in a palm oil coating to protect against ruminal fermentation. Coated urea contained 82% urea on DM basis (230% CP, 72.8% ruminal protection, and 92.9% digestibility), which has 18% less N than urea due to the vegetable oil (palm oil) coating of CU. The DGC+U treatment was used as a standard diet commonly used in Brazilian feedlots, providing recommendations of primary source of grain and roughage, additive utilized; processing method and inclusion level of grains, CP concentration, urea recommended level, and forage: concentrate ratio. In that case, it was considered the inventory of nutritional practices adopted by nutritionists in Brazilian feedlots (Silvestre & Millen, 2021). All the diets were formulated to provide sufficient energy, protein, minerals, and vitamins to provide NASEM (2016) requirements for Nellore steers in the finishing phase, with an estimated ADG of 1.5 kg/d. The feedlot mineral premix contained, per kilogram, 120 g Ca (minimum), 30 g P, 80 g Na, 50 g K, 68 g Mg, 25 g S, 1,220 mg Zn, 330 mg Cu, 950 mg Mn, 20 mg Co, 24 mg I, 6 mg Se, 67,000 IU vitamin A, 9,500 IU vitamin D3, 950 IU/kg vitamin E and 650 mg/kg monensin to the diet DM (equivalent to 20 mg/kg of diet DM).

4.5 Feeding management and measurements

Total mixed rations were manually mixed, offered ad libitum twice daily 0800 and 1600 h. Bulls had free choice access to feed and fresh water. Based on dry matter intake (DMI) of the previous 3-days,

the amount of fresh feed offered to each pen was adjusted so that refused feed should not exceed 5% of daily intake. Samples of all ingredients in each concentrate batch and corn silage and REC were collected daily throughout the study while one sample of the refusals from each pen were collected twice a week. A composed weekly representative sample was obtained and partially dried in a forced-air oven (55°C for 72h). Weekly DM contents of corn silage and REC were used to adjust the ingredient proportions of the diets if DM deviated by >3% from average. Weekly samples were grounded in a knife mill with a 1-mm screen. Laboratory DM of samples were determined by drying at 105°C for 16 h. DMI of each pen was determined as the difference between the amount of feed offered each day and refused at the end of each week, corrected for DM content of TMR and refusals.

4.6 Samples analysis

Samples of silage, feedstuffs, leftovers, and feces were ground in a knife mill to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen. Samples of each material ground through 1-mm sieves (silage, feedstuffs, leftovers, and feces) were analysed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012) for DM (dried overnight at 105°C; method INCT-CA no. G-003/1), ash (complete combustion in a muffle furnace at 600°C for 4 h; method INCT-CA no. M-001/1), N (Kjeldahl procedure; method INCT-CA no. N-001/1), NDF corrected for ash and protein (NDFap; using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein; method INCT-CA no. F-002/1). From samples processed through a 2-mm sieve, iNDF content was determined as the residual NDF remaining after 288 h of ruminal in situ incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY), according to Valente et al. (2011).

To describe diet particle size diet samples were collected weekly and separated using the Penn State Particle Separator, in which sieves were stacked in the following order: 19.0-mm sieve on top, 8.0-mm sieve second, 4-mm sieve, and then a plastic pan fitted to the bottom of the last sieve. The sieve set was placed on a flat surface, and approximately 400 g of diet sample was spread out on the top sieve. The sieve set was shifted horizontally on the flat surface 5 times, rotated one-fourth turn, and shifted 5 times again, according to Kononoff et al. (2003) and Gentry et al. (2016).

4.7 Animal performance

Once the bulls were allocated to their respective treatment groups, they were reweighed individually (non-fasted) at the end (d 100; after a 16-h solid-feed fasting) of the feed period between 0700 and 1000 h and before delivery of fresh feed. Shrunk BW at d 0 of feedlot period was estimated by using a non-linear equation developed for Zebu cattle to account for gut fill ($0.88 \times BW^{1.0175}$; Valadares Filho et al., 2016). Average daily gain (ADG) was calculated as the difference between the

initial and final shrunk BW divided by the number of days from the experiment, G:F ratio was calculated as ADG divided by DMI.

At the end of the trial, bulls were transported to a commercial abattoir (Supremo Carnes, Campo Belo, MG, Brazil) for harvesting. Pre-harvest handling was conducted in accordance with good animal welfare practices, and slaughtering procedures followed strict guidelines stablished and regulated by the Sanitary and Industrial Inspection Regulation for Animal Origin Products in Brazil (Brazil, 1997). Dressing percentage was calculated based on the final carcass weight and BW ratio after fasting. Net energy contents of the diets were calculated from growth performance and DMI based as described by Zinn et al. (2002).

The metabolizable energy (ME) was calculated using the following equations: Digestible energy (DE) = TDN × 0.04409; and ME = 0.82 × DE (NASEM, 2016). The net energy for maintenance (Em) required was calculated using the following equation $Em = 0.077 \times BW^{0.75}$ (where BW is the mean body weight; Lofgreen & Garrett, 1968) and multiplied by a correction factor of 0.9 for Bos indicus breeds (NASEM, 2016). The net energy required for gain (Eg) was calculated using the following equation $Eg = (0.0493 \times BW^{0.75}) \times ADG^{1.097}$ (NRC, 1984) to estimate net energy for maintenance (NEm) and net energy for gain (NEg) of the diets. Net energy of maintenance (NEm, Mcal/kg) of the diets was calculated from the quadratic formula, $x = (-b \pm \sqrt{(b^2 - 4ac)})/2a$, where a = –0.877 DMI, b = 0.877 Em + 0.41 DMI + Eg, and c = –0.41 Em (Zinn & Shen, 1998) and net energy of gain (NEg, Mcal/kg) was calculated as 0.877 NEm – 0.41.

On d 99 of feedlot period, measurements of the longissimus thoracis muscle area (LMA), back fat thickness (BFT), and rump fat thickness (RFT) were scanned by the right side using an Aloka 183 500-V machine (Corometrics Medical Systems, Wallingford, CT) with a linear probe (3.5-MHz, 17.2-cm linear array transducer). The LMA was measured on a transversal section in the 12th and 13th ribs, BFT was measured on a longitudinal section in the 12th rib, ¾ the length ventrally over the longissimus muscle. The RFT was taken at the junction of the biceps femoris and gluteus medius between the ischium and ileus and parallel to the vertebral column. The images analysis was performed using the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, IA, USA). Ultrasound images were collected and analyzed by a trained technician.

4.8 Blood sample collection and analysis

Blood samples on all bulls were obtained before feeding on d 49 and 99 of the trial. At each sampling point, 2 blood samples were collected from the jugular vein: one 10-mL treated tube containing separator gel and a coagulation accelerator (BD Vacuntainer® SST II Plus), and one 4-mL NaF/EDTA treated tubes (BD Vacutainer®, São Paulo, Brazil). Immediately after collection, the blood samples were

placed on ice and thereafter centrifuged at 2,500 × g for 15 min at 4°C to harvest the serum or plasma. Plasma and two samples of serum were recovered and stored at -20◦C.

To measure energy metabolism, NaF/EDTA treated tube of whole blood was used to measure glucose concentrations using a quantitative colorimetric kit (K082-2; Quibasa Química Básica Ltda; Belo Horizonte, MG, Brazil). The intra- and inter-assay coefficients of variation for glucose were 2.65% and 4.92%, respectively. The first serum sample was used to measure protein metabolism parameters (albumin, creatinine, total protein, and urea); enzymes and hepatic markers [alkaline phosphatase (ALP), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT)]. Albumin was analyzed by bromocresol green colorimetric method (K040-1); aspartate aminotransferase (AST; also called aspartate transaminase was determined using the AST/GOT Liquiform kit (K048-6) by the UV-IFCC continuous kinetic method; alanine aminotransferase (ALT; also called ALT/TGP) by the kinetic test (K049-6); creatinine was measured colorimetrically by end-point reaction and alkaline picrate – Jaffe´s method (K222-1); gamma glutamyltransferase (GGT; also called gamma-glutamyl transpeptidase) was quantified by the kinetic method with a modified Szasz procedure using the GamaGT Liquiform kit (Reference 105); total proteins was determined by biuret colorimetric end-point reaction (K031-1); and urea was determined by the enzymatic UV photometric method using fixed time kinetic (K056-1). All serum samples were analyzed colorimetrically with an automatic biochemical analyzer (BS200E, Mark Mindray; Model BS200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China). The second serum sample was used to evaluate IGF-I concentration. Concentrations of IGF-I were determined using an immunometric chemiluminescence immunoassay (Immulite 2000 IGF-I Assay, Erlangen, Germany).

4.9 Feeding behavior and sorting

Feeding behavior was evaluated by visual observation for 24 h at d 42 and 92 on the feedlot period. The behavioral categories evaluated were the eating time, ruminating time (RT), nonchewing behavior (NB), and drinking time (DT), according to definitions by Robles et al. (2014). Activities were observed and registered at 5 min intervals.

Diets and refusal samples of each pen were collected at the same days to establish particle-size distribution and frozen at -20°C for further analyses. Prior analysis, samples were defrosted at ambient temperature and divided into 3 sub-samples: sub-sample 1 for particle size analysis, sub-sample 2 for DM determination, and sub-sample 3 for chemical composition analysis. Particle size analysis performed by the PSPS, and particle size proportions of each diet are described in Table 2. Feed sorting index was calculated as the actual intake of each fraction size expressed as a percentage of the predicted intake, where predicted intake of each fraction equals the product of as-fed intake and as-fed fraction in the diet. Values less than 100% indicate selective refusals, greater than 100% indicate preferential consumption, and equal than 100% indicate no sorting (Leonardi & Armentano, 2003).

4.10 Apparent total tract digestibility

Apparent digestibility of nutrients was determined in two periods of three consecutive day, during the sampling collections (d 44–46 and d 94–96). Fecal spot samples were collected from each animal at the pen floor immediately after defecation and any dirt was carefully removed to avoid contamination. To ensure sample representation and homogeneity, samples were collected at different time points (0600, 1200, and 1800 h). This process was performed twice (total of 6 d), samples were composited by pen, homogenized, and 300 g (wet basis). Feedstuffs, orts, and fecal samples collected between these days were oven-dried (55ºC), ground using a Knife mill (model R-TE-650/1, Tecnal, Piracicaba, SP, Brazil) to pass through a 2-mm screen, and a subsample of approximately 50% of the ground material was ground to pass through a 1-mm screen to posterior chemical analysis.

4.11 Nitrogen balance

Nitrogen retention was estimated from individual animal performance, as previously described by Cole et al. (2008), using the following equations described at the BR-Corte for Zebu cattle (Valadares Filho et al., 2016):

$$SBW = 0.8800 \times BW^{1.0175}$$

$$EBW = 0.8126 \times SBW^{1.0134}$$

$$EBG = 0.9630 \times ADG^{1.0151}$$

$$RE = 0.061 \times (EBW^{0.75} \times EBG^{1.035})$$

$$ProtRe = 1.140 \times (RE/EBG)-1.137$$

$$NRe = ProtRe/6.25$$

where: SBW = shrunk body weight (kg); EBW = empty body weight (kg); EBG = empty body weight gain (kg/d); EBG = empty body gain (kg/d); RE = retained energy (Mcal/d); ProtRe = protein retained (kg); NRe = retained nitrogen (g/d). Urinary N excretion was calculated as the difference between N intake and fecal + retained N.

4.12 Enzymatic activities

At the end of the experiment, cattle were slaughtered as described above. After evisceration (within 45 min of stunning), the rumen, jejunum, and pancreas were sampled. Sample of rumen epithelium (4 cm^2) was collected from ventral sac and jejunum (middle of the first half non-duodenal small intestine) were rapidly excised from each bull after removing the digesta. Pancreas was sampled from the midparte region (5 g). Sterile tools and gloves were used to avoid contaminations. These

samples were washed with saline solution, minced, snap minced, snap frozen in liquid nitrogen promptly and subsequently stored at -80˚C until analysis.

For enzymatic activity analyses, fragments of these samples were weighed in a Falcon tube, and a PBS buffer solution was added for homogenization. The amount of buffer added was calculated based on the sample weight multiplied by 5. Using a Turrax device (IKA® T18 ULTRA-TURRAX® Basic Homogenizer, Wilmington, NC, USA), the samples were homogenized for 5 minutes. After this procedure, the samples were placed in 2 mL tubes and centrifuged for 20 to 30 minutes at 5000 rpm. Subsequently, the supernatant was retrieved and transferred to another tube, stored in a freezer at -4°C until the analyses were performed. The enzymes tested were α-amylase, maltase, and urease.

4.13 α-Amylase activity

To determine the α-amylase activity in the pancreas and jejunum, two test tubes were used, one as a control (without the sample) and the other as a sample tube (with the sample). For the control, 0.5 mL of the substrate (starch < 5 nmol/L along with a phosphate buffer < 500 nmol/L, stabilizer, and preservatives) was added and placed in a water bath for 2 minutes at 37°C. After homogenization, the substrate was incubated at 37°C for seven minutes and thirty seconds. Subsequently, 0.5 mL of the working reagent (color reagent – potassium iodide < 50 nmol/L, potassium iodate < 600 nmol/L, hydrochloric acid < 200 nmol/L, and stabilizer), along with 4 mL of deionized water, was added. In the tube designated for enzymatic activity detection, containing the sample, 0.5 mL of the substrate was also added, brought to a water bath for 2 minutes at 37°C, 10 µL of the sample was added, followed by incubation at 37°C for seven and a half minutes, and then 4 mL of deionized water was added. Absorbance was measured using a spectrophotometer at 660 nm (620 – 700 nm) after zeroing the device with deionized water.

4.14 Maltase activity

Maltose, starch buffer, and glucose oxidase were used as substrates to determine maltase activity in the jejunum and pancreas of the animals. 0.350 g of maltose diluted in 50 mL of starch buffer was used, incubated for 15 minutes at 37°C. Later, 200 µL of glucose oxidase was added, and the solution was incubated again at 37°C for 10 minutes. In a plate, samples were pipetted in triplicate, with 10 µL being the sample, 200 µL of glucose oxidase, and 100 µL of maltose. The reaction was read by measuring absorbance on a spectrophotometer.

4.15 Urease activity

Urease activity in the pancreas, jejunum, and rumen was determined through the following enzymatic assay: 1 mL of 3% urea solution buffered with 1 mL of 0.2 M phosphate buffer (pH = 0.7) was pipetted into a tube. 1 mL of enzymatic extract was added, and it was incubated at 55°C for 15

minutes. At the end of the incubation period, the tubes were placed on ice. The reaction was terminated by adding 1 mL of 0.66 N H_2SO_4, and 1 mL of 1 M sodium tungstate solution was pipetted to allow protein precipitation after centrifugation. Following centrifugation, the supernatant was recovered in new tubes and analyzed for NH_3-N, and enzyme activity was determined colorimetrically by measuring the formed quantity. It is assumed that the urea that disappears during incubation is hydrolyzed by urease.

4.16 Statistical analyses

Prior to all statistical analyses, the data were checked for normality and homoscedasticity. Data transformations were not necessary. Additionally, outliers were checked. One bull (REC+UC) was removed from the study within the first 6 week due for reasons unrelated to treatment (Phlegmon infection) and the corresponding data was removed prior to statistical analysis. All statistical analyses were conducted using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Pen was the experimental unit (all the bulls within a pen belong to the same treatment group) and bulls as replicates. The model was fitted with individual animal data and included the fixed effects of CPM, US, and the CPM × US interaction as well and a random effect of pen nested within treatment (St-Pierre, 2007). In addition to overall growth performance, ADG and G:F were analysed using a carcass-adjusted final BW calculated by dividing the HCW by the common dressing percentage of 55.63% (the mean dressing percentage over all replicates included on the study) to account for possible differences in gut-fill during weigh-out and allow for more accurate evaluation of cattle growth. The initial BW was used as a covariate for growth performance variables when was deemed significant at $P < 0.10$. When significant CPM × US interactions were detected ($P < 0.10$), pairwise comparisons of the simple effect means were conducted with the PDIFF option of the MIXED procedure of SAS.

The mean DMI of each pen was calculated during adaptation on d 1 to 6 (step 1), d 7 to 10 (step 2), and d 11 to 14 (step 3), and then weekly (12 weeks in total). Data collected for each pen for each week period were analysed as a mixed linear model with treatment, period (as a repeated measure) and the treatment × period as fixed effects and pen as the experimental unit. The variance components were used as the covariance structure and pen within treatment × period as subject for the repeated measures. The restricted maximum likelihood method was used for estimating variance components and the Kenward-Roger option was used to adjust the degrees of freedom. The variance and covariance error structures that were investigated included compound symmetry, heterogeneous compound symmetry, and autoregressive. The error structure with the lowest Akaike information criteria fit statistic was selected for the model. Data are reported as least square means and differences among treatments were determined using orthogonal contrasts for corn processing, urea source, and interactions effects. For the repeated measures model, the SLICE option was used when the treatment × period interaction was

significant to partition and test the simple main effects. Differences were considered significant at $P \leq 0.05$ and trends were discussed at $0.05 < P < 0.10$.

5. RESULTS

5.1 DMI

In the present study, no significant interactions were observed between CPM and US for growth performance and carcass traits (Table 2). Although there was no difference between treatments on daily DMI during the entire trial (Table 2). Fig. 1 illustrates some differences for the CPM × US treatments (treatment × time: $P < 0.01$). The DMI of bulls fed DGC+CU diets was higher than those fed REC+U diets during week 3, and tendency were verified at week 4 and 7 between these treatments ($P = 0.09$, 0.06). At step 3 and week 6, DMI tended to be lower in cattle fed REC+U ($P = 0.10$ and $P = 0.06$, respectively) than those fed DGC+CU. In the other weeks, DMI was not different between treatments ($P \geq 0.12$).

5.2 Growth performance and carcass traits

Initial BW was not different among treatments (Table 2), and final BW and ADG were both affected by CPM and US. In general, total DMI was not impacted by CPM ($P = 0.86$). However, when expressed in a BW basis, bulls fed REC-diets ate significantly less than those fed DGC-diets (21.1 vs. 21.9 g/kg BW; $P = 0.03$). Bulls fed REC-based diets had 9.2% greater carcass ADG ($P = 0.02$) and 7.0% greater carcass G:F ($P < 0.001$) compared to bulls fed DGC-based diets (Table 2). Corn grain processing method did not affect dressing and ultrasound measurements ($P \geq 0.26$), however bulls fed REC-diets presented higher HCW (326 vs. 316 kg; $P = 0.01$) compared with bulls fed DGC-based diets. Regarding US effects, CU inclusion increased ADG 7.8% ($P < 0.01$) and, because DMI was similar US treatments ($P = 0.58$), carcass-gain efficiency improved 6.9% ($P < 0.01$) of bulls compared with those fed U-diets (Table 2). Based on it, bulls fed CU-diets improved final BW ($P < 0.01$) and HCW ($P = 0.04$) in about 14 and 8 kg compared to bulls fed U-diets, respectively. Bulls fed CU had greater carcass-gain efficiency than U (102 vs. 92 g/kg DMI; $P < 0.01$). Additionally, rump muscle length was longer in bulls fed CU than U ($P = 0.02$).

5.3 Observed Dietary NE concentrations and fecal starch

There was no interaction between CPM and US on observed NE concentrations calculated from animal performance (Table 3; $P \geq 0.14$). Nevertheless, CPM and US affect these variables. Dietary NE intakes, which were calculated from growth performance measurements (BW, ADG, and DMI), were greater ($P \leq 0.01$) for bulls fed REC compared with bulls fed DGC. Observed NEm and NEg values (Mcal/kg) were respectively 7.7% and 10.0% greater ($P < 0.02$) for REC- than for DGC-based diets.

The observed NE:expected NE ratios were greater ($P < 0.01$) for REC- than DGC-based diets. Fecal starch was affected by CPM ($P < 0.01$). Mean fecal starch as a percentage of fecal DM was less ($P < 0.01$) for bulls fed REC diets (5.5%) than for bulls fed DGC diets (11.6%). As a result of lower fecal starch, fecal pH of bulls fed REC was about 15.8% greater than fecal pH of bulls fed DGC ($P < 0.01$). Estimates of NEm_{corn} and NEg_{corn} were greater for bulls fed REC than bulls fed DGC, reflecting similar patterns of fecal starch since values were calculated using its content.

Bulls fed CU had greater observed NEm and NEg than bulls fed U (2.08 vs. 2.15 Mcal/kg of NEm and 1.41 vs. 1.47 Mcal/kg of Neg, respectively; $P < 0.03$). Replacing U with CU resulted in a lower fecal starch concentration (7.5 vs. 9.5%; $P = 0.02$) and, consequently, a tendency to improve fecal pH ($P = 0.06$) was observed (6.43 vs. 6.25). Following the same pattern of fecal starch content and estimates of NEm_{corn} and NEg_{corn} was enhanced by the replacement of U with CU ($P = 0.01$).

5.4 Intake and total apparent digestibility of dietary components

Treatment means for voluntary intake and digestibility of dietary components are presented in Table 4. During the digestibility assay, CPM × US effects were not detected for voluntary intake ($P \geq 0.12$), but apparent digestibility of dietary constituents showed some interaction effects ($P < 0.10$). Bulls fed DGC+CU presented lower CP and NDF ($P < 0.04$) digestibility compared with those fed DGC+U, but there were no differences between bulls fed REC+U and REC+CU ($P > 0.30$). The REC-based diets resulted in greater overall digestibility of dietary components, reflecting in an increased digestible organic matter (DOM) intake ($P = 0.03$). The CU inclusion significant increased starch digestibility ($P = 0.05$; 95.0 vs. 93.8%).

5.5 Feeding behaviour and sorting

The feeding behaviour revealed an interaction between CPM and US on eating time ($P \leq 0.02$; Table 5), but no on sorting ($P \geq 0.52$). Bulls fed DGC+CU spend less time eating than those DGC+U (as expressed as min and min/kg DMI; $P \leq 0.02$), while bulls fed REC-based diets did not differ ($P = 0.12$). Bulls fed REC-diets sorted for long and medium, and sorted against fine particles compared to bulls fed DGC-diets ($P \leq 0.03$).

5.6 N utilization

The N intake, apparent N absorved, and total N excretion were not affected by CPM, US, and their interaction ($P \geq 0.26$). However, a tendency on fecal N ($P = 0.08$; Table 6) and their N intake and total N excretion ratios were found ($P \leq 0.05$). Fecal N excretion and their ratios to N intake and total N excretion were significantly greater in bulls fed DGC+CU than those fed DGC+U ($P \leq 0.04$), but for REC-diets there was no US differences ($P = 0.63$). Urine was the major route of N excretion in the Nellore bulls fed corn-based diets (about 80.0%). Feeding DGC+CU compared with DGC+U diet

reduced the proportion of N excreted in urine from 65 to 60% of N intake and from 80 to 74% of total N output. There was no difference between bulls fed REC-diets ($P \geq 0.21$, averaging 65 and 82%, respectively).

The replacement of U by CU in corn-based diets tended to decrease the estimated urinary N excretion ($P = 0.10$; 150 vs. 140 g/d). Either CPM as US affect N retention and its efficiencies ($P \leq 0.01$). Bulls fed REC-based diets and those with CU inclusion presented greater N retentions ($P < 0.01$) and its efficiencies of utilization ($P \leq 0.06$). In average, efficiencies of N retention (% of N intake) were improved about 10.0 and 8.6% in bulls fed REC and CU than those fed DGC and U ($P \leq 0.02$), respectively. Nevertheless, efficiency of N retention relative to apparent N absorbed was only significantly affected by US ($P < 0.01$). Bulls fed diets with CU were 10.9% more efficient than those fed U.

5.7 Blood metabolites

An interaction between CPM and US was only observed on GGT concentration ($P = 0.05$; Table 7). When bulls were fed with DGC-based diets, GGT concentration was greatest for those with U than CU inclusion, on the other hand, the opposite effect was observed when bulls were fed REC-based diets ($P = 0.05$). The others blood metabolites of energy metabolism, muscle body mass, and liver function were not influenced by CPM or US ($P \geq 0.15$).

5.8 Carbohydrate and nitrogen metabolism-related enzyme activities

Table 8 shows the enzymatic activities in the rumen, jejunum, and pancreas. The CPM, US, and their interaction did not affect total urease activity in the rumen (average of 268 μg of ammonia/hour hydrolyzed; $P \geq 0.58$). In the jejunum, there was a CPM × US interaction for protein concentration ($P = 0.08$) and for concentration of urease and total maltase activities ($P < 0.03$). Protein concentration was greater in jejunal tissue of bulls fed REC-based diets with CU inclusion compared to those fed REC+U ($P = 0.05$), whereas bulls fed DGC-based diets showed no difference ($P = 0.66$). Concentration of urease activity was greater in the jejunum of bulls fed DGC+CU than DGC+U ($P < 0.001$), and greater in bulls fed REC+U than REC+CU ($P < 0.01$). Conversely, total maltase activity was lower in the jejunum of bulls fed DGC+CU compared to DGC+U ($P = 0.04$), while no difference was observed between REC-diets ($P = 0.25$).

Total urease activity in the jejunum was lower in bulls fed REC-based diets compared to DGC-based diets (908 vs. 1,642 U, respectively; $P < 0.01$). Total α-amylase activity and its concentration in the jejunum were greater in bulls fed CU than U (552 vs. 429 U and 70 vs. 63 U/mg protein, respectively; $P < 0.02$). On the other hand, the concentration of jejunal maltase activity was 1.2 times greater in bulls fed U than CU ($P < 0.01$). In pancreatic tissue, no CPM × US interactions were observed ($P > 0.47$). Pancreatic protein concentration was greater in bulls fed DGC- than REC-based diets (12.0 vs. 8.9 mg/g;

$P < 0.01$). Consistent with increased α-amylase activity in the jejunum, bulls fed CU diets showed greater total (483 vs. 425 AU; $P = 0.05$) and concentration (49.8 vs. 39.7 AU/mg protein; $P < 0.01$) of α-amylase activity in pancreatic tissue than those fed U diets. Additionally, pancreatic α-amylase concentration was greater in bulls fed REC- than DGC-diets (50 vs. 40 AU/mg protein; $P < 0.001$). Similarly, total chymotrypsin activity tended to be 17.3% higher ($P = 0.10$), and chymotrypsin concentration increased approximately 1.6-fold (47 vs. 30 AU/mg protein; $P < 0.001$).

5.9 Meat quality

There were no effects of CPM × US interaction ($P > 0.16$), CPM ($P > 0.26$), or US ($P > 0.20$) on shear force, thawing loss, cooking loss, or total loss (Table 9). However, meat from bulls fed diets with coated urea (CU) tended ($P = 0.10$) to have greater total loss on day 14 (cooking and thawing) than those fed urea (U) (28% vs. 25%). A tendency for a CPM × US interaction ($P < 0.10$) was observed for lightness (L*), metmyoglobin (MMb), and oxymyoglobin (MbO_2) at day 14 (Tale 10). Meat from bulls fed DGC+U and REC+CU tended ($P < 0.09$) to exhibit greater L* and MMb than meat from bulls fed DGC+CU and REC+U, respectively. Conversely, meat from bulls fed DGC+U and REC+ULP showed lower MbO_2 at day 14 ($P < 0.10$) compared to those fed DGC+CU and REC+U, respectively. The CPM method tended to influence ($P = 0.10$) the hue angle (h*), with meat from bulls fed REC diets showing a tendency for greater values than those fed DGC diets. Other color parameters and pigment concentrations were not affected by the evaluated factors or their interactions ($P \geq 0.13$).

6. DISCUSSION

The study examined the interaction effects of CPM (DGC and REC-based diets) with different US (U vs. CU) when fed to finishing Nellore bulls. Limited data are available regarding the response of beef cattle to coated urea aiming to increase post-ruminal release, although there are some studies on the overall performance of grazing beef cattle (Souza et al., 2022; Reis et al., 2023) and dairy cattle (Rauch, 2021). To our knowledge, such an evaluation of replacing a U source by CU on the performance and carcass traits of finishing beef cattle is unique and novel information. However, the lack of CPM × US interactions for growth performance and N metabolism variables were not expected and consistent with our hypothesis (Table 2 and 6). Nevertheless, although dietary CP (13.5% CP), N intake (226 g/d), and total N excretion (184 g/d) were similar between treatments evaluated in this trial (Tables 1 and 6), interestingly we evidenced an interaction between CPM and US on route of N excretion (Table 6).

The potential loss of NH_3–N from manure depends on the amount and chemical form of N present in feces and urine. As in rumen, MPS in the hindgut is driven by the availability of fermentable energy reaching the lower digestive tract, which in turn depends on the site and extent of carbohydrate fermentation (Bierman et al., 1999; Adams et al., 2004). The reduced total-tract NDF digestibility

(50.1% vs. 56.8%; $P = 0.04$) in bulls fed DGC+CU compared to those fed DGC+U suggests a larger pool of fermentable energy available for microbial growth in the lower tract. This was likely due to the increased recycling N from the CU source, as the primary N supply for MPS in the lower tract originates from urea-N recycling via blood circulation. Consequently, improving the fecal N excretion and decreasing CP digestibility (80.6% vs. 84.7% in bulls fed DGC+CU and DGC+U, respectively; $P = 0.01$). However, CP digestibility reduction is not necessarily detrimental, as volatile fatty acids (VFA) produced during fiber fermentation in the lower tract, although contributing only marginally, still support the animal's overall energy status. Nevertheless, N incorporated into MPS is rendered unavailable for absorption and is ultimately excreted in feces. Regarding urinary excretion, cattle predominantly excrete N as urea in urine, which accounts for 60–80% of total N excretion (Erickson & Klopfenstein, 2010; Batista et al., 2016a; 2016b; Lino et al., 2025). In the companion metabolic study evaluating similar treatments to this trial, Lino et al. (2025) did not find differences for the proportion of urea-N excretion (~77.3%). In addition, plasma urea-N excretion was not affected by treatments (Table 5). Therefore, we did not expect differences for urinary urea-N excretion.

In the present study, the fecal N excretion ratio relative to N intake (20.8 vs. 16.7%; $P = 0.01$) and fecal N excretion ratio relative to total N excretion (25.7 vs. 20.3%; $P < 0.01$) were higher in bulls fed DGC+CU than in those fed DGC+U. However, contrary to the fecal N excretion pattern observed, we found lower urinary N excretion relative to N intake (60.2 vs. 65.3%; $P < 0.001$) and total N excretion (74.3 vs. 79.7%; $P < 0.01$) in bulls receiving these respective treatments. Fecal nitrogen is considered less readily mineralized and emitted as NH_3–N, with only 10% of fecal N converting to NH_3–N after 30 days of storage at 20°C (Whitehead & Raistrick, 1993). Additionally, urea-N and NH_3–N in urine represent the most susceptible forms of manure N that contribute to NH_3–N emissions (Cole et al., 2005). Therefore, shifting the primary route of N excretion from urine to feces may help mitigate the environmental impact of nutrient excretion in feedlot cattle. Considering this, we infer that replacing U with CU in a DGC-based diet may result in lower NH_3–N emissions and reduced N losses from manure. This reduction in NH_3–N volatilization may also contribute to a better N:P balance in manure, potentially enhancing its suitability for crop production (Koenig et al., 2003).

In Brazil and most South American countries, flint corn is the predominant corn grain used in cattle diets. Flint corn contains a greater proportion of vitreous endosperm and lower starch availability compared to dent corn (Correa et al., 2002). Historically, DGC has been the primary corn processing method adopted in Brazilian feedlots (Millen et al., 2009; Oliveira & Millen, 2014; Pinto & Millen, 2018; Silvestre & Millen et al., 2021; Monsalve & Millen, 2025). However, feedlot nutritionists have shown growing interest in ensiling flint corn using methods such as HMC or REC (Bernardes & Castro, 2019; Monsalve & Millen, 2025). Ensiling facilitates starch–protein matrix breakdown, increasing

starch digestibility and improving nutrient utilization (Mahanna, 2008; Hoffman et al., 2011; Silva et al., 2020a, 2020b; Owens et al., 1986).

Jacovaci et al. (2022) recently conducted a meta-analysis evaluating the effects of ensiling flint corn on feeding value and feedlot cattle performance (HMC and REC). Their results indicated that ensiling flint corn decreased DMI by 14.1%, but did not affect ADG, leading to an 18.3% increase in G:F compared to DGC. Contrary to their findings, our study showed no effects on total DMI (averaging 10.5 kg/d), but when expressed relative to BW, bulls fed REC-based diets consumed 3.8% less than those fed DGC-based diets. This result is consistent with findings from previous studies involving HMC- (Galyean et al., 1976; Huntington, 1997; Cooper et al., 2002) and REC-based diets (Silva et al., 2020; Godoi et al., 2021). Additionally, consistent with previous studies comparing U vs. CU (Tedeschi et al., 2002; Salami et al., 2020; Pacheco et al., 2021), we did not find differences on DMI between US. However, it is important to emphasize that the commercial CU sources evaluated in previous studies were formulated to deliver urea to the rumen in a slow-release manner, whereas the CU source investigated in the present study was designed to redirect most of the dietary urea from the rumen to the post-ruminal tract.

In high-concentrate diets, replacing DGC with REC or HMC frequently reduces DMI (Owens et al., 1997; Zinn et al., 2011) due to higher ruminal starch fermentability, which induces hypophagia. Increased starch fermentability leads to greater VFA production and absorption in the forestomach (e.g., propionate; Oba & Allen, 2003), promoting higher net portal flux of propionate in REC- vs. DGC-based diets. In the liver, an abundant propionate supply stimulates anaplerosis in the tricarboxylic acid (TCA) cycle and increases ATP production, which suppresses appetite, primarily by reducing meal size (Allen et al., 2009; Allen, 2020). Nevertheless, in a companion study with heifers fed similar diets, Lino et al. (2025) found no differences in the molar proportion of propionate, but reported a greater ruminal VFA pool in heifers fed REC-based diets, supporting its feed intake suppression effect.

Bulls fed REC-based diets exhibited sorting behavior favoring long, medium, and short particles, while sorting against fine particles, in contrast to bulls fed DGC-based diets. Sorting for larger particles in the diet can be considered a strategy to mitigate discomfort associated with low ruminal pH conditions (DeVries et al., 2008; DeVries et al., 2014). Although ruminal pH was not continuously monitored in this trial, bulls fed REC diets likely experienced longer periods under subclinical acidosis. In a companion study, Lino et al. (2025) measured ruminal pH at 2-h intervals and reported that heifers fed REC-based diets exhibited lower mean (6.40 vs. 6.61), minimum (5.80 vs. 6.01), and maximum (6.96 vs. 7.12) pH values compared with those fed DGC-based diets. This lower ruminal pH may explain why bulls sorted for larger particles, as fiber intake can help stabilize ruminal conditions. The CPM, such as ensiling (HMC and REC) and steam flaking, increase starch degradation in the rumen (Owens & Basalan, 2013), potentially leading to VFA accumulation and a reduction in ruminal pH (Lino et al.,

2025). This shift in fermentation dynamics may further influence sorting behavior against fine particles in bulls fed REC-based diets.

REC-based diets improved ADG (1.78 vs. 1.96 kg/day for DGC- vs. REC-based diets), resulting in greater G:F (170 vs. 187 g/kg DMI, respectively). These findings are consistent with previous studies conducted with Nellore bulls fed flint corn-based diets (Gouvêa et al., 2016; Marques et al., 2016). Hot carcass weight (HCW) was also increased in bulls fed REC-based diets. As previously reported, REC processing enhances starch digestibility (Theurer, 1986; Drouillard & Reinhardt, 2006) and increases NEg (Zinn et al., 2011), which contributes to greater estimated daily carcass gain, heavier final body weight, and improved carcass G:F (Tables 2 and 3). However, no significant differences were detected in other carcass characteristics. Feeding corn-based diets supplemented with CU, compared with U, led to increases in carcass weight (325 vs. 317 kg), carcass ADG (1.06 vs. 0.98 kg/day), and carcass G:F (102 vs. 92 g/kg DMI). Furthermore, bulls fed CU-based diets exhibited greater rump muscle length (11.4 vs. 10.8 cm) and numerically higher LMA (93 vs. 87 cm^2; P = 0.12) compared with those fed U-based diets (Table 2). These results indicate that replacing U with CU improved the nitrogen status of the animals, as evidenced by approximately 7% increases in both N retention and the proportion of N intake or N digested that was retained. Collectively, these findings support the use of CU over feed-grade urea at typical inclusion levels used in finishing diets for beef cattle in Brazilian feedlots (1.2% DM; Silvestre & Millen et al., 2021).

Ruminants have a distinct evolutionary advantage over other domesticated species, as they can efficiently utilize NPN for microbial protein synthesis, allowing for partial or even complete substitution of dietary protein sources. However, excessive N release in the form of ruminal NH_3-N, particularly in diets containing urea, can decrease animal performance and, in some cases, cause ammonia toxicity (Bartley et al., 1976; Huntington et al., 2006; Owens et al., 1980). Furthermore, the rapid ruminal hydrolysis of NPN sources may reduce the efficiency of N utilization, thereby increasing N excretion and NH_3-N volatilization from manure, which can result in negative environmental impacts (Calsamiglia et al., 2010).

To address these challenges, the livestock industry has developed coating technologies to produce urea derivatives that resist ruminal degradation, such as biuret (Waite & Wilson 1968; Löest et al. 2001), urea-formaldehyde (Prokop & Klopfenstein 1977), urea with linseed oil and talc (Forero et al. 1980), urea-oils (Owens et al., 1980), and polymer-coated urea (Optigen®; Alltech Inc., Nicholasville, KY, USA); These compounds have been shown to reduce post-prandial peaks of ruminal NH_3-N compared to urea (Komatsu & Sakaki, 1971; Smith, 1986; Veen & Bakker, 1977), thereby may improving the synchronization between fiber and carbohydrate degradation in the rumen, which may enhances microbial efficiency (Seo et al., 2013). These coated urea products consist of uniformly sized central particles made of concentrated rumen-degradable N compounds, encapsulated by a semi-

permeable membrane that encloses the core and allows for their gradual diffusion into the ruminal fluid. Nevertheless, a range of studies have evaluated the potential benefits of post-ruminal urea supplementation in ruminants (Egan, 1965; Becker et al., 1982; de Carvalho et al., 2020; Oliveira et al., 2020).

In sheep, post-ruminal urea infusion was shown to improve digestibility (Egan, 1965) and increase DMI (Becker et al., 1982; Egan, 1965). Recently, Oliveira et al. (2020) reported that DMI did not differ between continuous urea infusions into the rumen or abomasum of Nellore heifers, but the proportion of microbial N derived from recycled urea and efficiency of microbial N synthesis increased with abomasal infusion compared to ruminal infusion. In beef heifers, continuous abomasal urea infusion resulted in lower ruminal N-NH_3 concentrations, more stable ruminal pH, and increased total-tract NDF digestibility compared to a ruminal pulse dose of urea (de Carvalho et al., 2020). Collectively, these findings indicate that post-ruminal urea supplementation can contribute to N supply for MPS, whereas DMI responses in growing ruminants appear to be less influenced by the site of urea administration.

The high fermentability of the corn-based diets evaluated and the overlap of meals that occurs when animals are on ad libitum intake, can result in continuous fermentation and sufficient N requirements to support the fermentation (Pitt et al. 1996; Van Soest et al. 2000). Therefore, positive effects of CU on growth performance and carcass traits could be associated with the supplying of N compounds to rumen microbes and the adequacy of the available absorbed nutrients. In fact, we observed in the companion study (Lino et al., 2025) a lower peak value and a narrower range of ruminal NH_3-N and PUN concentrations over time in Nellore heifers fed corn-based diets with CU vs. U, similar to this trial. This indicates a slower and more consistent N release pattern, which can prevent excessive accumulation of free NH_3-N and increases of PUN, reducing N losses via absorption and hepatic conversion (Huntington & Archibeque, 1999; Taylor-Edwards et al., 2009). This is further supported by the tendency of lower estimated urinary N excretion (140 vs. 150 g/d) and their relations to N intake (62 vs. 65%) and N absorbed (75 vs. 78%) in bulls fed with CU compared with U. The mRNA expression of UT-B expression correlates with the urinary N excretion and, to our knowledge, it this is a novel finding. Renal UT-B is sometimes up-regulated with a low protein diet (Inoue et al., 2005). Although our experimental diets did not present low protein, we found a transcriptional up-regulation of mRNA expression of UT-B in bulls fed diets with CU. Therefore, bulls fed diets with inclusion of U increase N losses via urine due to reduced N reabsorption by the kidneys and probably decreased N recycling to GI tract (Marini et al., 2003; Inoue et al., 2005).

Nitrogen retention reflects lean tissue accretion, and the increase in N retention observed in Nellore cattle fed REC-based diets compared to those fed DGC-based diets (42.2 vs. 45.2 g/day) suggests a greater amount of energy retained. The proportion of N retention relative to N intake was also

higher in cattle fed REC compared to those fed DGC diets (20.3% vs. 18.4%). Bulls fed REC-based diets had greater DOM intake than those fed DGC-based diets (7.0 vs. 6.3 kg/day), while CP intake remained similar between CPM, averaging 1.33 kg/day. Considering the efficiencies of MP reported in the companion study (158 vs. 139 g/DOM intake and 803 vs. 631 g/kg CP intake; (Lino et al., 2025), the estimated daily microbial CP synthesis is approximately 1,070 g/day in bulls fed REC-based diets, compared to 870 g/day in those fed DGC-based diets. These findings suggest that bulls fed DGC-based diets may not have fully met their MP requirements at this stage of growth, leading to greater N retention and its efficiency relative to N intake in bulls fed REC-based diets due to increased protein demand.

The vitreousness of the flint corn grain used in this study averaged 86% (Lino et al., 2025), which is higher than values reported for Brazilian flint hybrids (Correa et al., 2002; Caetano et al., 2015; Gouvêa et al., 2016), where vitreousness typically ranges from 73–77%. Previous research (Hoffman et al., 2011; Mahanna, 2008; Silva et al., 2020b) suggests that ensiling-based processing methods (e.g., HMC, REC, and snaplage) promote starch–protein matrix breakdown, enhancing starch availability for microbial digestion. Silva et al. (2020) evaluated ground flint corn grains reconstituted with water to reach 65% DM. Their findings revealed that after 14 days of ensiling, insoluble N levels significantly decreased (67.7% vs. 54.3%), reaching stabilization after 120 days (31.8%). Therefore, REC-based diets expose a greater surface area to enzymatic action by ruminal microorganisms compared to DGC-based diets, promoting higher starch availability. Indeed, in a companion study, Lino et al. (2025) observed a significantly larger fast-degrading starch fraction (69.55% vs. 38.44%) in REC- compared to DGC-based diets. The starch–protein matrix degradation, coupled with optimal moisture retention inside the silo, reduces particle hydrophobicity, thereby enhancing water uptake capacity (Silva et al., 2020a, 2020b). This interplay of factors likely increases particle density and functional specific gravity (Lechner-Doll et al., 1991; Nocek & Kohn, 1987), potentially enhancing passage rate and post-ruminal starch flow for ensiled grain-based diets (Silva et al., 2020b; Lino et al., 2025).

As described above, ensiling-based processing methods such as REC promote starch–protein matrix breakdown and increase starch availability (Hoffman et al., 2011; Mahanna, 2008; Silva et al., 2020b). Although a greater starch degradation rate has been reported when REC replaces DGC in beef cattle diets, the extent of ruminal starch digestion has not been well established (Silva et al., 2020; Godoi et al., 2021; Lino et al., 2025). However, these authors observed an approximately 85% increase in starch digestion in the small intestine of Nellore cattle fed REC-based diets compared to those fed DGC-based diets, which may contribute to reduced fecal starch loss and increased total starch digestibility. In this present study, Nellore bulls fed REC-based diets exhibited higher fecal pH (6.80 vs. 5.87), lower fecal starch concentration (5.5 vs. 11.6%), and greater starch digestibility (96.8 vs. 92.0%). Similar findings have been reported in performance studies, where fecal starch levels decreased when flint corn was processed more extensively (Gouvêa et al., 2016; Caetano et al., 2019).

According to Zinn et al. (2002), cereal grain processing primarily aims to enhance starch digestibility throughout the digestive tract, thereby increasing the energy content of the grain. In this study, bulls fed REC-based diets exhibited greater DM, OM, CP, and starch digestibility. The increase in digestible organic matter (DOM) intake can be attributed exclusively to the effects of the non-fiber fraction of the diet, particularly starch. Starch degradability increases during ensiling, which supports the greater DOM intake observed in bulls fed REC-based diets. The NDF digestion may be impaired by the presence of digestible or soluble starch, as often occurs with grain silage (Owens et al., 2005). In a companion study, Nellore heifers fed REC-based diets exhibited a trend toward lower ruminal NDF digestion (Lino et al., 2025). However, as observed in the present study, total NDF digestibility was not affected by the CPM.

While increased starch utilization enhances the energy content of both the grain and the diet, the incremental improvement in total tract starch digestibility observed in this study does not fully account for the differences in growth performance between cattle fed REC- and DGC-based diets. The estimated NEm and gain NEg of corn grain were only 2.8% and 3.7% higher for REC compared to DGC. However, based on cattle growth performance data, observed diet NEm and NEg values increased by 5.8% and 7.5%, respectively, in bulls fed REC compared to those fed DGC-based diets. According to NASEM (2016), the estimated NEm and NEg for ensiled corn (HMC) are 2.25 and 1.56 Mcal/kg, while values for ground corn are 2.17 and 1.49 Mcal/kg, respectively. Similarly, Brazilian Tables of Feed Composition for Ruminants (CQBAL 4.0; Valadares Filho et al., 2023) reported NEm and NEg values of 2.28 and 1.59 Mcal/kg for REC and 2.23 and 1.54 Mcal/kg for DGC, respectively. These estimates indicate that NASEM's values for NEm and NEg are 3.7% and 4.7% greater for ensiled corn (HMC or REC) than for DGC, whereas CQBAL 4.0 estimates these values to be 2.2% and 3.2% higher. Based on bull performance data from the present study (considering mean BW, ADG, and DMI), NEm and NEg values were found to be 7.7% and 10.0% higher, respectively, in bulls fed REC-based diets compared to those fed DGC-based diets. Several researchers have reported that ensiled flint corn increases the NE value of grain compared to corn subjected to less intensive processing methods (Caetano et al., 2015; 2019). Although greater starch digestion is frequently cited as the primary reason for this increase in NE value, Owens & Basalan (2013) estimated that only 50% of the increase in grain energy can be attributed to differences in total starch digestibility. The remaining response is likely due to improved digestibility of other feed components, changes in ruminal fermentation leading to a higher proportion of propionate that reduces methane losses, and shifts in the site and efficiency of digestion. The observed NE-to-expected NE ratios exceeded 1 for both DGC- and REC-based diets, with the latter exhibiting a greater ratio, as shown in Table 3. Since no specific adjustment factor currently exists for energy availability based on corn grain vitreousness, existing equations for estimating flint corn NE values may underestimate the energy content of grain that has not undergone extensive processing (e.g., DGC or whole corn). Discrepancies between the NASEM (2016) values and those obtained in this study may be

attributed to differences in corn vitreousness, given that NASEM estimates for NEm and NEg are primarily derived from trials using dent corn, whereas this study utilized flint corn. Similarly, CQBAL 4.0 estimates are based on flint corn trials. However, it is important to consider that the NEm and NEg estimates in this study are based on a limited dataset of observations (n = 14), in contrast to the larger reference values published in both nutritional tables.

Additionally, a positive effect of CU was verified on observed dietary NEm and NEg (Table 3), which seems that this US could have promoted positive effects on starch digestion and/or metabolism. The pancreas produces a complement of enzymes, including α-amylase, which are responsible for the partial hydrolysis of starch in the small intestine. In high-concentrate feedlot diets, significant amounts of dietary starch reach the small intestine (Theurer, 1986), where there may be limitation in starch digestion potentially because of inadequate α-amylase secretion (Swanson & Harmon, 2002; Harmon et al., 2004).

The present results demonstrate that CPM and US not only exert independent effects on nutrient digestion and enzyme activity but also interact to modulate N utilization efficiency in finishing Nellore bulls. The US influenced jejunal enzyme secretion. The inclusion of CU stimulated pancreatic α-amylase and chymotrypsin activities and concentrations relative to U, consistent with the stimulatory role of amino acid supply from MCP on pancreatic enzyme synthesis (Swanson et al., 2008; Harmon, 2009). In contrast, diets containing U enhanced jejunal maltase activity, suggesting that rapid ruminal urea hydrolysis may augment intestinal microbial or mucosal carbohydrase responses when pancreatic digestion is limiting (Brake & Swanson, 2018).

We observed CPM × US interactions in jejunal mucosal protein and enzyme activities. Bulls fed REC+CU tended to exhibit the greatest jejunal tissue protein content, reflecting a synergistic effect of highly intestinal digestible starch and sustained NH_3-N release from CU on enterocyte growth (Trotta et al., 2021). In these animals, jejunal urease activity was lower than in REC+U, indicating more efficient microbial N salvage and reduced endogenous N degradation in the small intestine. Conversely, DGC+CU increased jejunal urease relative to DGC+U, suggesting that when starch is less digestible and fermenting, sustained N release may shift more ammonia into the intestine for microbial recycling. Total jejunal urease activity was markedly lower on REC vs. DGC (908 vs. 1,642 U), signifying that REC diets supply more true protein from microbial CP to the small intestine and diminish reliance on microbial ureolysis (Silva et al., 2020). Pancreatic tissue likewise reflected CPM effects: bull fed DGC-diets presents an increase on protein concentration over those fed REC-diets (12.0 vs. 8.9 mg/g), yet REC-diets elevated pancreatic α-amylase concentration and tended to increase chymotrypsin, aligning with enhanced postruminal nutrient flow (Godoi et al., 2021; Swanson et al., 2002). These findings support the model that greater intestinal starch and amino acid delivery upregulates exocrine pancreatic function, optimizing digestive capacity (Swanson et al., 2002; Harmon, 2009).

The probably greater intestinal starch digestion and elevated serum glucose over the day in bulls fed REC-diets, considering the result from the companion paper (Lino et al., 2025) likely promoted enhanced glycogen deposition in muscle tissue. Higher muscle glycogen content can accelerate postmortem glycolysis, reducing ultimate pH and contributing to paler meat with lower myoglobin (Mb) content and increased oxymyoglobin (MbO_2) proportion at early postmortem timepoints (day 0) (Kim et al., 2018; Picard et al., 2019). These biochemical changes may explain the tendency for greater yellowness (b*) and hue angle (h*) in meat from bulls fed REC-diets, as reduced Mb and higher MbO_2 content shift meat color toward a more oxygenated, yellow-red appearance (Mancini & Hunt, 2005). Additionally, the association of REC+CU appeared to intensify postmortem oxidation, as suggested by the increased lightness (L*) and metmyoglobin (MMb) content observed at day 14. Elevated MMb levels reflect pigment oxidation over time, which may have been influenced by oxidative stress or pro-oxidant conditions induced by the CU source (Jayasena et al., 2015).

The tendency for greater total loss (cooking and thawing) in bulls fed diets with CU compared to those with U (28 vs. 25%) could be associated with altered water-holding capacity, possibly due to modifications in muscle protein structure caused by oxidative processes or US effects on carcass metabolism (Huff-Lonergan & Lonergan, 2005). Interestingly, meat from bulls fed DGC+U and REC+ULP showed lower MbO_2 content at day 14 than that from DGC+CU and REC+U, respectively, indicating reduced color stability in the former combinations. These differences may relate to the interaction between CPM and US on the oxidative state of muscle pigments. Diets that promote higher glucose availability (REC) and post-ruminal urea release (CU) may maintain more favorable redox conditions postmortem, stabilizing MbO_2. Conversely, rapid nitrogen release (U or ULP) in combination with DGC or REC could exacerbate oxidative instability, promoting pigment oxidation. Together, these findings highlight the complex interplay between energy and nitrogen sources in shaping meat quality traits, particularly color development and stability during aging.

Beyond the ruminal degradation effects, metabolic or post-digestive effects of N can also positively affect the cattle performance. The N metabolic effects cannot be separately evaluated because an animal's metabolism is based on integration of different mechanisms, on availability of several substrates and metabolites and on a complex hormonal and biochemical signalling and regulation (Detmann et al., 2014). In this present trial, improvements on bulls' performance can also be attributed with a better adequate protein status (Egan, 1965a; Egan & Moir, 1965; Kempton et al., 1977). In theoretical terms, the expression 'N status' defines the quantitative and qualitative availability of N compounds for different physiological functions in animal metabolism, including functions associated with the metabolism of other compounds, such as energy (Leng et al., 1990; Detmann et al., 2014). When N status is improved, the metabolism can achieve a better adjustment. In other words, molecules of NPN can be direct towards other metabolic pathways, such as the urea cycle. Thus, amino acids

utilization for those pathways will decrease, which, in turn, improves the availability of metabolic precursors for protein synthesis (Detmann et al., 2014) and can result in a better N efficiency as probable occurred for bulls fed CU-diets. In fact, based on performance of bulls in the present study (mean BW and ADG) and by using BR-Corte (2016) equations to estimate efficiency of metabolizable energy (kg) and protein (k), we obtained a significant greater estimate in bulls fed CU- than U-diets ($P = 0.05$ and $P < 0.01$, respectively; data not reported). The average kg was 78.3% and 76.8%, and k was 47.3% and 47.1% for CU and U, respectively. Therefore, the increase in either kg or k could be related to the better N status of bulls fed CU-diets.

Contrary to the study by Souza et al. (2022), where the presence of the enzyme Gamma-Glutamyltransferase (GGT) tended to be lower in bulls supplemented with CU compared to those supplemented with U, in this study no differences were observed between the sources of urea. However, the highest GGT value was found in bulls fed DGC+U. According to Kaneko et al. (2008), the normal level of urea in the blood of ruminant animals should be between 20 and 30 mg/dL. In this study, as well as in the studies by Souza et al. (2022) and Reis et al. (2023), urea values were slightly higher than this range.

7. CONCLUSIONS

In summary, reconstituted corn grain silage markedly improved flint corn energy value on digestibility, growth performance, and feed efficiency compared with fine grinding corn, independently of the urea source. When cattle are finished on diets based on flint corn like the ones used in this study, independent of the processing method coated urea seems to be the optimal source of supplemental NPN.

Table 1. Ingredients and chemical and particle size analysis of the experimental diets

	Ground corn		Rehydrated and ensiled corn	
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea
Ingredient, % of DM				
Corn shredlage	28.0	28.0	28.0	28.0
Corn, dry ground	61.3	61.3	–	–
Corn, reconstituted and ensiled	–	–	61.3	61.3
Soybean meal	6.3	6.3	6.3	6.3
Feedlot premix[1]	3.0	3.0	3.0	3.0
Feed-grade urea	1.1	–	1.1	–
Coated urea[2]	–	1.3	–	1.3
Ammonium sulfate	0.1	0.1	0.1	0.1
Kaolin	0.2	–	0.2	–
Chemical composition, % of DM				
DM, % as-fed	60.7	60.7	50.7	50.7
CP	13.8	13.6	13.4	13.3
Ruminally degradable protein	9.2	7.2	9.1	7.2
Starch	55.0	55.1	58.2	58.2
NDF	22.4	22.4	20.9	20.9
Ether extract	3.4	3.6	3.4	3.6
Particle size analysis[3], % as-fed				
Long (> 19 mm)	2.7 ± 0.26	2.5 ± 0.22	2.7 ± 0.23	2.6 ± 0.26
Medium (8-19 mm)	23.8 ± 0.72	22.8 ± 1.14	23.2 ± 0.90	22.8 ± 0.90
Short (4-8 mm)	10.2 ± 0.84	14.1 ± 0.80	8.5 ± 0.64	14.4 ± 0.84
Fine (< 4 mm)	63.3 ± 1.29	60.6 ± 1.61	65.5 ± 1.56	60.2 ± 1.63
Particles > 4 mm	36.7 ± 1.29	39.4 ± 1.61	34.5 ± 1.56	39.8 ± 1.63

[1]Feedlot premix (BellPeso Essencial, Bellman, Trouw Nutrition Brazil) provided an additional 120 g/kg Ca (minimum), 30 g/kg P, 80 g/kg Na, 50 g/kg K, 68 g/kg Mg, 25 g/kg S, 1,220 mg/kg Zn, 330 mg/kg Cu, 950 mg/kg Mn, 20 mg/kg Co, 24 mg/kg I, 6 mg/kg Se, 67,000 IU/kg vitamin A, 9,500 IU/kg vitamin D3, 950 IU/kg vitamin E and 650 mg/kg Monensin to the diet DM.

[2] Produced by SIPENA SAS, St Malo, France.

[3] Particle size distribution of TMR measured using the Penn State Particle Separator with 3 sieves (19, 8, and 4 mm). SD (n = 12 weeks for particle sizes)

Table 2. Effects of corn grain processing method (CPM) and urea source (US), and their interactions on growth performance and carcass characteristics of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Growth performance[1]								
Initial BW,[1] kg	392	391	393	391	18.5	0.98	0.95	0.96
Final BW,[1] kg	562	579	578	589	5.5	0.02	<0.01	0.66
ADG,[3] kg	1.71	1.87	1.87	1.99	0.06	0.02	<0.01	0.66
DMI,[4] kg	10.2	10.7	10.4	10.3	0.37	0.86	0.58	0.44
DMI, g/kg	21.5	22.2	21.1	21.1	0.34	0.03	0.40	0.29
Feed efficiency, g/kg	166	174	178	192	3.2	<0.01	<0.01	0.31
Carcass characteristics								
HCW, kg	312	321	323	329	3.7	0.01	0.04	0.64
Dressing, %	55.5	55.5	55.8	55.7	0.42	0.46	0.89	0.95
Average daily gain, kg/d	0.93	1.02	1.04	1.09	0.04	0.01	<0.01	0.66
Carcass gain:feed, g/kg	92	96	96	105	2.00	<0.01	<0.01	0.56
Longissimus muscle area, cm^2	87.3	88.8	87.2	96.8	3.58	0.28	0.13	0.27
12th fat tickness, mm	3.3	3.3	4.0	3.6	0.37	0.62	0.20	0.55
Rump muscle lenght, cm	10.8	11.3	10.8	11.4	0.22	0.96	0.02	0.92
12^{th} rib fat thickness, mm	5.4	5.1	5.8	5.8	0.48	0.26	0.73	0.78

[1] Initial and final individual BW measured live and after 16 h of feed restriction.

[2] Carcass-adjusted values. Adjusted (Adj.) final BW was estimated by dividing HCW by the overall average dressing percentage obtained for treatments (55.63%) and so, adjusted ADG and feed efficiency were calculated.

[3]Calculated using initial and final BW (after 16 h of feed and water restriction).

[4]Recorded from each pen (7 pens/treatment) and divided by the number of animals within each pen, and expressed as kg animal/d.

Table 3. Effects of corn processing method (CPM) and urea source (US), and their interactions on dietary net energy (NE) concentrations and fecal characteristics of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
NE_m intake,[1] Mcal/d	20.6	22.3	22.9	23.8	1.01	0.07	0.20	0.68
NE_g intake,[1] Mcal/d	13.8	15.1	15.7	16.5	0.74	0.04	0.17	0.72
Observed NE[2]								
NE_m, Mcal/kg	2.00	2.07	2.15	2.23	0.031	<0.001	0.03	0.76
NE_g, Mcal/kg	1.35	1.40	1.48	1.55	0.027	<0.001	0.03	0.76
Oberved NE:expected NE[3]								
NE_m	1.01	1.04	1.05	1.09	0.015	<0.01	0.05	0.78
NE_g	1.01	1.05	1.07	1.12	0.020	<0.01	0.05	0.78
Fecal pH	5.71	6.03	6.78	6.82	0.095	<0.001	0.06	0.14
Fecal starch, %	13.1	10.1	6.0	4.9	0.87	<0.001	0.01	0.23
Estimated fecal analysis[4]								
NEm_{corn}, Mcal/kg	2.21	2.27	2.36	2.38	0.043	<0.001	0.01	0.23
NEg_{corn}, Mcal/kg	1.58	1.63	1.71	1.73	0.038	<0.001	0.01	0.23

[1] Calculated using observed NE values based on equation described by Zinn and Shen (1998).

[2] Calculated using cattle growth performance data based on the equation proposed by Zinn and Shen (1998).

[3] Expected NE values were estimated with the equations proposed by NASEM (2016); solution type = empirical level) with addition of monensin as feed additive and using the total digestible nutrient values, which had been calculated with the equation proposed by Weiss et al. (1992).

[4] Estimated from fecal starch as described by Zinn et al. (2002).

Table 4. Effects of corn grain processing method (CPM) and urea source (US), and their interactions on voluntary intake and digestibility of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Intake, kg/d								
DM	9.5	10.1	9.5	9.6	0.55	0.52	0.42	0.60
OM	9.1	9.6	9.1	9.2	0.53	0.57	0.39	0.59
Starch	5.1	5.3	5.4	5.5	0.31	0.35	0.45	0.70
NDF	2.3	2.5	2.3	2.2	0.09	0.11	0.55	0.34
CP	1.4	1.4	1.3	1.3	0.11	0.12	0.94	0.56
DOM	6.1	6.4	6.9	7.1	0.48	0.03	0.48	0.94
Apparent total tract Digestibility, % of intake								
DM	66.1	64.2	73.0	74.8	1.08	<0.01	0.95	0.10
OM	68.0	66.1	75.0	76.6	1.07	<0.01	0.92	0.12
CP	84.7	80.6	86.9	87.3	2.74	<0.01	0.09	0.05
NDF	56.8	50.1	51.0	53.4	4.20	0.58	0.34	0.04
Starch	91.2	92.7	96.5	97.2	1.31	<0.01	0.05	0.44

DOM= digestible organic matter.

Table 5. Effects of corn processing method (CPM) and urea source (US), and their interactions on behaviour and activity, and sorting behaviour of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Eating								
Min/d	211	188	182	203	8.8	0.46	0.92	0.02
Min/kg DMI	19	16	17	19	0.9	0.93	0.52	<0.01
Ruminating								
Min/d	195	185	195	165	15.6	0.53	0.21	0.52
Min/kg DMI	18	16	18	16	1.6	0.98	0.20	0.81
Chewing[1]								
Min/d	405	373	378	366	17.5	0.32	0.22	0.56
Min/kg DMI	37	32	35	34	1.9	0.98	0.17	0.27
Drinking, min/d	8	8	6	8	1.2	0.34	0.46	0.70
Standing, min/d	170	175	150	182	15.5	0.68	0.26	0.39
Lying, min/d	856	882	889	870	24.6	0.67	0.88	0.38
Sorting behaviour[2]								
Long (>19 mm)	115	116	128	134	5.4	<0.01	0.55	0.78
Medium (8-19 mm)	114	116	129	125	4.5	<0.01	0.80	0.52
Short (4-8 mm)	104	102	113	107	3.4	0.03	0.27	0.54
Fine (< 4 mm)	95	96	85	86	2.1	<0.001	0.80	0.97

[1]Chewing = eating + rumination

[2]Values < 100% indicate selective refusals (sorting against), > 100% indicate preferential consumption (sorting for), and = 100% indicate no sorting.

Table 5. Effects of corn processing method (CPM) and urea source (US) in retention and route of nitrogen (N) excretion in Nellore bulls.

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
N intake, g/d	227	230	229	220	8.32	0.48	0.90	0.32
N absorbed, g/d	188	183	192	182	6.16	0.81	0.23	0.76
Total N excretion, g/d	186	190	185	173	7.9	0.26	0.59	0.33
Fecal N, g/d	38	49	34	32	3.5	<0.01	0.24	0.08
% N intake	17	21	15	14	1.1	<0.001	0.10	0.05
% total N excreton	20	26	18	18	1.2	<0.001	0.04	0.04
Urine N, g/d	148	139	151	141	5.4	0.72	0.10	0.78
% N intake	65	60	66	64	0.9	0.02	<0.01	0.08
% total N excreton	80	74	82	82	1.2	<0.001	0.04	0.04
N retained, g/d	40	44	44	46	1.1	<0.01	<0.01	0.56
% intake	18	19	19	21	0.7	0.01	0.02	0.43
% absorbed	21	24	23	25	0.7	0.15	<0.01	0.92

Table 7. Effects of corn processing method (CPM) and urea source (US), and their interactions on blood hormones and metabolites of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
IGF-I,	372.7	380.5	372.1	352.3	9.67	0.15	0.36	0.24
Energy metabolism								
Glucose, mg/dL	109.6	115.7	122.0	109.3	5.27	0.46	0.57	0.20
Muscle body mass								
Creatinine, mg/dL	1.52	1.58	1.62	1.61	0.05	0.23	0.61	0.42
Urea, mg/dL	36.0	37.0	35.8	34.6	2.35	0.65	0.83	0.52
Liver function								
ALP, U/L	540.4	522.7	515.7	480.9	31.3	0.52	0.62	0.50
AST/GOT, U/L	82.3	85.1	83.2	82.9	5.48	0.93	0.90	0.86
GGT, U/L	21.7	18.0	16.3	19.6	1.59	0.35	0.95	0.05
Total protein, g/dL	6.75	6.81	6.88	6.86	0.16	0.54	0.80	0.78
Albumin, g/dL	3.22	3.23	3.23	3.27	0.04	0.62	0.46	0.75
Globulin, g/dL	3.52	3.57	3.69	3.60	0.15	0.51	0.98	0.62

Table 8. Effects of corn processing method (CPM) and urea source (US), and their interactions on the activity of digestive enzymes in the rumen, jejunum, and pancreas of Nellore bulls

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item[1]	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Rumen								
Urease, μg of NH_3 hydrolized	230	274	265	305	57.0	0.62	0.57	0.58
Jejunum								
Protein, mg/ 100g	7.27	7.53	6.38	8.43	0.746	0.61	0.23	0.08
Urease, AU	1,589	1,695	944	872	135.9	<0.001	0.43	0.70
mg of protein	214	241	148	100	3.9	<0.001	0.94	<0.001
Amylase, AU	450	514	407	590	48.9	0.53	0.01	0.14
mg of protein	63	69	64	71	4.8	0.12	0.02	0.85
Maltase, AU	97	78	92	99	6.2	0.21	0.54	0.03
mg of protein	13	11	14	12	0.7	0.10	<0.01	0.92
Pancreas								
Protein, mg/100 mg	12.3	11.7	9.5	8.4	0.72	<0.001	0.26	0.72
Amylase, AU	433	504	417	462	29.5	0.34	0.05	0.65
mg of protein	35	44	44	55	2.2	<0.001	<0.001	0.62
Chymotrypsin	350	354	432	393	21.5	0.10	0.65	0.47
mg of protein	29	30	46	48	1.9	<0.001	0.04	0.84

[1]AU = arbitrary units.

Table 9. Effects of corn processing method (CPM) and urea source (US) on shear force and meat losses in Nellore bulls.

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Shear force								
Day 0	7.33	7.39	6.91	8.22	0.65	0.71	0.23	0.28
Day 14	4.80	4.50	4.51	4.65	0.54	0.88	0.86	0.63
Shear force area								
Day 0	19.72	19.38	18.09	22.15	1.78	0.71	0.24	0.16
Day 14	14.99	15.22	14.50	14.80	1.67	0.75	0.85	0.97
Thawing loss								
Day 0	1.31	1.13	1.28	1.56	0.27	0.40	0.83	0.35
Day 14	9.92	10.13	8.88	11.71	1.34	0.82	0.20	0.27
Cooking weight loss								
Day 0	23.45	20.91	19.83	20.90	3.62	0.45	0.76	0.45
Day 14	18.54	19.54	16.28	18.70	1.65	0.26	0.56	0.83
Total loss								
Day 0	24.60	21.97	21.00	22.26	3.79	0.50	0.78	0.43
Day 14	26.60	27.66	23.75	28.22	1.85	0.48	0.10	0.30

Table 10. Effects of corn processing method (CPM) and urea source (US) on beef color parameters of Nellore bulls.

	Ground corn		Rehydrated and ensiled corn			*P*-values		
Item	Feed-grade urea	Coated urea	Feed-grade urea	Coated urea	SEM	CPM	US	CPM × US
Lightness (L*)								
Day 0	33.89	34.71	34.51	36.21	1.10	0.29	0.21	0.65
Day 14	37.05	36.19	36.21	39.27	1.97	0.34	0.35	0.10
Redness (a*)								
Day 0	20.38	20.71	21.78	22.38	1.98	0.25	0.72	0.92
Day 14	18.10	17.71	18.53	16.91	1.11	0.84	0.31	0.53
Yellowness (b*)								
Day 0	12.75	13.30	14.54	15.08	1.79	0.15	0.66	0.99
Day 14	12.12	11.95	12.68	12.82	1.19	0.29	0.98	0.81
Chroma (C*)								
Day 0	24.02	24.60	26.18	26.97	2.62	0.21	0.70	0.95
Day 14	21.84	21.43	22.53	21.32	1.23	0.79	0.45	0.71
Hue angle (h*)								
Day 0	31.93	32.74	33.77	34.04	1.12	0.10	0.72	0.75
Day 14	34.00	34.26	34.56	37.49	1.52	0.23	0.24	0.38
Metmyoglobin (MMb)								
Day 0	29.62	29.02	29.27	29.43	0.48	0.94	0.60	0.38
Day 14	33.62	30.29	29.02	34.69	2.16	0.75	0.95	0.01
Myoglobin (Mb)								
Day 0	9.45	9.12	5.96	6.76	1.88	0.05	0.59	0.68
Day 14	10.38	9.67	11.14	9.28	1.58	0.65	0.29	0.45
Oxymyoglobin (MbO2)								
Day 0	61.36	62.26	65.17	64.22	2.12	0.13	0.75	0.60
Day 14	56.06	60.11	59.90	56.09	1.88	0.94	0.79	0.02

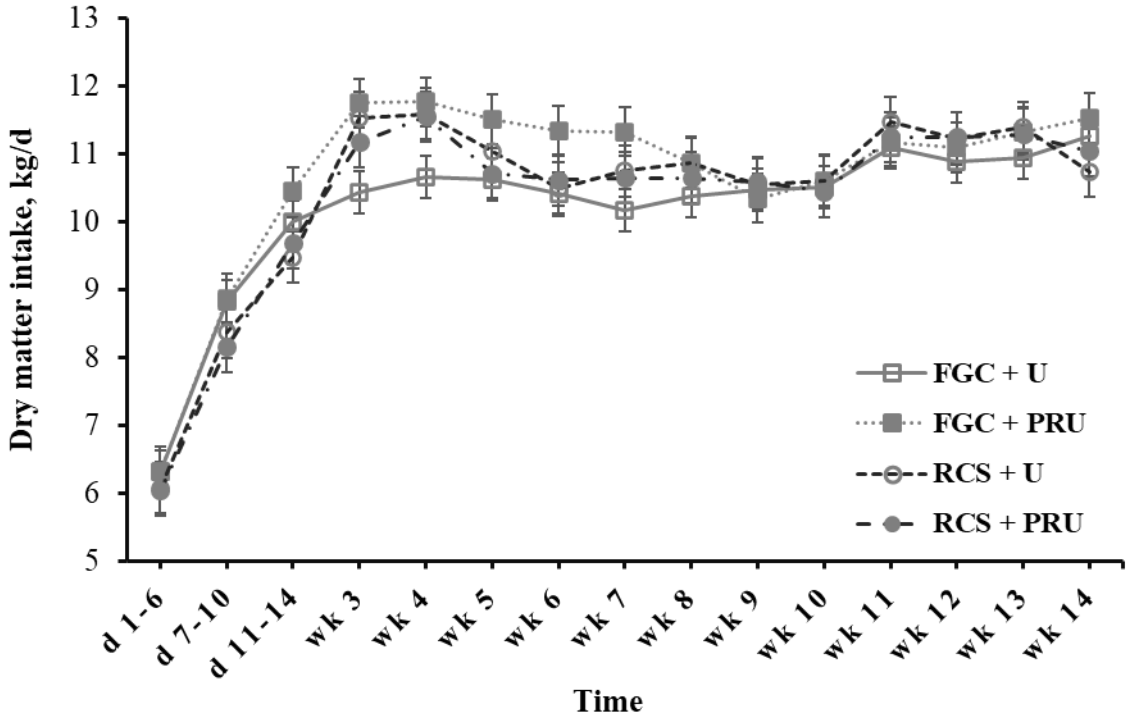


Figure 1. Weekly DMI change by Nellore feedlot bulls fed diets containing finely ground corn (DGC) or reconstituted corn grain silage (REC) and supplemented with feed-grade urea (U) or coated urea (CU) during a 100-d feedlot period. Time ($P < 0.001$), treatment ($P = 0.81$), and treatment × time ($P < 0.01$). Pooled SEM = 0.43; n = 7 pens per treatment.

8. REFERENCES

ABDOUN, K.; STUMPFF, F.; MARTENS, H. Ammonia and urea transport across the rumen epithelium: a review. *Animal Health Research Reviews*, v. 7, n. 1-2, p. 43-59, 2006.

ADAMS, J. R.; FARRAN, T. B.; ERICKSON, G. E.; KLOPFENSTEIN, T. J.; MACKEN, C. N.; WILSON, C. B. Effect of organic matter addition to the pen surface and pen cleaning frequency on nitrogen mass balance in open feedlots. *Journal of Animal Science*, 82: 2153–2163, 2004.

ALLEN, M. Control of feed intake by hepatic oxidation in ruminant animals: integration of homeostasis and homeorhesis. *Animal*, v. 14, n. S1, p. s55-s64, 2020.

ALLEN, M.; BRADFORD, B.; OBA, M. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. *Journal of animal science,* v. 87, n. 10, p. 3317-3334, 2009.

ARCHIBEQUE, S. L.; FREETLY, H. C.; COLE, N. A.; FERRELL, C. L. The influence of oscillating dietary protein concentrations on finishing cattle. II. Nutrient retention and ammonia emissions. *Journal of Animal Science,* v. 85, n. 6, p. 1496-1503, 2007.

ATKINSON, R.; TOONE, C.; LUDDEN, P. Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on site and extent of digestion and ruminal characteristics in lambs fed low-quality forage. *Journal of animal science*, v. 85, n. 12, p. 3322-3330, 2007.

BACH, A.; CALSAMIGLIA, S.; STERN, M. D. Nitrogen metabolism in the rumen. *Journal of Dairy Science,* v. 88, p. E9-E21, 2005.

BATISTA, E. D.; DETMANN, E.; TITGEMEYER, E. C.; VALADARES FILHO, S. C.; VALADARES, R. F. D.; PRATES, L. L.; RENNÓ, L. N.; PAULINO, M. F. Effects of varying ruminally undegradable protein supplementation on forage digestion, nitrogen metabolism, and urea kinetics in Nellore cattle fed low-quality tropical forage. *Journal of Animal Science*, 94: 201–216, 2016.

BATISTA, E. D.; DETMANN, E.; GOMES, D. I.; RUFINO, L. M. A.; PAULINO, M. F.; VALADARES FILHO, S. C.; FRANCO, M. O.; SAMPAIO, C. B.; REIS, W. L. S. Effect of protein supplementation in the rumen, abomasum, or both on intake, digestibility, and nitrogen utilisation in cattle fed high-quality tropical forage. *Animal Production Science*, 57: 1993–2000, 2016.

BECKER, K.; MOCTAR, M.; PFEFFER, E. Investigations in growing lambs on the utilization of urea infused into the abomasum [Untersuchungen an wachsenden Lämmern über die Verwertung von in den Labmagen infundiertem Harnstoff]. *Archiv für Tierernährung*, v. 32, p. 27–32, 1982.

BENTON, J. R.; KLOPFENSTEIN, T. J.; ERICKSON, G. E. Effects of corn moisture and length of ensiling on dry matter digestibility and rumen degradable protein. *Nebraska Beef Cattle Report*. 2005.

BERNARDES, T.; CASTRO, T. PSXII-12 Silages and roughage sources in the Brazilian beef feedlots. *Journal of Animal Science*, v. 97, Suppl. 3, p. 411-411, 2019.

BIERMAN, S.; ERICKSON, G. E.; KLOPFENSTEIN, T. J.; STOCK, R. A.; SHAIN, D. H. Evaluation of nitrogen and organic matter balance in the feedlot as affected by level and source of dietary fiber. *Journal of Animal Science*, 77: 1645–1653, 1999.

BRAKE, D. W.; SWANSON, K. C. Ruminant nutrition symposium: Effects of postruminal flows of protein and amino acids on small intestinal starch digestion in beef cattle. *Journal of Animal Science*, v. 96, n. 2, p. 739–750, 2018.

CABRITA, A. R. J.; DEWHURST, R. J.; ABREU, J. M. F.; FONSECA, A. J. M. Evaluation of the effects of synchronising the availability of N and energy on rumen function and production responses of dairy cows – a review. *Animal Research*, v. 55, n. 1, p. 1-24, 2006.

CAETANO, M.; GOULART, R. S.; SILVA, S. D. L.; DROUILLARD, J. S.; LEME, P. R.; LANNA, D. P. D. Effect of flint corn processing method and roughage level on finishing performance of Nellore-based cattle. *Journal of Animal Science*, 93(8): 4023–4033, 2015.

CAETANO, M.; GOULART, R. S.; RIZZO, P. M.; SILVA, S. D. L.; DROUILLARD, J. S.; LEME, P. R.; LANNA, D. P. D. Impact of flint corn processing method and dietary starch concentration on finishing performance of Nellore bulls. *Animal Feed Science and Technology*, 251: 166–175, 2019.

CALSAMIGLIA, S.; FERRET, A.; REYNOLDS, C. K.; KRISTENSEN, N. B.; VAN VUUREN, A. M. Strategies for optimizing nitrogen use by ruminants. *Animal,* v. 4, p. 1184–1196, 2010.

CAMARGO, K. D. V.; MESSANA, J. D.; SILVA, L. G.; GRANJA-SALCEDO, Y. T.; DIAS, A. V. L.; ALVES, K. L. G. C.; BERCHIELLI, T. T. Intake, metabolism parameters, and performance of growing beef cattle on pasture supplemented with different rumen undegradable protein with different amino acid profile. *Animal Feed Science and Technology*, v. 286, p. 115258, 2022.

CARVALHO, P. C.; DOELMAN, J.; MARTÍN-TERESO, J. Post-ruminal non-protein nitrogen supplementation as a strategy to improve fibre digestion and N efficiency in the ruminant. *Journal of Animal Physiology and Animal Nutrition*, v. 104, n. 1, p. 64-75, 2020.

CASPER, D. P.; MAIGA, H. A.; BROUK, M. J.; SCHINGOETHE, D. J. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. *Journal of Dairy Science*, 82(8): 1779–1790, 1999.

CHERDTHONG, A.; WANAPAT, M. Development of urea products as rumen slow-release feed for ruminant production: a review. *Australian Journal of Basic and Applied Sciences*, v. 4, n. 8, p. 2232-2241, 2010.

COLE, N. A.; CLARK, R. N.; TODD, R. W.; RICHARDSON, C. R.; GUEYE, A.; GREENE, L. W.; et al. Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. *Journal of Animal Science*, 83: 722–731, 2005.

COLE, N. A.; TODD, R. W. Opportunities to enhance performance and efficiency through nutrient synchrony in concentrate-fed ruminants. *Journal of Animal Science*, 86(suppl_14): E318–E333, April 2008.

COOPER, R. J.; MILTON, C. T.; KLOPFENSTEIN, T. J.; SCOTT, T. L.; WILSON, C. B.; MASS, R. A. Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle. *Journal of Animal Science*, 80(3): 797–804, 2002.

CORONA, L.; OWENS, F. N.; ZINN, R. A. Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. *Journal of Animal Science*, v. 84, n. 11, p. 3020-3031, 2006.

CORREA, C. E. S.; SHAVER, R. D.; PEREIRA, M. N.; LAUER, J. G.; KOHN, K. Relationship between corn vitreousness and ruminal in situ starch degradability. *Journal of Dairy Science*, v. 85, n. 11, p. 3008-3012, 2002.

DETMANN, E.; VALENTE, É. E.; BATISTA, E. D.; HUHTANEN, P. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. *Livestock Science*, 162: 141–153, 2014.

DETMANN, E.; CECON, P. R.; PAULINO, M. F.; VALADARES FILHO, S. C.; HENRIQUES, L. T.; DETMANN, K. D. S. C. Rumen variables evaluated through continuum mathematical functions. *Pesquisa Agropecuária Brasileira,* v. 42, p. 1651-1657, 2007.

DETMANN, E.; SOUZA, M.; VALADARES FILHO, S. D. C.; QUEIROZ, A. C.; BERCHIELLI, T. T.; SALIBA, E. O. S.; AZEVEDO, J. A. G. Métodos para análise de alimentos-INCT. *Viçosa, MG, Brazil: Suprema Gráfica*, 2012.

DEVRIES, T. J.; SCHWAIGER, T.; BEAUCHEMIN, K. A.; PENNER, G. B. The duration of time that beef cattle are fed a high-grain diet affects feed sorting behavior both before and after acute ruminal acidosis. *Journal of Animal Science*, 92(4): 1728–1737, 2014.

DEVRIES, T. J.; DOHME, F.; BEAUCHEMIN, K. A. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. *Journal of Dairy Science*, 91(10): 3958–3967, 2008.

DEWHURST, R. J.; DAVIES, D. R.; MERRY, R. J. Microbial protein supply from the rumen. *Animal Feed Science and Technology*, v. 85, n. 1-2, p. 1-21, 2000.

DROUILLARD, J.; REINHARDT, C. Flaked grain variables: Control points and evaluation of flaked grains. *Proceedings of the Cattle Grain Processing Symposium*, Tulsa, OK, 2006. p.81-87.

EGAN, A. The fate and effects of duodenally infused casein and urea nitrogen in sheep fed on a low-protein roughage. *Australian Journal of Agricultural Research*, v. 16, n. 2, p. 169-177, 1965a.

EGAN, A.; MOIR, R. Nutritional status and intake regulation in sheep. I. Effects of duodenally infused single doses of casein, urea, and propionate upon voluntary intake of a low-protein roughage by sheep. *Australian Journal of Agricultural Research*, v. 16, n. 3, p. 437-449, 1965

ERICKSON, G.; KLOPFENSTEIN, T. Nutritional and management methods to decrease nitrogen losses from beef feedlots. *Journal of Animal Science*, 88(suppl_13): E172–E180, 2010.

FERRARETTO, L. F.; CRUMP, P. M.; SHAVER, R. D. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. *Journal of Dairy Science*, v. 96, n. 1, p. 533-550, 2013.

FORERO, O.; OWENS, F. N.; LUSBY, K. S. Evaluation of slow-release urea for winter supplementation of lactating range cows. *Journal of Animal Science*, v. 50, p. 532–538, 1980.

GALYEAN, M.; WAGNER, D.; JOHNSON, R. Site and extent of starch digestion in steers fed processed corn rations. *Journal of Animal Science*, v. 43, n. 5, p. 1088-1094, 1976.

GENTRY, W. W.; WEISS, C. P.; MEREDITH, C. M.; MCCOLLUM, F. T.; COLE, N. A.; JENNINGS, J. S. Effects of roughage inclusion and particle size on performance and rumination behavior of finishing beef steers. *Journal of Animal Science*, 94(11): 4759–4770, 2016.

GERVÁSIO, J. R. S. Reidratação e ensilagem de grãos de milho com diferentes granulometrias e inclusões na dieta para bovinos de corte. *Tese (Doutorado em Ciência Animal) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária e Zootecnia*, Botucatu, SP. 2021.

GIUBERTI, G.; GALLO, A.; MASOERO, F.; FERRARETTO, L. F.; HOFFMAN, P. C.; SHAVER, R. D. Factors affecting starch utilization in large animal food production system: A review. *Starch-Stärke,* v. 66, n. 1-2, p. 72-90, 2014.

GODOI, L. A.; SILVA, B. C.; SILVA, F. A. S.; PUCETTI, P.; PACHECO, M. V. C.; SOUZA, G. A. P.; LAGE, B. C.; RENNÓ, L. N.; SCHOONMAKER, J. P.; VALADARES FILHO, S. C. Effect of flint

corn processing methods on intake, digestion sites, rumen pH, and ruminal kinetics in finishing Nellore bulls. *Animal Feed Science and Technology,* v. 271, p. 114775, 2021.

GOUVÊA, V. N.; BATISTEL, F.; SOUZA, J. D.; CHAGAS, L. J.; SITTA, C.; CAMPANILI, P. R. B.; SANTOS, F. A. P. Flint corn grain processing and citrus pulp level in finishing diets for feedlot cattle. *Journal of Animal Science*, 94(2): 665–677, 2016.

HAILEMARIAM, S.; ZHAO, S.; HE, Y.; WANG, J. Urea transport and hydrolysis in the rumen: A review. *Animal Nutrition*, 7(4): 989–996, 2021.

HALL, M. B.; HOOVER, W. H.; JENNINGS, J. P.; WEBSTER, T. K. M. A method for partitioning neutral detergent-soluble carbohydrates. *Journal of the Science of Food and Agriculture,* v. 79, n. 15, p. 2079-2086, 1999.

HALL, M. B.; HUNTINGTON, G. B. Nutrient synchrony: Sound in theory, elusive in practice. *Journal of Animal Science,* v. 86, suppl. 14, p. E287-E292, 2008.

HARMON, D. L. Understanding starch utilization in the small intestine of cattle. *Asian-Australasian Journal of Animal Sciences,* v. 22, n. 7, p. 915–922, 2009.

HARMON, D. L.; YAMKA, R. M.; ELAM, N. A. Factors affecting intestinal starch digestion in ruminants: a review. *Canadian Journal of Animal Science,* v. 84, n. 3, p. 309–318, 2004.

HÄUSSNER, S.; et al. Rumen-undegradable urea product and method for producing same. *European Patent Office, International Application No. PCT/EP2018/076234*, 2020.

HOFFMAN, P. C.; ESSER, N. M.; SHAVER, R. D.; COBLENTZ, W. K.; SCOTT, M. P.; BODNAR, A. L.; SCHMIDT, R. J.; CHARLEY, R. C. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *Journal of Dairy Science*, v. 94, n. 5, p. 2465-2474, 2011.

HRISTOV, A. N.; BANNINK, A.; CROMPTON, L. A.; HUHTANEN, P.; KREUZER, M.; MCGEE, M.; YU, Z. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. *Journal of Dairy Science*, v. 102, n. 7, p. 5811-5852, 2019.

HUBER, J.; KUNG JR, L. Protein and nonprotein nitrogen utilization in dairy cattle. *Journal of Dairy Science*, v. 64, n. 6, p. 1170-1195, 1981

HUFF-LONERGAN, E.; LONERGAN, S. M. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, v. 71, n. 1, p. 194–204, 2005.

HUNTINGTON, G. B. Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. *Canadian Journal of Animal Science*, v. 69, n. 1, p. 215-223, 1989.

HUNTINGTON, G. B. Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science,* v. 75, n. 3, p. 852-867, 1997.

HUNTINGTON, G. B. Uptake and transport of nonprotein nitrogen by the ruminant gut. *Federation Proceedings*, 45(8): 2272–2276, July 1986.

HUNTINGTON, G. B.; ARCHIBEQUE, S. L. Practical aspects of urea and ammonia metabolism in ruminants. *Proceedings of the American Society of Animal Science*, p. 1-11, 1999.

HUNTINGTON, G. B.; HARMON, D. L.; RICHARDS, C. J. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. *Journal of Animal Science*, v. 84, n. suppl. 13, p. E14-E24, 2006

ILLIUS, A. W.; JESSOP, N. S. Metabolic constraints on voluntary intake in ruminants. *Journal of Animal Science*, v. 74, n. 12, p. 3052-3062, 1996.

INOUE, H.; KOZLOWSKI, S. D.; KLEIN, J. D.; BAILEY, J. L.; SANDS, J. M.; BAGNASCO, S. M. Regulated expression of renal and intestinal UT-B urea transporter in response to varying urea load. *American Journal of Physiology - Renal Physiology*, 289(2): F258–F262, 2005.

JACOVACI, F. A.; SALVO, P. A. R.; JOBIM, C. C.; DANIEL, J. L. P. Effect of ensiling on the feeding value of flint corn grain for feedlot beef cattle: A meta-analysis. *Revista Brasileira de Zootecnia*, v. 50, e20200111, 2021.

JAYASENA, D. D.; KIM, H. J.; YONG, H. I.; PARK, S.; KIM, K.; CHOE, W.; JO, C. Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: Effects on pathogen inactivation and meat-quality attributes. *Food Microbiology*, 46: 51–57, 2015.

JOHNSON, R. R. Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. *Journal of Animal Science*, v. 43, n. 1, p. 184-191, 1976.

JUNGES, D.; MORAIS, G.; SPOTO, M. H. F.; SANTOS, P. S.; ADESOGAN, A. T.; NUSSIO, L. G.; DANIEL, J. L. P. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. *Journal of Dairy Science*, 100(11): 9048–9051, 2017.

KANEKO, J. J.; HARVEY, J. W.; BRUSS, M. L. Clinical Biochemistry of Domestic Animals. 6. ed. San Diego: *Academic Press*, 2008.

KEIM, J. P.; ANRIQUE, R. Nutritional strategies to improve nitrogen use efficiency by grazing dairy cows. *Chilean Journal of Agricultural Research*, v. 71, n. 4, p. 623, 2011.

KEMPTON, T.; NOLAN, J.; LENG, R. Principles for the use of non-protein nitrogen and by-pass proteins in diets of ruminants. *World Animal Review*, 1977

KENNEDY, P.; MILLIGAN, L. The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants: a review. *Canadian Journal of Animal Science*, v. 60, n. 2, p. 205-221, 1980.

KIM, Y. H. B.; MA, D.; SETYABRATA, D.; FAROUK, M. M.; LONERGAN, S. M.; HUFF-LONERGAN, E.; HUNT, M. C. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science*, 144: 74–90, 2018.

KOENIG, K. M.; BEAUCHEMIN, K. A.; RODE, L. M. Effect of grain processing and silage on microbial protein synthesis and nutrient digestibility in beef cattle fed barley-based diets. *Journal of Animal Science*, 81(4): 1057–1067, April 2003.

KOMATSU, E.; SAKAKI, Y. A new nonprotein nitrogen source IBDU for ruminants. *Chem. Economy Eng.* Rev, v. 3, n. 4, p. 43-50, 1971

KONONOFF, P.; HEINRICHS, A.; LEHMAN, H. The effect of corn silage particle size on eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. *Journal of Dairy Science*, v. 86, n. 10, p. 3343-3353, 2003

KOZLOSKI, G. V. Bioquímica dos ruminantes. *Santa Maria: Fundação de Apoio à Tecnologia e Ciência – Editora UFSM*, 2017.

LAPIERRE, H.; LOBLEY, G. E. Nitrogen recycling in the ruminant: A review. *Journal of Dairy Science*, v. 84, p. E223-E236, 2001.

LECHNER-DOLL, M.; KASKE, M.; ENGELHARDT, W. Factors affecting the mean retention time of particles in the forestomach of ruminants and camelids. In: *Physiological aspects of digestion and metabolism in ruminants*. Elsevier. p. 455–482, 1991.

LENG, R. Factors affecting the utilization of 'poor-quality'forages by ruminants particularly under tropical conditions. *Nutrition research reviews*, v. 3, n. 1, p. 277-303, 1990.

LEONARDI, C.; ARMENTANO, L. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *Journal of Dairy Science*, v. 86, n. 2, p. 557-564, 2003

LÖEST, C. A.; TITGEMEYER, E. C.; DROUILLARD, J. S.; LAMBERT, B. D.; TRATER, A. M. Urea and biuret as nonprotein nitrogen sources in cooked molasses blocks for steers fed prairie hay. *Animal Feed Science and Technology,* v. 94, n. 3–4, p. 229–238, 2001

LOFGREEN, G.; GARRETT, W. A system for expressing net energy requirements and feed values for growing and finishing beef cattle. *Journal of animal science*, v. 27, n. 3, p. 793-806, 1968.

MAHANNA, B. Bottom line: Renewed interest in snaplage displayed. *Feedstuffs*, 80(50): 1–3, 2008.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. *Meat Science*, v. 71, n. 1, p. 100–121, 2005.

MAPATO, C.; WANAPAT, M.; CHERDTHONG, A. Effects of urea treatment of straw and dietary level of vegetable oil on lactating dairy cows. *Tropical Animal Health and Production,* v. 42, p. 1635-1642, 2010.

MARINI, J. C.; VAN AMBURGH, M. E. Nitrogen metabolism and recycling in Holstein heifers. *Journal of Animal Science*, v. 81, n. 3, p. 545–552, 2003.

MARQUES, R. S.; CHAGAS, L. J.; OWENS, F. N.; SANTOS, F. A. P. Effects of various roughage levels with whole flint corn grain on performance of finishing cattle. *Journal of Animal Science*, 94(1): 339–348, 2016.

MCALLISTER, T. A.; PHILLIPPE, R. C.; RODE, L. M.; CHENG, K. J. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. *Journal of Animal Science*, v. 71, n. 1, p. 205-212, 1993.

MCDONALD, P.; EDWARDS, R. A.; GREENHALGH, J. F. D.; MORGAN, C. A.; SINCLAIR, L. A.; WILKINSON, R. G. *Animal nutrition*. 7th ed. Harlow, England: Pearson Education Limited, 2011.

MENEZES, A. C. B.; VALADARES FILHO, S. C.; PACHECO, M. V.; PUCETTI, P.; SILVA, B. C.; ZANETTI, D.; CATON, J. S. Oscillating and static dietary crude protein supply. I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls. *Translational Animal Science*, 3(4): 1205–1215, 2019.

MILLEN, D. D.; PACHECO, R. D. L.; ARRIGONI, M. D. B.; GALYEAN, M. L.; VASCONCELOS, J. T. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. *Journal of Animal Science*, v. 87, n. 10, p. 3427-3439, 2009.

MILLEN, D. D.; PACHECO, R. D. L.; DA SILVA CABRAL, L.; CURSINO, L. L.; WATANABE, D. H. M.; RIGUEIRO, A. L. N. Ruminal acidosis. In: *Rumenology*, p. 127-156, 2016.

MILLER, W. J. The Use of Nonprotein Nitrogen (NPN) for Dairy Cattle. In: MILLER, W. J. (Ed.). *Dairy Cattle Feeding and Nutrition*. San Diego: Academic Press. p. 62–73, 1979.

MONSALVE, J. G.; MILLEN, D. D. A snapshot of nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil in 2023. *Frontiers in Veterinary Science*, v. 12, p. 1518571, 2025.

MUDD, A. The use of non-protein nitrogen in cattle diets. In: *Symposium on Recent Developments in the Use of New Sources of Protein, Essential Amino Acids and Non-Protein Nitrogen with Special Reference to Ruminants*. Geneva, Switzerland, 10 Jan 1977.

NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE. *Nutrient requirements of beef cattle. 8. ed*. Washington, DC: The National Academies Press, 2016.

NATIONAL RESEARCH COUNCIL - NRC. *Nutrient Requirements of Beef Cattle*. Washington, DC: National Academy Press, 1984.

NICHOLS, K.; DE CARVALHO, I. P. C.; RAUCH, R.; MARTÍN-TERESO, J. Unlocking the limitations of urea supply in ruminant diets by considering the natural mechanism of endogenous urea secretion. *Animal*, 16: 100537, 2022.

NOCEK, J. E.; KOHN, R. A. Initial particle form and size on change in functional specific gravity of alfalfa and timothy hay. *Journal of Dairy Science*, v. 70, n. 9, p. 1850–1863, 1987.

OBA, M.; ALLEN, M. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. *Journal of Dairy Science*, v. 86, n. 1, p. 174-183, 2003.

OLIVEIRA, C. V.; SILVA, T. E.; BATISTA, E. D.; RENNÓ, L. N.; SILVA, F. F.; DE CARVALHO, I. P.; DETMANN, E. Urea supplementation in rumen and post-rumen for cattle fed a low-quality tropical forage. *British Journal of Nutrition*, v. 124, n. 11, p. 1166-1178, 2020.

OLIVEIRA, C.; MILLEN, D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. *Animal Feed Science and Technology*, v. 197, p. 64-75, 2014.

OWENS, F. N.; LUSBY, K. S.; MIZWICKI, K.; FORERO, O. Slow ammonia release from urea: rumen and metabolism studies. *Journal of Animal Science*, 50(3): 527–531, 1980.

OWENS, F. N. Corn grain processing and digestion. *Pioneer Hi-Bred International*, Johnston, IA, 2005.

OWENS, F. N.; SECRIST, D. S.; HILL, W. J.; GILL, D. R. The effect of grain source and grain processing on performance of feedlot cattle: a review. *Journal of Animal Science*, 75(3): 868–879, 1997.

OWENS, F. N.; BASALAN, M. Grain processing: gain and efficiency responses by feedlot cattle. In: *Plains Nutrition Council Spring Conference.* Proceedings. 2013. p. 76-100.

OWENS, F. N.; QI, S.; SAPIENZA, D. A. Invited Review: Applied protein nutrition of ruminants—Current status and future directions. *The Professional Animal Scientist*, v. 30, n. 2, p. 150-179, 2014.

OWENS, F. N.; ZINN, R. A.; KIM, Y. K. Limits to starch digestion in the ruminant small intestine. *Journal of Animal Science*, v. 63, n. 5, p. 1634-1648, 1986.

PACHECO, R. F.; MACHADO, D. S.; VIANA, A. F. P.; TEIXEIRA, J. S.; MILANI, L. Comparison of the effects of slow-release urea vs conventional urea supplementation on some finishing cattle parameters: A meta-analysis. *Livestock Science*, 250, 2021.

PEREIRA, M. N.; PEREIRA, R. A. N.; BITENCOURT, L. L.; DIAS, J.; LOPES, N. M.; ZACARONI, O. D. F. Silagem de milho reidratado na alimentação do gado leiteiro. *Informe Agropecuário – Empresa de Pesquisa Agropecuária de Minas Gerais*, v. 34, n. 277, 2013.

PICARD, B.; GAGAOA, M.; AL JAMMAS, M.; BONNET, M. Beef tenderness and intramuscular fat proteomic biomarkers: Effect of gender and rearing practices. *Journal of Proteomics*, v. 200, p. 1–10, 2019.

PINTO, A. C.; MILLEN, D. D. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: the 2016 Brazilian survey. *Canadian Journal of Animal Science*, v. 99, n. 2, p. 392-407, 2018

PITT, R. E.; PELL, A. N.; FOX, D. G.; CROSS, T. L.; BARRY, M. C. Remodeling the Cornell model. In: *Proceedings of the Cornell Nutrition Conference for Feed Manufacturers*. New York State College of Agriculture & Life Sciences, Cornell University, Rochester, NY. p. 142–149, 1996.

PROKOP, M. J.; KLOPFENSTEIN, T. J. Slow ammonia release urea. *Nebraska Beef Cattle Report,* No. EC 77-218, 1977.

RAUCH, R.; MARTÍN-TERESO, J.; DANIEL, J. B.; DIJKSTRA, J. Dietary protein oscillation: Effects on feed intake, lactation performance, and milk nitrogen efficiency in lactating dairy cows. *Journal of Dairy Science*, 104(10): 10714–10726, 2021.

REIS, I. A.; SOUZA, M. G. D.; GRANJA-SALCEDO, Y. T.; CARVALHO, I. P. C. D.; PORCIONATO, M. A. D. F.; PRADOS, L. F.; DE RESENDE, F. D. Effect of post-ruminal urea supply on growth performance of grazing Nellore young bulls at dry season. *Animals*, 13(2): 207, 2023.

REYNOLDS, C. K.; KRISTENSEN, N. B. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. *Journal of Animal Science*, 86(suppl_14): E293–E305, 2008.

ROBLES, V.; GONZÁLEZ, L. A.; FERRET, A.; MANTECA, X.; CALSAMIGLIA, S. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high-concentrate diets. *Journal of Animal Science,* v. 85, n. 10, p. 2538–2547, 2007.

ROONEY, L. W.; PFLUGFELDER, R. L. Factors affecting starch digestibility with special emphasis on sorghum and corn. *Journal of Animal Science,* v. 63, n. 5, p. 1607-1623, 1986.

SALAMI, S. A.; MORAN, C. A.; WARREN, H. E.; TAYLOR-PICKARD, J. A meta-analysis of the effects of slow-release urea supplementation on the performance of beef cattle. *Animals*, 10(4): 657, 2020.

SANTOS, F. A. P.; DA SILVA MARQUES, R.; DÓREA, J. R. R. Grain processing for beef cattle. In: *Rumenology,* p. 213-241, 2016.

SCOTT, K. A.; PENNER, G. B.; MUTSVANGWA, T. Influence of forage level and corn grain processing on whole-body urea kinetics, and serosal-to-mucosal urea flux and expression of urea transporters and aquaporins in the ovine ruminal, duodenal, and cecal epithelia. *Journal of Animal Science,* v. 98, n. 4, skaa098, 2020.

SEAL, C. J.; PARKER, D. S.; AVERY, P. J. The effect of forage and forage–concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric- and portal-drained viscera in growing steers. *British Journal of Nutrition*, v. 67, n. 3, p. 355-370, 1992.

SEO, J. K.; KIM, M. H.; YANG, J. Y.; KIM, H. J.; LEE, C. H.; KIM, K. H.; HA, J. K. Effects of synchronicity of carbohydrate and protein degradation on rumen fermentation characteristics and microbial protein synthesis. *Asian-Australasian Journal of Animal Sciences*, 26(3): 358–365, 2013.

SILVA, B. D. C.; PACHECO, M. V. C.; GODOI, L. A.; ALHADAS, H. M.; PEREIRA, J. M. V.; RENNO, L. N.; VALADARES FILHO, S. D. C. Reconstituted and ensiled corn or sorghum grain:

Impacts on dietary nitrogen fractions, intake, and digestion sites in young Nellore bulls. *PLoS One*, 15(8): e0237381, 2020.

SILVA, B. C.; PACHECO, M. V. C.; GODOI, L. A.; SILVA, F. A. S.; ZANETTI, D.; MENEZES, A. C. B.; VALADARES FILHO, S. C. In situ and in vitro techniques for estimating degradation parameters and digestibility of diets based on maize or sorghum. *The Journal of Agricultural Science*, 158(1–2): 150–158, 2020.

SILVESTRE, A. M.; MILLEN, D. D. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia,* v. 50, p. 1-25, 2021.

SINCLAIR, L. A.; BLAKE, C. W.; GRIFFIN, P.; JONES, G. H. The partial replacement of soyabean meal and rapeseed meal with feed grade urea or a slow-release urea and its effect on the performance, metabolism and digestibility in dairy cows. *Animal*, 6(6): 920–927, 2012.

SMITH, R. Utilization of NPN-Supplements, other than Urea, by Ruminants. *Archives of Animal Nutrition,* v. 36, n. 2-3, p. 175-182, 1986.

SOUZA, M. G. D.; REIS, I. A.; CARVALHO, I. P. C. D.; PORCIONATO, M. A. D. F.; PRADOS, L. F.; GRANJA-SALCEDO, Y. T.; RESENDE, F. D. D. Effects of post-ruminal urea supplementation during the seasonal period on performance and rumen microbiome of rearing grazing Nellore cattle. *Animals*, 12(24): 3463, 2022.

ST-PIERRE, N. Design and analysis of pen studies in the animal sciences. *Journal of dairy science*, v. 90, p. E87-E99, 2007.

SWANSON, K. C.; KELLY, N.; WANG, Y. J.; KO, M.; HOLLIGAN, S.; FAN, M. Z.; MCBRIDE, B. W. Pancreatic mass, cellularity, and α-amylase and trypsin activity in feedlot steers fed increasing amounts of a high-moisture-corn based diet. *Canadian Journal of Animal Science*, 88(2): 303–308, 2008.

SWANSON, K. C.; HARMON, D. L. Dietary influences on pancreatic alpha-amylase expression and secretion in ruminants. In: ZABIELSKI, R.; GREGORY, P. C.; WESTROM, B. (Eds.). *Biology of the intestine in growing animals*. Amsterdam: Elsevier Science. p. 515–537, 2002.

SWANSON, K. C.; MATTHEWS, J. C.; WOODS, C. A.; HARMON, D. L. Postruminal administration of partially hydrolyzed starch and casein influences pancreatic alpha-amylase expression in calves. *Journal of Nutrition*, v. 132, n. 3, p. 376–381, 2002.

TAMMINGA, S.; VAN HELLEMOND, K. The protein requirements of dairy cattle and developments in the use of protein, essential amino acids and non-protein nitrogen in the feeding of dairy cows. 1977

TAYLOR, C. C.; ALLEN, M. S. Corn grain endosperm type and brown midrib three corn silage: Feeding behavior and milk yield of lactating cows. *Journal of Dairy Science*, v. 88, n. 4, p. 1425-1433, 2005.

TAYLOR, J. R.; EMMAMBUX, M. N. Developments in our understanding of sorghum polysaccharides and their health benefits. *Cereal Chemistry,* v. 87, n. 4, p. 263-271, 2010.

TAYLOR-EDWARDS, C. C.; HIBBARD, G.; KITTS, S. E.; MCLEOD, K. R.; AXE, D. E.; VANZANT, E. S.; KRISTENSEN, N. B.; HARMON, D. L. Effects of slow-release urea on ruminal digesta characteristics and growth performance in beef steers. *Journal of Animal Science*, v. 87, n. 1, p. 200–208, 2009.

TEDESCHI, L. O.; BACKER, M. J.; KETCHEN, D. J.; FOX, D. G. Performance of growing and finishing cattle supplemented with a slow-release urea product and urea. *Canadian Journal of Animal Science*, 82(4): 567–573, 2002.

THEURER, C. B. Grain processing effects on starch utilization by ruminants. *Journal of Animal Science,* v. 63, n. 5, p. 1649-1662, 1986.

THEURER, C. B.; HUNTINGTON, G. B.; HUBER, J. T.; SWINGLE, R. S.; MOORE, J. A. Net absorption and utilization of nitrogenous compounds across ruminal, intestinal, and hepatic tissues of growing beef steers fed dry-rolled or steam-flaked sorghum grain. *Journal of Animal Science*, 80(2): 525–532, 2002.

THOMAS, P. C. Microbial protein synthesis. *Proceedings of the Nutrition Society*, v. 32, n. 2, p. 85-91, 1973.

TROTTA, R. J.; SWANSON, K. C. Prenatal and postnatal nutrition influence pancreatic and intestinal carbohydrase activities of ruminants. *Animals*, v. 11, n. 1, p. 171, 2021

VALADARES FILHO, S. C.; COSTA E SILVA, L. F.; ROTTA, P. P.; GIONBELLI, M. P.; CHIZZOTTI, M. L.; MARCONDES, M. I.; PRADOS, L. F. *Exigências nutricionais de zebuínos puros e cruzados – BR-CORTE 2023.* Viçosa, MG: Universidade Federal de Viçosa, 2023.

VALADARES FILHO, S. C.; COSTA E SILVA, L. F.; GIONBELLI, M. P.; ROTTA, P. P.; MARCONDES, M. I.; CHIZZOTTI, M. L.; PRADOS, L. F. *Exigências nutricionais de zebuínos puros e cruzado – BR-Corte*. v. 1. Viçosa: Universidade Federal de Viçosa, 2016.

VALADARES FILHO, S.C.; LOPES, S.A.; SILVA, B.C.; CHIZZOTTI, M.L.; BISSARO, L.Z. *CQBAL 4.0. Tabelas Brasileiras de Composição de Alimentos para Ruminantes*. 2018.

VALENTE, T. N. P.; DETMANN, E.; QUEIROZ, A. C. D.; VALADARES FILHO, S. D. C.; GOMES, D. I.; FIGUEIRAS, J. F. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. *Revista Brasileira de Zootecnia*, 40: 2565–2573, 2011.

VALKENERS, D.; THEWIS, A.; PIRON, F.; BECKERS, Y. Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls. *Journal of Animal Science*, v. 82, n. 6, p. 1818-1825, 2004.

VAN SOEST, P. J. *Nutritional ecology of the ruminant*. Ithaca: Cornell University Press, 1994.

VAN SOEST, P. J.; VAN AMBURGH, M. E.; TEDESCHI, L. O. Rumen balance and rates of fiber digestion. *In: Proceedings of the Cornell Nutrition Conference for Feed Manufacturers (pp. 150–166).* New York State College of Agriculture & Life Sciences, Cornell University, Rochester, NY, 2000.

VEEN, W.; BAKKER, I. T. The course of ammonia concentration in the rumen fluid of lactating dairy cows after the intake of corn silage with or without added urea or 1.1.-diureido isobutane. *Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde*, v. 38, n. 1-6, p. 255-260, 1977.

WAITE, R.; WILSON, A. G. The composition of rumen fluid from cows fed biuret and urea. *Journal of Dairy Research*, 35(2): 203–212, 1968.

WHITEHEAD, D.C., RAISTRICK, N. The volatilization of ammonia from cattle urine applied to soils as influenced by soil properties. *Plant Soil* 148, 43–51, 1993.

WICKERSHAM, T. A.; TITGEMEYER, E. C.; COCHRAN, R. C.; WICKERSHAM, E. E. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *British Journal of Nutrition*, 101(2): 225–232, 2008.

YANG, W. Z.; BEAUCHEMIN, K. A. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. *Journal of Dairy Science*, v. 89, p. 217–228, 2006.

ZINN, R. A.; OWENS, F. N.; WARE, R. A. Flaking corn: processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *Journal of Animal Science*, v. 80, n. 5, p. 1145-1156, 2002.

ZINN, R. A.; BARRERAS, A.; CORONA, L.; OWENS, F. N.; PLASCENCIA, A. Comparative effects of processing methods on the feeding value of maize in feedlot cattle. *Nutrition Research Reviews*, 24(2): 183–190, 2011.

ZINN, R. A.; SHEN, Y. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. *Journal of Animal Science*, 76(5): 1280–1289, 1998.