frontiers | Research Topics

Feedlot cattle nutrition and metabolism

Edited by
Danilo Domingues Millen, Pedro Henrique Vilela Carvalho and Rodrigo Marques

Published in
Frontiers in Veterinary Science
Frontiers in Animal Science

ISSN 1664-8714
ISBN 978-2-8325-3018-4
DOI 10.3389/978-2-8325-3018-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Feedlot cattle nutrition and metabolism

Topic editors

Danilo Domingues Millen — São Paulo State University, Brazil

Pedro Henrique Vilela Carvalho — University of California, Davis, United States

Rodrigo Marques — Montana State University, United States

Citation

Millen, D. D., Carvalho, P. H. V., Marques, R., eds. (2023). *Feedlot cattle nutrition and metabolism*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3018-4

Table of contents

frontiers | Frontiers in Veterinary Science

TYPE Editorial
PUBLISHED 27 June 2023
DOI 10.3389/fvets.2023.1239768

OPEN ACCESS

EDITED AND REVIEWED BY
Domenico Bergero,
University of Turin, Italy

*CORRESPONDENCE
Danilo Domingues Millen
✉ danilo.millen@unesp.br

RECEIVED 13 June 2023
ACCEPTED 19 June 2023
PUBLISHED 27 June 2023

CITATION
Millen DD (2023) Editorial: Feedlot cattle nutrition and metabolism.
Front. Vet. Sci. 10:1239768.
doi: 10.3389/fvets.2023.1239768

Editorial: Feedlot cattle nutrition and metabolism

Danilo Domingues Millen*

Department of Animal Science, São Paulo State University, Jaboticabal, São Paulo, Brazil

KEYWORDS

acidosis, cattle, feedlot, heat stress, additives

Editorial on the Research Topic
Feedlot cattle nutrition and metabolism

Like other ruminants, cattle evolved as herbivores by consuming forages (grasses and legumes), characterized by the high cell wall content. As a result, the rumen microbiota was shaped over the centuries by fermenting structural carbohydrates, turning the rumen into an environment rich in fiber-utilizing microorganisms, including bacteria, protozoa, and fungi. However, high levels of productivity cannot be achieved when cattle are consuming forage-based diets, and to increase beef production, cattle are often fed and finished in feedlot operations.

Feedlot cattle are often fed highly fermentable diets to achieve rapid rates of gain and improve feed efficiency. However, this type of diet causes excessive rates of acid production in the rumen, which can overwhelm the ability of cattle to regulate ruminal pH, and consequently may increase the risk of ruminal acidosis. Based on this fact, the objective of this Research Topic was to collect suitable papers to improve our knowledge and understanding of rumen function and metabolism in order to mitigate acidosis and improve the performance of feedlot cattle.

Most articles on this Research Topic addressed the effects of some type of feed additive, or a combination of them, to manipulate ruminal fermentation and maximize either the short-chain fatty acids production in the rumen or the animal performance. Combinations of feed additives to prevent ruminal disorders and increase productivity included: monensin and narasin (Baggio et al.), monensin and essential oils (Silva et al.), monensin and virginiamycin (Rigueiro et al.). Sodium monensin is the most used feed additive in feedlot diets around the world, however, due to its negative effect on dry matter intake, the association with other feed additives may lead to a synergistic effect to improve cattle health and performance.

Moreover, the search for an alternative to replacing sodium monensin in feedlot diets, since its use as a growth promoter was banned in some countries, was also the objective of some of the authors on this Research Topic. Polyclonal antibodies failed on controlling ruminal acidosis when compared to monensin (Pacheco, Souza, et al.); however,

virginiamycin was as effective as monensin to prevent rumen pH decline when cattle were adapted for 14 days (Squizatti et al.). Furthermore, a blend of essential oils and 25-hydroxyvitamin D3 seems to be an alternative to replace monensin in feedlot diets offered to Holstein steers (Latack et al.).

Interestingly, two papers addressed the effect of different molecules on reducing heat stress in feedlot cattle, which is literally a hot topic in production systems that are not even close to the natural habitat cattle are used to. Carvalho et al. reported that the use of NutraGen supplement induced significant changes in the metabolism of the steers, whereas Pacheco, Oliveira Gusmão, et al. concluded that lysolecithin enhances feedlot performance and has the potential to increase diet intake during very hot days.

In summary, this Research Topic contributed to improving the current knowledge of feedlot cattle nutrition and metabolism by providing an enormous amount of new relevant data that certainly are useful worldwide.

Author contributions

DM idealized the Research Topic and wrote the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

frontiers | Frontiers in Animal Science

ORIGINAL RESEARCH
published: 14 June 2022
doi: 10.3389/fanim.2022.904503

Effects of Rumen-Protected Creatine Pyruvate on Meat Quality, Hepatic Gluconeogenesis, and Muscle Energy Metabolism of Long-Distance Transported Beef Cattle

Guwei Lu[1], Yanjiao Li[2], Kang Mao[1], Yitian Zang[3], Xianghui Zhao[2], Qinghua Qiu[2], Mingren Qu[1] and Kehui Ouyang[2]*

[1] Jiangxi Province Key Laboratory of Animal Nutrition, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China, [2] Jiangxi Province Key Laboratory of Animal Nutrition/Animal Nutrition and Feed Safety Innovation Team, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China, [3] College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China

OPEN ACCESS

Edited by:
Otávio R. Machado Neto,
São Paulo State University, Brazil

Reviewed by:
Mateus P. Gionbelli,
Universidade Federal de Lavras, Brazil
John Michael Gonzalez,
University of Georgia, United States

***Correspondence:**
Yanjiao Li
yanjiaoli221@163.com

Specialty section:
This article was submitted to Animal Nutrition, a section of the journal Frontiers in Animal Science

Received: 25 March 2022
Accepted: 05 May 2022
Published: 14 June 2022

Citation:
Lu G, Li Y, Mao K, Zang Y, Zhao X, Qiu Q, Qu M and Ouyang K (2022) Effects of Rumen-Protected Creatine Pyruvate on Meat Quality, Hepatic Gluconeogenesis, and Muscle Energy Metabolism of Long-Distance Transported Beef Cattle. Front. Anim. Sci. 3:904503. doi: 10.3389/fanim.2022.904503

Pre-slaughter long-distance transport resulted in a rapid depletion of muscle glycogen and led to a higher rate of dark, firm and dry (DFD) meat. Therefore, enhancing muscle glycogen reserves is critical for beef cattle prior to transportation. Creatine pyruvate (CrPyr) can provide simultaneous pyruvate and creatine and both are proven to promote the glycogen reserves. This study aimed to investigate the effects of transport treatment and dietary supplementation of rumen-protected (RP)-CrPyr on the meat quality, muscle energy metabolism, and hepatic gluconeogenesis of beef cattle. Twenty 18 month-old male Simmental crossbred cattle (659 ± 16 kg) were allotted 4 treatments based on a 2 × 2 factorial arrangement with two RP-CrPyr levels (140 g/d or 0 g/d) and two transport treatments (12 h or 5 min): ST_CrPyr0, ST_CrPyr140, LT_CrPyr0 and LT_CrPyr140. Three cattle per group were slaughtered after 30 days of feeding. The interaction of transport and RP-CrPyr had a significant effect on the muscle $pH_{45\ min}$, redness, glycogen content, GP, and AMP level ($P < 0.05$). Compared with short-distance transport, long-distance transport increased the muscle $pH_{45\ min}$ value, redness, yellowness, drip loss, creatine level ($P < 0.05$), decreased muscle glycogen content, glycolytic potential (GP), and liver glucose amount ($P < 0.05$). Supplementation of RP-CrPyr decreased the activities of creatine kinase and lactate dehydrogenase in serum, muscle $pH_{24\ h}$ value, redness, yellowness, lactate content, AMP level, and AMP/ATP ($P < 0.05$), increased the muscle glycogen content, GP, hexokinase activity, ATP and ADP levels, and ATP/ADP, liver pyruvate and glucose contents, activity of pyruvate carboxylase in the liver of cattle than those in the nonsupplemented treatments ($P < 0.05$). These results indicated that dietary RP-CrPyr supplementation might be favorable to improve meat quality and regulatory capacity of energy metabolism of beef cattle suffering long-distance transport followed with recovery treatment by increasing muscle glycogen storage, energy supply, and hepatic gluconeogenesis.

Keywords: beef cattle, creatine pyruvate, meat quality, muscle glycogen, transport stress

INTRODUCTION

The separation of breeding and slaughtering is the dominant model of the beef cattle industry in China. The beef cattle are inevitably subjected to stress during the centralized transportation process. Transport stress has been reported to increase the physical energy consumption of beef cattle, resulting in a decrease in muscle glycogen content at slaughter. The insufficient muscle glycogen cannot produce enough lactic acid and H^+ and leads to a high ultimate pH (pHu) in postmortem muscle, which results in dark, firm, and dry (DFD) meat. Higher rates of DFD meat cause substantial economic losses to the beef cattle industry (Ferguson and Warner, 2008; Ponnampalam et al., 2017). In Australia, the incidence of "Dark Cutting" (DC) beef is close to 10%, resulting in a potential loss about AU $36 million to the cattle industry, which equates to $0.45/kg hot carcass weight. The DC meat in the US was estimated to cost about $165 million to the beef industry in the year 2000, an amount equal to an estimated $5.43 per fed beef animal harvested (Ponnampalam et al., 2017). In China, the average incidence of DFD in beef cattle was as high as 10.07%, of which a major factor that affects the rate of DFD meat is pre-slaughter transport time and quality (Zhao, 2013). Ponnampalam et al. (2017) summarized that short-distance transport (< 400 km) has little effect on the postmortem muscle pHu value of beef cattle, while long-distance transport can increase the pHu value by 0.1 to 0.2 pH units. Therefore, alleviating pre-slaughter long-distance transport stress is an important guarantee to produce high-quality beef.

Pre-slaughter nutritional management is an efficient technological means to regulate energy metabolism, relieve transportation stress, reduce depletion of muscle glycogen, and improve beef quality in transported beef cattle (Ponnampalam et al., 2017). Creatine pyruvate (CrPyr, $C_7H_{13}N_3O_5$, 219.20) is a new multifunctional nutrient that contains pyruvate and creatine at a ratio of 40:60 (Jäger et al., 2011). CrPyr can provide pyruvate and creatine, which are both the products of normal cellular metabolism and plays critical roles in the process of energy metabolism. Pyruvate is a pivotal component in intermediary metabolism (glucose metabolism, lipid metabolism, and amino acid metabolism), can regulate energy metabolism through gluconeogenesis/glycolysis pathway and tricarboxylic acid cycle (Chen et al., 2011). Guo et al. (2009) reported that supplement of calcium pyruvate for 3 weeks could improve liver and muscle glycogen content at the start of exhaustive exercise. Shetty et al. (2012) showed that exogenous pyruvate could enhance internal glycogen stores and therefore increase the energy buffering capacity of hippocampal slices under low glucose condition. Additional studies have reported that pyruvate could enhance hypoxia and even anaerobic tolerance of cells by restoring pyruvate dehydrogenase complex activity and increasing glycolytic enzymes activity (Sharma et al., 2009; Hu et al., 2016). Creatine, as an important material for the synthesis of energy storage substance phosphocreatine, is the backup guarantee of skeletal muscle ATP production (Allen, 2012). Creatine supplementation could promote the absorption of creatine by human and animal skeletal muscle, increase the content of creatine and phosphocreatine in muscle, enhance the energy buffering effects of phosphogen system, and improve the energy metabolism of skeletal muscle (Schoch et al., 2006). Moreover, creatine supplementation could facilitate glycogen accumulation in muscle of mammalian and reduce the glycogen depletion under normal physiological conditions or during exercise (Ceddia and Sweeney, 2004; Ju et al., 2005).

Interestingly, the effect of CrPyr feeding on muscle energy metabolism of broilers was significantly higher than that of its separate active ingredients, pyruvate and creatine (Chen et al., 2012). A previous study discovered that CrPyr supplementation was helpful for athletes to further promote aerobic capacity and improve endurance performance (Jäger et al., 2008). Chen (Chen et al., 2011) discovered that CrPyr supplementation has a positive influence on enhancing the activity of creatine kinase and phosphocreatine concentration in broilers muscle and increased muscle glycogen reserve by reducing glycogenolysis *via* decreasing the activity of phosphorylase-b. Recently, another study reported that *in ovo* feeding of CrPyr could promote broilers liver and muscle energy reserves on the day of hatch and enhance glycolysis in chest through increasing the activity of glycolytic enzyme (Zhao, 2017). However, the information about the impacts of CrPyr on energy metabolism and whether CrPyr can relieve the glycogen consumption in the transported beef cattle is largely unknown. We hypothesized that CrPyr might affect meat quality by influencing the muscle energy metabolism and hepatic gluconeogenesis that beef cattle suffer during transportation. Therefore, the present study was designed to test this hypothesis.

MATERIALS AND METHODS

Animal Care

This experiment was approved by the Committee for the Care and Use of Experimental Animals at Jiangxi Agricultural University (JXAULL-2021-10).

Animal Treatments and Experimental Diets

Experimental design of experimental cattle has been described in a joint experiment (Mao et al., 2022). Briefly, a total of twenty 18-mouths-old male Chinese Simmental crossbred cattle, raised under identical feeding and environmental conditions, weighing 659 ± 16 kg, with similar genetic backgrounds were used in this study. They were allotted four treatments based on a 2 × 2 factorial arrangement with two supplemental levels of RP-CrPyr (140 g/d/head or 0 g/d/head) in basal diets and two treatments of transport (12 h or 5 min that were ST_CrPyr0 (0 g/d/head RP-CrPyr + 5 min transport), ST_CrPyr140 (140 g/d/head RP-CrPyr + 5 min transport), LT_CrPyr0 (0 g/d/head RP-CrPyr + 12 h transport), LT_CrPyr140 (140 g/d/head RP-CrPyr + 12 h transport). For long-distance transport, we decided 12 h because one previous study indicated that 12 h of transportation pre-slaughter resulted in decreases in slaughter performance and beef quality of Simmental crossbred cattle (Lu et al., 2015) and for short-distance transport we decided 5min

because transport to the abattoir near the farm (1 km) took approximately 5 min. Three cattle from each treatment were randomly slaughtered and samples were collected after feeding for 30 days. The cattle in the short-distance transport group were fed at 04:00 pm on September 16, 2020, loaded at 04:00 am on September 17, 2020, and after 5 min of transportation the cattle reached the abattoir near the farm (1 km). After unloading, cattle were given 2 hours rest and then subjected to slaughter by electric shock (at 06:00 am on September 17, 2020). The cattle in the long-distance transport group were fed at 06:00 am on September 16, 2020. The transport journey was from Xuchang (01:00 pm on September 16, 2020) to Nanyang (01:00 am on September 17, 2020), Henan Province, which took 12 h (mean velocity of 60-70 km/h). During the 12 h of transport, the temperature and humidity were 22–32°C and 30–72%, respectively. The cattle were allowed 2 h to rest and then subjected to slaughter by electric shock (03:00 am on September 17, 2020).

CrPyr (purity of 99.9%) was bought from Shanghai Jinli Technology Co., Ltd. (Shanghai, China). The coating processing was completed by Hangzhou King Techina Feed Co., Ltd (Hangzhou, China), the content of CrPyr in the final RP-CrPyr was 40%, 12 h of rumen bypass ratio was 82.8%, and 12 h of intestinal fluid dissolution was 78.6%. RP-CrPyr was added in concentrate and fed twice a day (06:00 am and 04:00pm). Concentrate was given after the rice straw was fed. All animals were placed individual pens in a closed cowshed with available access to clean water during the entire experimental period. **Table 1** shows the ingredients and nutrient levels of the experimental diet.

Sample Collection

Blood sampling was performed from cattle by jugular vein puncture before slaughter and then placed in evacuated anticoagulated tubes and stored at room temperature. Serum was obtained by centrifugation (3000 g, 10 min, 4°C) and then stored at −20°C to measure indices. Shortly afterwards, cattle were slaughtered after being stunned by electric shock. Immediately, the liver sample and the *M. longissimus thoracis* (LT) muscle (taken from the last rib of the left-half carcass) was stored at −80°C to analyze the indices including hepatic gluconeogenesis and muscle energy metabolism. About 5 cm thickness steaks of LT, without extra-muscular fat and connective tissues from the last rib of the left-half carcass, were collected within 30 min after slaughter. After muscle $pH_{45\ min}$ determination, these chops were vacuum-packed at 4°C to measure muscle $pH_{24\ h}$, color, drip loss, and texture analyzer determination.

TABLE 1 | Composition and nutrient levels of experimental diet (air-dry basis, %).

Ingredients	Content	Nutrient levels	Content
Wheat Straw	40.0	Dry matter	87.2
Corn	43.7	Crude protein	13.5
wheat bran	3.66	Crude fat	2.47
Soybean meal	9.12	Ash	7.86
Sodium bicarbonate	0.420	Neutral detergent fiber	36.2
Premix[a]	3.06	Acid detergent fiber	15.8
Total	100		

The premix [1] (per kg of diet) is: 80000 IU of vitamin A, 20000 IU of vitamin D3, 280 mg of vitamin E, 4100 mg of Fe, 1100 mg of Mn, 800 mg of Zn, 265 mg of Cu, 120 g of Ca and 35 g of P.

Serum Parameters Determination

The activities of creatine kinase (CK) and lactate dehydrogenase (LDH) and the pyruvate concentration in serum were determined by commercial CK, LDH and pyruvate kits (Nanjing Jiancheng Bioengineering Institute, Nangjing, China) according to kit instructions. The serum glucose concentration was determined by a commercial glucose kit (Shanghai RongSheng Biotech Co. Ltd., Shanghai, China). The serum creatine concentration was measured by 1'-Hydroxy-2'-acetonaphthone colorimetry. Firstly, a standard curve was performed using different concentrations of creatine. Briefly, 1 mL of the reaction system contained 250 μL of 5% $ZnSO_4$, 250 μL of 5% $Ba(OH)_2$ and 500 μL of different dilutions of creatine standard was mixed and centrifuged (3000 r/min for 10 min) to get supernatant. Then, 0.5 mL of supernatant was pooled with 2.5 mL of chromogenic reagent (ten-fold volume of 7 g/L a-naphthol solution mixed with one-fold volume of 0.1% biacetyl reagent, 1 L of 7 g/L a-naphthol solution contained 128 g of anhydrous Na_2CO_3, 60 g of NaOH, 7 g of a-naphthol). After 5 min of rest, the mixture was incubated at 30°C for 30 min. Following incubation, 2.5 mL of distilled water was added and absorbance was measured at 520 nm. Finally, the solubility curve was drawn. For serum creatine content determination, the appropriate amount of serum sample was taken and distilled water was added to it, sonicated for 20 min, shocked for 15 min, and centrifuged (4000 r/min for 10 min) to get supernatant. Subsequently, 250 μL of the supernatant was collected and 250 μL of distilled water, 250 μL of 5% $ZnSO_4$, and 250 μL of 5% $Ba(OH)_2$ were added and followed by the protocol described above. Lastly, the absorbance values were recorded and quantified using standard curves established.

Meat Quality Measurements

Muscle $pH_{45\ min}$ and $pH_{24\ h}$ were determined using an insertiontype portable pH meter (HI99163N, Hanna, Padova, Italy). Triplicates were done for each chop at different areas and averaged to calculate the results.

Color of the meat was determined using a spectrocolorimeter (WSC-S, Shanghai, China) at 24 h postmortem. The meat color was presented in (CIE) L* (lightness), a* (redness), and b* (yellowness). The illuminant and observer angle were D65 and 10°, respectively. The L*, a*, and b* values were collected from three different areas of each steak with freshly cut surface (exposure to air for 20 min).

To measure drip loss, approximately 30 g of rectangle muscle were cut along the fibre direction at 24 h postmortem. The samples were suspended in a polyethylene plastic bag to avoid any contact with the bag. Samples were re-weighed to calculate the drip loss after 24 h at 4°C.

Texture profile analysis (TPA) values of cooked samples were determined using a CT3 texture analyzer (Brookfield, Middleboro, MA, USA). Briefly, at 48 h postmortem, LT muscle samples were cut in approximately 2.5 cm thick pieces. The pieces were individually vacuumed packaged then placed in

a hot-water bath (80°C) to reach a center temperature of 75°C. The packaged samples were cooled down to room temperature by running water, then removed from bags, and wiped dry with absorbent paper. The blot-dried samples were cut in 1.0 cm thick pieces to ensure a flat surface. Finally, the meat pieces were placed beneath the probe and analyzed for five TPA parameters: hardness, cohesiveness, springiness, gumminess, and chewiness. Each sample was measured thrice, and the relevant parameters were designed as follows: the probe model was TA3/100, the pre-measurement speed was 2 mm/s, the test speed was 0.5 mm/s, the post-measurement speed was 0.5 mm/s, the sample height was 10 mm, and the trigger force was 5 g.

Muscle Glycolytic Potential and Glycolytic Enzyme Activities

The measurement of glycogen was conducted as described by a previous study (Zhang et al., 2009). The glycogen was hydrolysed to glucose by reaction with amyloglucosidase and the glucose-6-phosphate was catalysed to glucose by reaction with glucose-6-phosphatase. Finally, the glucose determination operations were carried out according to a commercial glucose kit (Shanghai RongSheng Biotech Co. Ltd.). The lactate content was performed using a commercial diagnostic kit (Nanjing Jiancheng Bioengineering Institute) in accordance with kit instructions. The glycolytic potential (GP) was calculated according to the formula from Monin and Sellier (1985): GP = 2 × glycogen + lactate.

For muscle glycolytic enzyme activities determination, 0.5 g frozen LT muscle sample was homogenized in 4.5 mL ice-cold physiological saline for 1 min in an ice bath and centrifuged (2,700 g, 10 min, 4°C) to get supernatants. The supernatants of LT muscle were subjected to the determination of the activities of phosphofructokinase (PFK), hexokinase (HK), pyruvate kinase (PK), and LDH. The concentration of total protein in LT muscle was determined by TP kit (Nanjing Jiancheng Bioengineering Institute) according to kit instructions. The activities of PFK, HK, PK, and LDH were determined by commercial PFK, HK, PK, and LDH kits (Nanjing Jiancheng Bioengineering Institute). The enzyme activities in LT muscle were normalized by total protein concentration in LT muscle according to kit instructions.

Phosphocreatine, Creatine, and Adenosine Phosphates Determination

The phosphocreatine (PCr), creatine, and adenosine phosphates (ATP, ADP, and AMP) contents of LT muscle were assayed using a high-performance liquid chromatography (HPLC) according to previous study (Li et al., 2017). The reference standard samples used in the analysis were creatine anhydrous (98%), creatine phosphate disodium salt (97%), adenosine 5'-stiphosphate disodium salt, adenosine 5'-diphoshate, adenosine 5'-monophosphate. All of the standard samples were purchased from Shanghai Yuan Mu Biotechnology Co., Ltd (Shanghai, China). The extracted muscle sample solution was prepared as described previously (Monin and Sellier, 1985) and separated on a Shimadzu LC-2030 Plus HPLC (Shimadzu, Kyoto, Japan) equipped with a YMC-Pack ODS-AQ column (250 mm × 4.6 mm I.D., 5 μm, YMC Co., Ltd., Kyoto, Japan) at 25°C for PCr and creatine determination and at 30°C for ATP, ADP, and AMP determination. The mobile phase of creatine and PCr determination was HPLC methyl cyanides and phosphate buffer (29.4 mM potassium dihydrogen orthophosphate,1.15 mM tetra-butylammonium hydrogen sulphate, 2%/98%), and adenosine phosphates determination was HPLC grade methanol and phosphate buffer (2.5 mM tetra-butylammonium hydrogen sulphate, 0.06 M dipotassium hydrogen orthophosphate, 0.04 M potassium dihydrogen orthophosphate, 13.5%/86.5%). The UV detection for creatine and PCr was at 210 nm and adenosine phosphates was at 254 nm. The flow-rate was set to 1.0 mL/min and an auto-sequence injection was set for sample measurement. Peaks were identified and quantified using standard curves.

Liver Pyruvate, Glucose, and Glycogen Concentration, and Enzyme Activity Determination

The contents of pyruvate and glycogen were determined by commercial pyruvate and glycogen kits (Nanjing Jiancheng Bioengineering Institute) according to kit instructions. The glucose concentration was determined by a commercial glucose kit (Shanghai RongSheng Biotech Co. Ltd.). For liver enzyme activities determination, 0.5 g frozen liver sample was homogenized in 4.5 mL ice-cold physiological saline for 1 min in ice bath and centrifuged (2,700 g, 10 min, 4°C) to get supernatants. The supernatants of liver were subjected to the determination of the activities of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G-6-pase), and pyruvate carboxylase (PC). The concentration of total protein in liver was determined by TP kit (Nanjing Jiancheng Bioengineering Institute) according to kit instructions. The activities of PEPCK, G-6-pase, and PC were determined by commercial PEPCK kit (Nanjing Jiancheng Bioengineering Institute), commercial G-6-pase and PC kits (Shanghai enzyme linked Biotechnology Co., Ltd). Except for PEPCK, other enzyme activities in the liver were normalized by total protein concentration in the liver according to kit instructions.

Statistical Analysis

Data processing was performed using SPSS (version 17.0, IBM, Armonk, NY, USA). All treatment groups were analyzed by analysis of univariate using the general linear model (GLM) as a 2 × 2 factorial arrangement with transport, RP-CyPry and their interactions as the main effects. The results are shown as the mean and standard error mean (SEM). Differences among means were determined using Tukey's multiple range test was done when the interaction was significant. The level of statistical significance was set at $P < 0.05$.

RESULTS

Serum Parameters

As presented in **Table 2**, RP-CrPyr and transport had no interaction on serum parameters. The beef cattle in the RP-CrPyr-supplemented group had a lower activities of serum CK

TABLE 2 | Effects of transport and rumen-protected creatine pyruvate on the serum indicators of beef cattle.

Item	Treatment[1]				SEM	*P*-value		
	Long-distance transport		Short-distance transport					
	RP-CrPyr$_{140}$	RP-CrPyr$_0$	RP-CrPyr$_{140}$	RP-CrPyr$_0$		Transport	RP-CrPyr	Transport × RP-CrPyr[2]
CK, U/mL	0.146	1.00	0.195	0.895	0.0584	0.813	0.001	0.528
LDH, U/mL	1.08	1.30	1.02	1.20	0.0278	0.170	0.007	0.735
Glucose, mmol/L	4.32	4.17	4.16	4.14	0.102	0.681	0.685	0.771
Pyruvate, μmol/mL	0.326	0.292	0.259	0.273	0.0174	0.331	0.638	0.400
Creatine, μmol/L	155	149	155	157	4.114	0.649	0.803	0.629

[1] *A 2 × 2 factorial arrangement with 2 supplemental levels of rumen-protected creatine pyruvate (RP-CrPyr) in basal diets (0 or 140 g/d for each cattle) and 2 transport treatment (transport 5 min or 12 h before slaughter).*

[2] *Transport × RP-CrPyr, the interaction between Transport and RP-CrPyr.*

SEM, stand error of mean; CK, creatine kinase; LDH, lactate dehydrogenase.

(0.170 vs. 0.947 U/mL, $P < 0.01$) and LDH (1.05 vs. 1.25 U/mL, $P < 0.01$) than unsupplemented cattle. However, transport treatment has no significant effects on serum CK and LDH activities. The contents of serum pyruvate, glucose, and creatine appeared to be unaffected by transport or RP-CrPyr ($P > 0.05$).

Meat Quality

The results of meat quality can be seen in **Table 3**. Except for $pH_{45\ min}$ value and redness, RP-CrPyr and transport had no significant interaction influence on other meat quality characteristics. Compared with cattle in the nontransport group, the $pH_{45\ min}$ value (6.36 vs. 6.20, $P < 0.01$), drip loss (1.81% vs. 1.08%, $P < 0.05$), redness (34.6 vs. 27.9, $P < 0.01$), and yellowness (22.1 vs. 15.8, $P < 0.05$) of LT muscle were significantly increased in group transport. Dietary RP-CrPyr supplementation decreased the LT muscle $pH_{24\ h}$ value (5.47 vs. 5.59, $P < 0.05$), redness (28.2 vs. 34.3, $P < 0.01$), and yellowness (16.1 vs. 21.7, $P < 0.05$) of beef cattle than those in the unsupplemented groups. RP-CrPyr or transport had no effect on the meat color and texture properties of LT muscle ($P > 0.05$).

Muscle Glycolytic Potential and Glycolytic Enzyme Activity

As shown in **Table 4**, there is a significant transport × RP-CrPyr interaction influence on muscle glycogen and GP. However, there is no notable RP-CrPyr and transport interaction on glycogen, glucose, lactate, and glycolytic enzymes. Compared with the beef cattle in nontransport groups, the muscle glycogen content (37.3 vs. 48.3 μmol/g, $P < 0.05$) and GP (118 vs. 139 μmol/g, $P < 0.01$) of beef cattle decreased significantly in the transport groups. In addition, no notable differences were identified in the contents of muscle glucose and lactate between the nontransport and transport treatment ($P > 0.05$). Dietary supplementation of RP-CrPyr increased the muscle glycogen content (53.5 vs. 32.1 μmol/g) and GP (146 vs. 110 μmol/g) ($P < 0.01$), and decreased the muscle lactate content (39.2 vs. 46.0 μmol/g, $P < 0.01$) of beef cattle compared to those in the RP-CrPyr unsupplemented groups. Glycolytic enzymes, except for muscle HK activity (7.64 vs. 2.49 U/mgprot, $P < 0.01$), were significantly increased by RP-CrPyr supplementation compared to those in the unsupplemented groups, and the activities of PFK, PK, and LDH were not affected by transport or RP-CrPyr supplementation.

TABLE 3 | Effects of transport and rumen-protected creatine pyruvate on the *M. longissimus thoracis* meat quality of beef cattle.

Item	Treatment[1]				SEM	*P*-value		
	Long-distance transport		Short-distance transport					
	RP-CrPyr$_{140}$	RP-CrPyr$_0$	RP-CrPyr$_{140}$	RP-CrPyr$_0$		Transport	RP-CrPyr	Transport × RP-CrPyr[2]
$pH_{45\ min}$	6.27^b	6.46^a	6.24^b	6.16^b	0.0185	0.002	0.171	0.008
$pH_{24\ h}$	5.47	5.69	5.47	5.50	0.0277	0.182	0.033	0.217
Lightness (L*)	33.2	25.0	30.9	30.4	1.07	0.498	0.075	0.107
Redness (a*)	29.2^b	40.1^a	27.2^b	28.6^b	0.742	0.002	0.003	0.012
Yellowness (b*)	17.8	26.4	14.5	17.1	0.998	0.014	0.024	0.165
Drip loss, %	1.67	1.96	1.20	0.970	0.0868	0.030	0.874	0.165
Hardness, kg	0.600	0.840	0.560	0.790	0.145	0.877	0.438	0.995
Cohesiveness	0.610	0.710	0.700	0.660	0.0192	0.646	0.482	0.113
Springiness, mm	0.240	0.270	0.310	0.270	0.0170	0.357	0.850	0.357
Gumminess, kg	0.450	0.600	0.380	0.440	0.0846	0.540	0.552	0.812
Chewiness, mJ	10.8	16.0	14.0	25.2	3.43	0.391	0.267	0.679

[a,b] *Means within a row with no common superscript differ significantly ($P < 0.05$).*

[1] *A 2 × 2 factorial arrangement with 2 supplemental levels of rumen-protected creatine pyruvate (RP-CrPyr) in basal diets (0 or 140 g/d for each cattle) and 2 transport treatment (transport 5 min or 12 h before slaughter).*

[2] *Transport × RP-CrPyr, the interaction between Transport and RP-CrPyr.*

SEM, stand error of mean.

TABLE 4 | Effects of transport and rumen-protected creatine pyruvate on the contents of glycogen and lactate, glycolytic potential, and glycolytic enzymes activity in *M. longissimus thoracis* of beef cattle.

Item	Treatment[1]				SEM	*P*-value		
	Long-distance transport		Short-distance transport					
	RP-CrPyr$_{140}$	RP-CrPyr$_0$	RP-CrPyr$_{140}$	RP-CrPyr$_0$		Transport	RP-CrPyr	Transport × RP-CrPyr[2]
Glycogen, μmol/g	44.6^b	30.0^c	62.4^a	34.3^c	1.41	0.004	0.001	0.043
Lactate, μmol/g	38.5	47.6	40.0	44.5	0.871	0.664	0.004	0.225
GP, μmol/g	128^b	108^c	165^a	113^{bc}	2.88	0.006	0.001	0.025
HK, U/mgprot	7.15	2.30	8.26	2.69	0.458	0.412	0.001	0.668
PFK, U/mgprot	16.8	10.5	24.3	14.0	2.28	0.264	0.103	0.673
PK, U/gprot	451	510	461	626	43.0	0.482	0.227	0.555
LDH, U/kgprot	3.04	3.43	2.96	2.69	0.139	0.177	0.827	0.275

^{a,b} Means within a row with no common superscript differ significantly (P < 0.05).

[1] A 2 × 2 factorial arrangement with 2 supplemental levels of rumen-protected creatine pyruvate (RP-CrPyr) in basal diets (0 or 140 g/d for each cattle) and 2 transport treatment (transport 5 min or 12 h before slaughter).

[2] Transport × RP-CrPyr, the interaction between Transport and RP-CrPyr.

SEM, stand error of mean; GP (glycolytic potential), 2 × (glycogen) + lactate (Monin and Sellier, 1985); LDH, lactate dehydrogenase; PFK, phosphofructokinase; PK, pyruvate kinase; HK, hexokinase.

Phosphocreatine, Creatine, and Adenosine Phosphates in Muscle

The results of muscle PCr, creatine, and adenosine phosphates are showed in **Table 5**. Except for muscle AMP content, RP-CrPyr and transport had no significant interaction on the other characteristics. Compared with the beef cattle in nontransport groups, the beef cattle in transport groups had higher muscle creatine content (22.4 vs. 21.2 μmol/g, $P < 0.05$). Dietary RP-CrPyr supplementation significantly decreased the level of muscle AMP (67.4 vs. 76.8 μg/g, $P < 0.01$), muscle AMP/ATP (0.13 vs. 0.68, $P < 0.05$), and significantly increased the levels muscle ATP (579 vs. 217 μg/g), muscle ADP (330 vs. 238 μg/g), and muscle ATP/AMP (1.59 vs 0.790) ($P < 0.05$).

Hepatic Gluconeogenesis

As shown in **Table 6**, RP-CrPyr and transport had no significant interaction influence on any of these parameters. Compared with the beef cattle in nontransport groups, the beef cattle with transport treatment had lower liver glucose content (0.171 vs. 0.209 mmol/g, $P < 0.05$). Dietary supplementation of RP-CrPyr significantly increased the concentrations of liver pyruvate (41.8 vs. 29.9 μmol/gprot, $P < 0.01$) and glucose (0.213 vs. 0.167 mmol/g, $P < 0.05$), and the level of PC (19.2 vs. 13.7 ng/mgprot, $P < 0.05$) compared with the beef cattle in RP-CrPyr nosupplementation groups. Transport or RP-CrPyr had no effect on the liver glycogen content and the levels of G-6-Pase and PEPCK ($P > 0.05$).

DISCUSSION

The results of our previous study indicated that transport significantly decreased the catalase activity and total antioxidant capacity in serum, which shows that transport leads to oxidative damage to beef cattle (Mao et al., 2022). It is widely known that oxidative damage might be harmful to the

TABLE 5 | Effects of transport and rumen-protected creatine pyruvate on the contents of phosphocreatine, creatine, and adenosine phosphates in *M. longissimus thoracis* of beef cattle.

Item	Treatment[1]				SEM	*P*-value		
	Long-distance transport		Short-distance transport					
	RP-CrPyr$_{140}$	RP-CrPyr$_0$	RP-CrPyr$_{140}$	RP-CrPyr$_0$		Transport	RP-CrPyr	Transport × RP-CrPyr[2]
PCr, μmol/g	2.07	2.37	2.18	2.16	0.0530	0.637	0.223	0.176
Creatine, μmol/g	21.7	23.2	21.5	21.0	0.230	0.027	0.315	0.068
ATP, μg/g	592	168	567	267	69.0	0.794	0.030	0.667
ADP, μg/g	288	249	373	227	17.2	0.391	0.027	0.162
AMP, μg/g	64.3^b	83.2^a	70.5^b	70.4^b	1.35	0.256	0.008	0.008
ATP/ADP	1.68	0.630	1.49	0.950	0.136	0.829	0.020	0.379
AMP/ATP	0.126	0.774	0.135	0.588	0.108	0.693	0.034	0.664

^{a,b} Means within a row with no common superscript differ significantly (P < 0.05).

[1] A 2 × 2 factorial arrangement with 2 supplemental levels of rumen-protected creatine pyruvate (RP-CrPyr) in basal diets (0 or 140 g/d for each cattle) and 2 transport treatment (transport 5 min or 12 h before slaughter).

[2] Transport × RP-CrPyr, the interaction between Transport and RP-CrPyr.

SEM, stand error of mean; PCr, phosphocreatine; Crm cretaine; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate.

TABLE 6 | Effects of transport and rumen-protected creatine pyruvate on the hepatic gluconeogenesis of beef cattle.

Item	Treatment[1]				SEM	*P*-value		
	Long-distance transport		Short-distance transport					
	RP-CrPyr$_{140}$	RP-CrPyr$_0$	RP-CrPyr$_{140}$	RP-CrPyr$_0$		Transport	RP-CrPyr	Transport × RP-CrPyr[2]
Pyruvate, μmol/gprot	42.4	25.7	41.2	34.1	1.33	0.215	0.002	0.110
Glucose, mmol/g	0.187	0.155	0.239	0.180	0.00719	0.028	0.014	0.370
Glycogen, mg/g	17.5	15.9	17.9	17.8	1.27	0.657	0.740	0.783
PC, ng/mgprot	22.4	13.5	16.1	13.9	0.926	0.148	0.017	0.114
PEPCK, U/kg	86.1	83.4	80.2	87.9	3.19	0.913	0.708	0.442
G-6-Pase, ng/mgprot	5.22	5.18	5.79	5.53	0.325	0.499	0.830	0.868

[1] *A 2 × 2 factorial arrangement with 2 supplemental levels of rumen-protected creatine pyruvate (RP-CrPyr) in basal diets (0 or 140 g/d for each cattle) and 2 transport treatment (transport 5 min or 12 h before slaughter).*

[2] *Transport × RP-CrPyr, the interaction between Transport and RP-CrPyr.*

SEM, stand error of mean; PC, pyruvate carboxylase; PEPCK, Phosphoenolpyruvate carboxykinase; G-6-pase, glucose-6-phosphatase.

cellular and alter membrane permeability and thus, the stress enzymes such as CK and LDH are released into the serum through leakage arising from altered membrane permeability (Mader, 2003; Sattler and Fürll, 2004). Consistently, previous studies indicated that serum alanine aminotransferase, aspartate aminotransferase, CK, and LDH activities were increased in transported wild ungulates due to tissue damage, poor muscular tissue perfusion, reduced heat dissipation, hypoxia, and fatigue (López-Olvera et al., 2006). In this study, serum CK and LDH activities were lower in the RP-CrPyr-supplemented group which indicates that oxidative damage was minimized. Correspondingly, our previous study presented that RP-CrPyr reduced oxidative stress, to some extent, by tending to decrease the serum MDA concentration (Mao et al., 2022). Creatine and pyruvate are both energetic and antioxidant substances (Rieger et al., 2017), RP-CrPyr can be absorbed in the form of pyruvate and creatine in the small intestine after ingestion, thereby enhancing the antioxidant properties of beef cattle. Blood glucose level is used as a metabolic indicator of energy status in cattle. Transportation without feed and water for 2 days caused a decrease in serum glucose of steer calves immediately after transportation (Takemoto et al., 2018). While, another study reported that the blood glucose of bulls increased significantly after 9 h of transport (Earley et al., 2013). Our result was different with both of these study that there was no difference in serum glucose between the long-distance transport and short-distance transport. This may be due to the differences in the transportation time, which can be summarized as: 2 days of transport results in a decrease, 9 h of transport results in an increase, and 12 h of transport with 2 h of recovery did not cause change in blood glucose. Moreover, the present study showed that RP-CrPyr supplementation had no effect on serum pyruvate and creatine contents. For this, one possible explanation might be that the pyruvate and creatine entered the tissues from blood when sampling.

It is well known that pre-slaughter stress results in depletion of glycogen stores in the muscle, further limits post-mortem glycolysis and the formation of lactic acid, and leads to a high pH_u (Ponnampalam et al., 2017; Shange et al., 2019). Among which, transport stress is one of the major factors. In the present study, the transport × RP-CrPyr interaction significantly affected the $pH_{45\ min}$ value of muscle, which seems to be associated with the observed interaction for glycogen and GP in muscle (**Table 4**). This suggests that RP-CrPyr could restore the long-distance transport-induced rice in $pH_{45\ min}$,by increasing muscle glycogen content. Moreover, long-distance transport followed with recovery treatment significantly increased the $pH_{45\ min}$ value and decreased glycogen and GP, while had no effect on $pH_{24\ h}$ value. This situation may be assumed because of the reduced muscle glycogen retention related to transport stress but it was not low enough to result in high $pH_{24\ h}$. Indeed, it was suggested the relationship between initial muscle glycogen content and ultimate pH is only linear at very low levels of glycogen (Marína et al., 2003). Similarly, Fernandez et al. (1996) reported that there was no significant change in the pH at 48 h postmortem while 11 h of transport significantly reduced muscle GP and increased pH at 4 h postmortem. Moreover, a previous study reported beef meat that has a $pH_{24\ h}$ greater than 5.5 is considered a result of pre-slaughter glycogen depletion and comes with a lack of glycogen in muscle that accumulated adequate lactate concentration (Kannan et al., 2002). Consistently in this study, the muscle from long-distance transported beef cattle without RP-CrPyr supplementation had $pH_{24\ h}$ greater than 5.5 ($pH_{24\ h}$ = 5.69). Meat color is the most important component in physical appearance and is used by consumers to evaluate the quality and freshness of meat. When the color of meat changes from bright red (oxymyoglobin, the oxygenated form of the muscle pigment myoglobin) to brown (metmyoglobin), it is well known that a* values are most closely related to the oxymyoglobin content of the meat (Renerre et al., 1996) while L* will decrease with the increase of a* and the flesh color will gradually become dark. Transport stress is accompanied by oxidative stress, which leads to the decline of muscle antioxidant capacity and increase in oxymyoglobin content of meat. Consistently, the long-distance transported beef cattle without RT-CrPyr (antioxidants) supplementation had lower L* value and higher a* value, while long-distance transported beef cattle fed with RT-CrPyr increased L* value and decreased a* value. In addition, our findings showed that long-distance transport followed with recovery treatment significantly increased muscle drip loss. This result was in accordance with at

least one study (Honkavaara et al., 2003) but in contrast to others (Fernandez et al., 1996; Xin et al., 2018).

In the current study, RP-CrPyr supplementation reduced the $pH_{24\ h}$ value and this was attributed to RP-CrPyr increasing the muscle glycogen content and glycolytic potential at the early postmortem period, providing sufficient glycogen and lactate for the reduction of muscle pH at 24 h postmortem. Previous research indicated that exogenous pyruvate, creatine, and CrPyr could promote glycogen stores in muscle of mammalian (Ceddia and Sweeney, 2004; Ju et al., 2005; Guo et al., 2009; Chen et al., 2012; Shetty et al., 2012;), therefore, the muscle glycogen level in cattle fed RP-CrPyr was higher in this study. During early postmortem metabolism, the phosphagen system and glycolysis contribute to the maintenance of ATP levels (Scheffler and Gerrard, 2007). Creatine supplementation has been shown to promote the increase in intramuscular PCr, which could improve the energy supply substrates of the phosphagen system, relatively reduce glycogenolysis, and thereby decrease lactate production (Li et al., 2018). Consistent with this, the present study shows that RP-CrPyr increased muscle ATP reserve and decreased the muscle lactate content at early postmortem. Glycolytic enzymes are the key factors regulating glycolytic flow and lactate accumulation in muscle (Scheffler and Gerrard, 2007). Oxidative stress causes the inactivation of key glycolytic enzymes in mammalian cells (Reichmann et al., 2018). Our previous study showed that RP-CrPyr could reduce oxidative stress induced by transport stress to some extent (Mao et al., 2022). Therefore, in this study the enhanced activity of glycolytic enzyme HK by RP-CrPyr might be attributed to the antioxidant properties of CrPyr. Similarly, Zhao et al. (2018) reported that *in ovo* feeding of CrPyr increased glycolytic enzyme activity and promoted glycolysis in chest of broilers. Bortoluzzi et al. (2019) found that creatine, plus pyruvate supplementation, prevented the oxidative stress induced decrease in HK and PK cell activities in the brain of rats subjected to chemically-induced phenylketonuria.

For ruminants, the gluconeogenesis in liver accounts for 80% of endogenous glucose (Aschenbach et al., 2010). Propionate, glucogenic amino acids, lactate, glycerol, and pyruvate are all substrates for hepatic gluconeogenesis in ruminants. Previous research has found that supplementation of gluconeogenic precursors increased glycogen content in muscle and liver *via* stimulating the rate of gluconeogenesis (Volpi-Lagreca and Duckett, 2017). Our results show that RP-CrPyr supplementation increase the liver pyruvate content and provide an additional source of substrate for gluconeogenesis, which in turn increases hepatic glucose production and glucose levels. In the process of gluconeogenesis, PC is the primary key enzyme for the conversion of pyruvate to oxaloacetic acid, which is then converted to phosphoenolpyruvate through PEPCK, undergoes a series of non-enzymatic reactions to generate glucose-6-phosphate, and finally catalyzes by G-6-Pase enzyme to produce glucose. The PC, PEPCK, and G-6-Pase are the rate-limiting enzymes responsible for gluconeogenesis (Aschenbach et al., 2010), and their activity is regulated transcriptionally and post-transcriptionally, such the supply of gluconeogenic precursors to the liver as well as by insulin and glucagon (She et al., 1999; Bobe et al., 2009). In this study, we found that RP-CrPyr supplementation increased the activity of PC. Similarly, Zhao et al. (2018) reported that *in ovo* feeding of CrPyr up-regulated the mRNA expression levels of PC and PEPCK in broilers. Hence, the increased hepatic gluconeogenic enhanced the glucose generation, which might be useful to the muscle glycogen deposition.

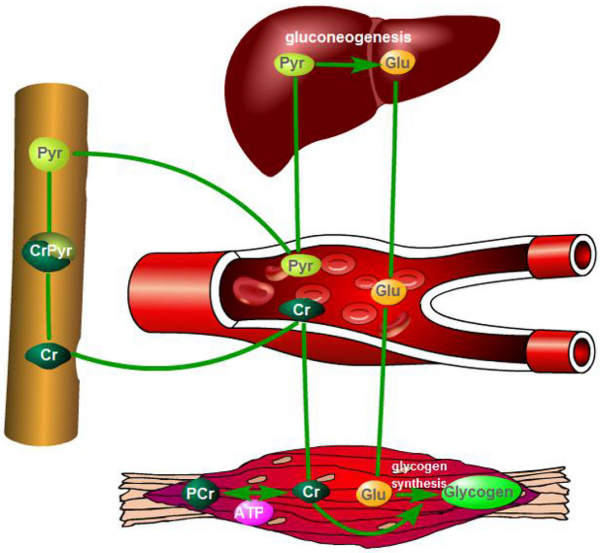


FIGURE 1 | The possible mechanisms diagram of creatine pyruvate promoting beef muscle glycogen deposit. CrPyr, creatine pyruvate; Cr, creatine; Py, pyruvate; Glu, glucose; ATP, adenosine triphosphate.

In conclusion, dietary supplementation, with RP-CrPyr, improved meat quality and energy metabolism regulatory capacity in beef cattle suffering from long-distance transport followed with recovery treatment. This may show that RP-CrPyr attributes to increased muscle glycogen storage and energy supply and hepatic gluconeogenesis (**Figure 1**).

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the study data are owned by Jiangxi Province Key Laboratory of Animal Nutrition. Access to these data would require additional approval beyond that of the authors. Requests to access these datasets should be directed to Dr. Yanjiao Li, yanjiaoli221@163.com.

ETHICS STATEMENT

This experiment was approved by the Committee for the Care and Use of Experimental Animals at Jiangxi Agricultural University (JXAULL-2021-10).

AUTHOR CONTRIBUTIONS

GL and YL designed the overall study. GL, KM, YZ, XZ, QQ, MQ, and KO performed experiments. GL and YL wrote the

manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (No. 32160814), the Jiangxi Provincial Youth Scientist Foundation (20202BAB215010), and the China Agriculture Research System of MOF and MARA (CARS-37).

ACKNOWLEDGMENTS

The authors appreciate all the helps from our colleagues and collaborators.

REFERENCES

Allen, P. J. (2012). Creatine Metabolism and Psychiatric Disorders: Does Creatine Supplementation Have Therapeutic Value? *Neurosci. Biobehav. Rev.* 36 (5), 1442–1462. doi: 10.1016/j.neubiorev.2012.03.005

Aschenbach, J. R., Kristensen, N. B., Donkin, S. S., Hammon, H. M., and Penner, G. B. (2010). Gluconeogenesis in Dairy Cows: The Secret of Making Sweet Milk From Sour Dough. *IUBMB. Life.* 62 (12), 869–877. doi: 10.1002/iub.400

Bobe, G., Velez, J. C., Beitz, D. C., and Donkin, S. S. (2009). Glucagon Increases Hepatic mRNA Concentrations of Ureagenic and Gluco-Neogenic Enzymes in Early-Lactation Dairy Cows. *J. Dairy. Sci.* 92, 5092–5099. doi: 10.3168/jds.2009-2152

Bortoluzzi, V. T., Brust, L., Preissler, T., de Franceschi, I. D., and Wannmacher, C. M.D. (2019). Creatine Plus Pyruvate Supplementation Pre-Vents Oxidative Stress and Phosphotransfer Network Disturbances in the Brain of Rats Subjected to Chemically-Induced Phe-Nylketonuria. *Metab. Brain. Dis.* 34 (6), 1649–1660. doi: 10.1007/s11011-019-00472-7

Ceddia, R. B., and Sweeney, G. (2004). Creatine Supplementation Increases Glucose Oxidation and AMPK Phosphorylation and Reduces Lactate Production in L6 Rat Skeletal Muscle Cells. *J. Physiol.* 555 (2), 409–421. doi: 10.1113/jphysiol.2003.056291

Chen, J., Huang, J., Deng, J., Ma, H., and Zou, S. (2012). Use of Comparative Proteomics to Identify the Effects of Creatine Pyruvate on Lipid and Protein Metabolism in Broiler Chickens. *Vet. J.* 193 (2), 514–521. doi: 10.1016/j.tvjl.2012.01.034

Chen, J., Wang, M., Kong, Y., Ma, H., and Zou, S. (2011). Comparison of the Novel Compounds Creatine and Pyruvateon Lipid and Protein Metabolism in Broiler Chickens. *Animal* 5 (7), 1082–1089. doi: 10.1017/S1751731111000085

Earley, B., Drennan, M., and O'Riordan, E. G. (2013). The Effect of Road Transport in Comparison to a Novel Environment on the Physio-Logical, Metabolic and Behavioural Responses of Bulls. *Res. Vet. Sci.* 95, 811–818. doi: 10.1016/j.rvsc.2013.04.027

Ferguson, D. M., and Warner, R. D. (2008). Have We Underestimated the Impact of Pre-Slaughter Stress on Meat Quality in Ruminants? *Meat. Sci.* 80 (1), 12–19. doi: 10.1016/j.meatsci.2008.05.004

Fernandez, X., Monin, G., Culioli, J., Legrand, I., and Quilichini, Y. (1996). Effect of Duration of Feed Withdrawal and Transportation Time on Muscle Characteristics and Quality in Friesian-Holstein Calves. *J. Anim. Sci.* 74 (7), 1576–1583. doi: 10.2527/1996.7471576x

Guo, Y., Gong, D., Li, X, Cui, L. L., Wei, M., Hu, J. Y., et al. (2009). Effects of Calcium Pyruvate Supplemen-Tation on Exercise Ability in Mice. *J. Dalian. Med. Univ.* 31 (2), 145–147,161.

Honkavaara, M., Rintasalo, E., Ylönen, J., and Pudas, T. (2003). Meat Quality and Transport Stress of Cattle. *Dtsch. Tierarztl. Wochenschr.* 110 (3), 125–128.

Hu, S., Lin, Z. L., Zhao, Z. K., Liu, R., Ma, L., Luo, H. M., et al. (2016). Pyruvate Is Superior to Citrate in Oral Rehy-Dration Solution in the Protection of Intestine *via* Hypoxia Inducible Factor-1 Activation in Rats With Burn Injury. *JPEN. J. Parenter. Enteral. Nutr.* 40 (7), 924–933. doi: 10.1177/0148607115577817

Jäger, R., Metzger, J., Lautmann, K., Shushakov, V., Purpura, M., Geiss, K. R., et al. (2008). The Effects of Creatine Pyruvate and Creatine Citrate on Performance During High Intensity Exercise. *J. Int. Soc Sports. Nutr.* 5 (1), 1–9. doi: 10.1007/s00726-011-0874-6

Jäger, R., Purpura, M., Shao, A., Inoue, T., and Kreider, R. B. (2011). Aalysis of the Efficacy, Safety, and Regulatory Status of Novel Forms of Creatine. *Amino. Acids* 40 (5), 1369–1383. doi: 10.1007/s00726-011-0874-6

Ju, J. S., Smith, J. L., Oppelt, P. J., and Fisher, J. S. (2005). Creatine Feeding Increases GLUT4 Expression in Rat Skeletal Muscle. *Am. J. Physiol. Endocrinol. Metab.* 288 (2), E347–E352. doi: 10.1152/ajpendo.00238.2004

Kannan, G., Chawan, C. B., Kouakou, B., and Gelaye, S. (2002). Inflfluence of Packaging Method and Storage Time on Shear Value and Mechanical Strength of Intramuscular Connective Tissue of Chevon. *J. Anim. Sci.* 80, 2383–2389. doi: 10.1051/gse:2002028

Li, Y. J., Gao, T., Li, J. L., Zhang, L., Gao, F., and Zhou, G. H. (2017). Effects of Dietary Starch Types on Early Postmortem Muscle Energy Metabolism in Finishing Pigs. *Meat. Sci.* 133, 204–209. doi: 10.1016/j.meatsci.2017.07.008

Li, J., Zhang, L., Fu, Y., Li, Y., Jiang, Y., Zhou, G., and Gao, F. (2018). Creatine Monohydrate and Guanidinoacetic Acid Supplementation Affects the Growth Performance, Meat Quality, and Creatine Metabolism of Finishing Pigs. *J. Agric. Food Chem.* 66 (38), 9952–9959. doi: 10.1021/acs.jafc.8b02534

López-Olvera, J. R., Marco, I., Montané, J., and Lavín, S. (2006). Transport Stress in Southern Chamois (Rupicapra Pyrenaica) and its Modulation by Acepromazine. *Vet. J.* 172 (2), 347–355. doi: 10.1016/j.tvjl.2005.06.007

Lu, C., Cao, Y., Li, J., Gao, Y., Li, Q., Yang, J., et al. (2015). Effects of Preslaughter Transportation Stress on Slaughter Performance and Beef Quality of Meet Cattle. *Chin. J. Vet. Sci.* 35 (12), 2045–2048. doi: 10.16303/j.cnki.1005-4545.2015.12.30

Mader, T. L. (2003). Environmental Stress in Confined Beef Cattle. *J. Anim. Sci.* 81 (E. Suppl. 2), E110–E119. doi: 10.2527/2003.8114_suppl_2E110x

Mao, K., Lu, G., Li, Y., Zang, Y., Zhao, X., Qiu, Q., et al. (2022). Effects of Rumen-Protected Creatine Pyruvate on Blood Bio-Chemical Parameters and Rumen Fluid Characteristics in Transported Beef Cattle. *BMC. Vet. Res.* 18 (1), 1–15. doi: 10.1186/s12917-021-03134-y

Marıía, G. A., Villarroel, M., Sañudo, C., Olleta, J. L., and Gebresenbet, G. (2003). Effect of Transport Time and Ageing on Aspects of Beef Quality. *Meat. Sci.* 65 (4), 1335–1340. doi: 10.1016/S0309-1740(03)00054-8

Monin, G., and Sellier, P. (1985). Pork of Low Technological Quality With a Normal Rate of Muscle pH Fall in the Immediate Post-Mortem Period: The Case of the Hampshire Breed. *Meat. Sci.* 13, 49–63. doi: 10.1016/S0309-1740(85)80004-8

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., Bekhit, A. E.D., et al. (2017). Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review Compr. *Rev. Food Sci. Food Saf.* 16 (3), 400–430. doi: 10.1111/1541-4337.12258

Reichmann, D., Voth, W., and Jakob, U. (2018). Maintaining a Healthy Proteome During Oxidative Stress. *Mol. Cell.* 69 (2), 203–213. doi: 10.1016/j.molcel.2017.12.021

Renerre, M., Dumont, F., and Gatellier, P.. (1996). Antioxidant Enzyme Activities in Beef in Relation to Oxidation of Lipid and Myoglobin. *Meat. Sci.* 43, 111–121. doi: 10.1016/0309-1740(96)84583-9

Rieger, E., de Franceschi, I. D., Preissler, T., and Wannmacher, C. M.D. (2017). Neuroprotective Effect of Creatine and Pyruvate on Enzyme Ac-Tivities of Phosphoryl Transfer Network and Oxidative Stress Alterations Caused by Leucine Administration in Wistar Rats. *Neurotox. Res.* 32, 575–584. doi: 10.1007/s12640-017-9762-5

Sattler, T., and Fürll, M. (2004). Creatine Kinase and Aspartate Aminotransferase in Cows as Indicators for Endometritis. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.* 51 (3), 132–137. doi: 10.1111/j.1439-0442.2004.00612.x

Scheffler, T. L., and Gerrard, D. E. (2007). Mechanisms Controlling Pork Quality Development: The Biochemistry Controlling Postmortem Energy Metabolism. *Meat. Sci.* 77 (1), 7–16. doi: 10.1016/j.meatsci.2007.04.024

Schoch, R. D., Willoughby, D., and Greenwood, M. (2006). The Regulation and Expression of the Creatine Transporter: A Brief Review of Creatine Supplementation in Humans and Animals. *J. Int. Soc Sports. Nutr.* 3 (1), 60–66. doi: 10.1186/1550-2783-3-1-60

Shange, N., Gouws, P., and Hoffman, L. C. (2019). Changes in Ph, Colour and the Microbiology of Black Wildebeest (Connochaetes Gnou) Longissimus Thoracis Et Lumborum (LTL) Muscle With Normal and High (DFD) Muscle pH. *Meat. Sci.* 147, 13–19. doi: 10.1016/j.meatsci.2018.08.021

Sharma, P., Benford, B., Li, Z. Z., and Ling, G. S. (2009). Role of Pyruvate Dehydrogenase Complex in Traumatic Brain Injury and Meas-Urement of Pyruvate Dehydrogenase Enzyme by Dipstick Test. *J. Emerg. Trauma. Shock.* 2 (2), 67–72. doi: 10.4103/0974-2700.50739

She, P., Lindberg, G. L., Hippen, A. R., Beitz, D. C., and Young, J. W. (1999). Regulation of Messenger Ribonucleic Acid Expression for Gluconeogenic Enzymes During Glucagon Infusions Into Lactating Cows. *J. Dairy. Sci.* 82, 1153–1163. doi: 10.3168/jds.S0022-0302(99)75338-5

Shetty, P. K., Sadgrove, M. P., Galeffi, F., and Turner, D. A. (2012). Pyruvate Incubation Enhances Glycogen Stores and Sustains Neuronal Function During Subsequent Glucose Deprivation. *Neurobiol. Dis.* 45 (1), 177–187. doi: 10.1016/j.nbd.2011.08.002

Takemoto, S., Funaba, M., and Matsui, T. (2018). Effect of Niacin Supplementation in Long-Distance Transported Steer Calves. *Anim. Sci. J.* 89, 1442–1450. doi: 10.1111/asj.13084

Volpi-Lagreca, G., and Duckett, S. (2017). Supplementation of Glycerol or Fructose *via* Drinking Water to Grazing Lambs on Tissue Gly-Cogen Level and Lipogenesis. *J. Anim. Sci.* 95, 2558–2575. doi: 10.2527/jas.2017.1449

Xin, L. I., Xia, A. Q., Chen, L. J., Du, M. T., Li, C., Ning, K., et al. (2018). Effects of Lairage After Transport on Post Mortem Muscle Glycolysis, Protein Phosphorylation and Lamb Meat Quality. *J. Integr. Agric.* 17 (10), 2336–2344. doi: 10.1016/S2095-3119(18)61922-7

Zhang, L., Yue, H. Y., Zhang, H. J., Xu, L., Wu, S. G., Yan, H. J., et al. (2009). Transport Stress in Broilers: I. Blood Metabolism, Glycolytic Potential, and Meat Quality. *Poultry. Sci.* 88, 2033–2041. doi: 10.3382/ps.2009-00128

Zhao, Q. (2013). *Survey on DFD Beef Incidences in China and Specification in Pre-Slaughter Management of Cattle. [Master's Thesis]* (Taian, China: Shandong Agricultural University).

Zhao, M. M. (2017). *Regulatory Mechanism of in Ovo Feeding of Creatine Pyruvate on Energy Metabolism and Muscle Development of Broiler Chickens. [Master's Thesis]* (Nanjing, China: Nanjing Agricultural University).

Zhao, M., Gong, D., Gao, T., Zhang, L., Li, J., Lv, P. A., et al. (2018). In Ovo Feeding of Creatine Pyruvate Increases the Glycolysis Pathway, Glucose Transporter Gene Expression, and AMPK Phosphorylation in Breast Muscle of Neonatal Broilers. *J. Agric. Food Chem.* 66 (29), 7684–7691. doi: 10.1021/acs.jafc.8b02557

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 01 December 2022
DOI 10.3389/fvets.2022.1032532

OPEN ACCESS

EDITED BY
Bangyuan Wu,
China West Normal University, China

REVIEWED BY
Flavio Santos,
University of São Paulo, Brazil
Yosra Ahmed Soltan,
Alexandria University, Egypt

*CORRESPONDENCE
Pedro H. V. Carvalho
pcarvalho@ucdavis.edu

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 30 August 2022
ACCEPTED 15 November 2022
PUBLISHED 01 December 2022

CITATION
Latack BC, Carvalho PHV and Zinn RA (2022) The interaction of feeding an eubiotic blend of essential oils plus 25-hydroxy-vit-D3 on performance, carcass characteristics, and dietary energetics of calf-fed Holstein steers. *Front. Vet. Sci.* 9:1032532. doi: 10.3389/fvets.2022.1032532

The interaction of feeding an eubiotic blend of essential oils plus 25-hydroxy-vit-D3 on performance, carcass characteristics, and dietary energetics of calf-fed Holstein steers

Brooke C. Latack[1], Pedro H. V. Carvalho[2]* and Richard A. Zinn[2]

[1]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Holtville, Holtville, CA, United States, [2]Department of Animal Science, University of California, Davis, Davis, CA, United States

Bans on the use of ionophores in several regions of the world has led to a need to identify alternative feed additivies to be added in cattle diets. Essential oil blends have been identified as a potential alternative to ionophores in feedlot diets. The objective of this study was to evaluate the effects of a supplemental a blend of essential oils and 25-hydroxyvitamin D3 on growth performance, energetic efficiency, and carcass characteristics in calf-fed Holstein steers. Ninety Holstein steer calves (123 ± 7 kg; 4 months old) were randomly assigned to 18 pens (5 steers/pen; 6 pens/treatment). Dietary treatments consisted of a steam-flaked corn-based diet supplemented with (DM basis): (1) no additives (CON); (2) 30 mg/kg DM of monensin (MON); (3) 200 mg/kg DM of a mixture of essential oils plus 25-hydroxyvitamin D3 (EO+HYD). There were no treatment effects ($P > 0.05$) on initial, intermediate and final cattle live weight; moreover, cattle had similar ($P > 0.05$) average daily gain (ADG) and dry matter intake (DMI) among dietary treatments. However, during the first 112 days of feed, calf-fed Holstein steers supplemented with EO+HYD had a greater ($P \leq 0.05$) gain to feed ratio (G/F) than cattle fed the control diet but similar ($P > 0.05$) G/F to cattle supplemented with MON. However, there was no effect ($P > 0.05$) of dietary treatments on 112 to 286 d and the overall G/F ratio of calf-fed Holstein steers. Calf-fed Holstein steers supplemented with EO+HYD had greater ($P \leq 0.05$) estimated net energy for maintenance (NEm) and net energy for gain (NEg) based on cattle growth performance than cattle fed the CON diet. Cattle supplemented with MON had an intermediate and similar ($P > 0.05$) NEm and NEg compared to the other two dietary treatments. However, when observed vs. expected NEm and NEg were calculated, cattle supplemented with MON and EO+HYD had greater efficiency of dietary energy utilization than cattle fed the CON diet. Calf-fed Holstein steers supplemented with MON had greater ($P < 0.05$) fat thickness than EO+HYD supplemented steers, and both were intermediate ($P \geq 0.05$) to that of cattle fed the CON diet. There were no other

effects ($P > 0.05$) on kidney, pelvic and heart fat, longissimus area, marbling score, and retail yield. The health status of cattle and liver abscesses or liver scars at slaughter were similar ($P > 0.05$). We conclude that supplementing calf-fed Holstein steers with MON or EO+HYD for over 285 days increased dietary net energy utilization for maintenance and gain of the diet by 3 and 4%, respectively, compared to non-supplemented steers.

KEYWORDS

essential oil, monensin, cattle, feedlot, Holstein

Introduction

Monensin (MON) is among the most commonly used ionophores in the growing-finishing diets for feedlot cattle (1). Enhancements in gain efficiency and digestive function (2) are partially attributable to selective action on the gram-positive bacteria in the rumen, potentially decreasing ruminal acetate:propionate molar ratio and methane energy losses (3, 4), and reduction in maintenance energy requirements (5). However, the present ban on ionophore supplementation within the European Union (6), and the potential trend elsewhere, have led to the quest for alternatives to conventional antibiotic supplements. Among those alternatives are plant-extracted essential oils. As with the ionophores, essential oil (EO) supplementation may reduce acetate:propionate molar ratio, methane production, and ruminal protein degradation (7). Moreover, recent studies have reported that growing-finishing cattle fed high energy diets supplemented with EO or MON had similar growth performance and carcass characteristics effects (8–10). However, the broader antimicrobial activities of EO within the rumen (7, 11, 12) may also lead to negative responses in organic matter digestion and cattle growth performance.

Another alternative feed additive that has been recently explored in the literature is the supplementation of 25-hydroxy-vitamin-D3 (HYD).

It has been reported that cattle supplementation with HYD increased average daily gain (13), carcass weight (14), and dressing percentage (15) compared to cattle receiving a diet without supplementation of HYD. However, these studies did not aimed to include an EO plus HYD in their dietary treatments. Crina® Ruminants is a eubiotic blend composed of thymol, eugenol, limonene, and vanillin, while HyD® is the 25-hydroxyvitamin D3, an active metabolite of vitamin D3 (DSM Nutritional Products, El Salto, Jalisco, MX) for 286 days. Therefore, the effect of the combination of EO + HYD on cattle growth performance and carcass characteristics of calf-fed Holstein steers that stay on feed for long periods is still lacking.

According to the latest National Beef Quality Assurance Audit, the percent of Holstein steers fed for slaughter in the United States increased around 60% from 2011 to 2016 (16, 17). In 2016, Holsteins represented 16% of total cattle fed for slaughter in the U.S. (17). Calf-fed Holstein steers enter the feedlot at characteristically light weights (115–180 kg) represents most of the cattle fed in southwest desert region in the U.S., where they are fed for periods typically over 285 days (18, 19). However, studies evaluating the effects of EO + HYD supplementation on the growth performance of calf-fed Holstein steers fed grain-based diets over 285 days are limited. Therefore, the objective of this study was to evaluate the interaction of supplemental Crina® Ruminants and HyD® (EO+HYD) on growth performance, energetic efficiency, and carcass characteristics in calf-fed Holstein steers fed a conventional growing-finishing diet.

Materials and methods

All procedures involving animal care and management were in accordance with and approved by the University of California, Davis, Animal Use and Care Committee (protocol # 18811 and 18812).

Ninety Holstein steer calves (123 ±7 kg; 4 months old) originating from Tulare, California, were received at the University of California Desert Research Center, Holtville, CA. Upon arrival, calves were vaccinated for IBR, BVD, PI_3, and B.R.S.V. (Bovi-shield® Gold 4, Zoetis Animal Health, New York, NY), clostridial (UltraChoice® 8, Zoetis Animal Health, New York, NY), gram-negative septicemic diseases (Endovac-Beef; IMMVAC, Inc. Columbia, MO), treated for parasites (Dectomax® Injectable, Zoetis Animal Health, New York, NY) and injected (S.C.) with 400 mg Tulathromycin (Draxxin, Zoetis, Kalamazoo, MI). Steers were randomly assigned to 18 pens (5 steers/pen). Pens were 79 m^2 with 27 m^2 overhead shade, automatic waterers and 4.22 m fence-line feed bunks. During the initial 28-d adjustment period, all calves were fed the same non-supplemented (control) basal diet. On d 28, calves received Endovac-Beef and Ultra Choice 8 booster vaccinations, injected with 1,000,000 IU vitamin A (Vitamin AD, Huvepharma, Inc., St. Joseph, MO), weighed, and the trial was initiated. Dietary treatments are shown in Table 1, consisting of a steam-flaked

TABLE 1 Composition of experimental diets (DM basis).

Item	Basal diet[a]
Ingredient composition, % DM	
Sudangrass hay	8.00
Alfalfa hay	4.00
Tallow	2.50
Molasses, cane	4.00
Distillers Grains w/solubles	17.50
Steam flaked corn	61.07
Urea	0.80
Dicalcium phosphate	0.15
Limestone	1.56
Magnesium oxide	0.12
TM Salt[b]	0.30
Dry matter, %	89.2
Net energy for maintenance, Mcal/kg	2.20
Net energy for gain, Mcal/kg	1.53
Crude protein, %	14.9
Rumen degradable intake protein, %	60.0
Rumen undegradable intake protein, %	40.0
Ether extract, %	7.17
Ash, %	5.89
Nonstructural CHO, %	53.9
Neutral Detergent Fiber, %	20.5
Calcium, %	0.80
Phosphorus, %	0.40
Potassium, %	0.83
Magnesium, %	0.28
Sulfur, %	0.20

[a]Basal diet supplemented with: CON: control no antibiotic; MON, supplemented with 30 mg/kg (0.018% of diet, DM basis) monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN); EO+HyD: 200 mg/kg DM (0.018% of diet, DM basis) Crina® (dosage 0.1g/kg D.M.) plus HYD® (dosage 0.1mg/kg D.M.). Crina® + (D.S.M. Nutritional Products) is a eubiotic blend composed of thymol, eugenol, limonene, and vanillin combined with HyD® (25-hydroxyvitamin D3) supplemented to provide.
[b]Trace mineral salt contained: CoSO4, 0.068%; CuSO4, 1.04%; FeSO4, 3.57%; ZnO, 0.75%; MnSO4, 1.07%; KI, 0.052%; and NaCl, 93.4%.

corn-based diet supplemented with (DM basis): (1) no additives; (2) 30 mg/kg DM of monensin; (3) 200 mg/kg DM of a mixture of Crina® (dosage 0.1g/kg DM) plus HyD® (dosage 0.1 mg/kg DM). Diets were prepared weekly and stored in plywood boxes in front of each pen. Steers were allowed *ad libitum* access to water and dietary treatments. Fresh feed was provided daily. On days 112 and 224, all steers were injected subcutaneously with 500,000 IU vitamin A (Vital E-A + D, Stuart Products, Bedford, TX) and implanted with Revalor-S (Intervet, Millsboro, DE). The health status of cattle was monitored daily by trained personnel for signs of illness or pinkeye. Cattle with signs of illness were pulled out, classified as morbid, and treated with an antimicrobial if the rectal temperature was ≥39.5°C. Antimicrobial treatments were conducted following a veterinarian's recommendation. A post-treatment interval of 3 days was implemented after the first and second treatments. If cattle remained morbid after the third treatment and the prognosis of a full recovery was unlikely, cattle were removed from the study.

Steer full body weight (BW) was recorded every 28 days until the end of the experiment (day 286) to monitor live weight changes. Steers were not denied feed or water before weighing. In the determination of average daily gain (ADG), interim and final weights were reduced by 4% to account for digestive tract fill (20). On June 21, 18 steers (1 steer per pen) were orally administered a SmaX-tec intraruminal boluse. SmaX-tec animal care technology® enables the continuous (every 10 min) real-time display of ruminal temperature. The data were measured with the help of specific antennas (smaX-tec animal care technology®, Graz, Austria). From July 12 through December 6, respiration rate measures (breaths per minute) for these same steers, were observed weekly at 1,100 h (5 h following the morning feeding) by trained personnel.

Carcass measurements

Hot carcass weights were obtained from all steers at slaughter (286 days on trial). After carcasses were chilled for 48 h, the following measurements were obtained: (1) longissimus muscle area (ribeye area), taken by direct grid reading of the muscle at the twelfth rib; (2) subcutaneous fat over the ribeye muscle at the twelfth rib taken at a location 3/4 the lateral length from the chine bone end; (3) kidney, pelvic and heart fat (KPH) as a percentage of hot carcass weight, and (4) marbling score (21). Assessment of liver scaring and liver scores were obtained from all steers at the time of slaughter.

Estimation of dietary net energy (NE)

Daily energy gain (EG; Mcal/d) was calculated by the equation: $EG = ADG^{1.097}\ 0.0557W^{0.75}$, where W is the mean shrunk B.W. (kg; (22)) Maintenance energy (EM) was calculated by the equation: $EM = 0.086W^{0.75}$. Dietary net energy for gain (NEg) was derived from net energy for maintenance (NEm) by the equation: NEg = 0.877 NEm – 0.41 (5). Dry matter intake (DMI) is related to energy requirements and dietary NEm according to the equation: DMI = (EM/NEm) + (EG/(0.877NEm – 0.41). From this relationship, dietary NE can be resolved by means of the quadratic formula: $x = (-b - \sqrt{}b2 - 4ac) / 2c$, where: x = NEm, $a = -0.42$ EM, $b = 0.887$ EM + 0.41 DMI + EG, and $c = -0.887$ DMI (23).

Weather measurement and temperature and humidity index (THI) estimation

Climatic variables (ambient temperature and relative humidity) were obtained every hour from an on-site weather station (California Irrigation Management Information System; Meloland Station) throughout the experimental period. The temperature humidity index (THI) was calculated using the following formula THI = (0.8 × Ta) + [(H/100) × (Ta – 14.4)] + 46.4, where Ta is air temperature (°C) and H is relative humidity (24, 25); Min = minimum; Max = maximum.

Statistical design and analysis

The trial was analyzed as a completely random design, using pens as experimental units. Treatment effects were separated using Fisher's Least Significant Difference test. Treatment effects were considered significant when $P \leq 0.05$ and were identified as trends when $P > 0.05$ and ≤ 0.10. (Stastistix 10, Analytical Software, Tallahassee, FL).

Results

Treatment effects on growth performance and dietary NE are shown in Table 2. There were no treatment effects ($P > 0.05$) on initial, intermediate (112 d), and final (286 d) live weight. Holstein steers supplemented with MON or EO+HYD had similar ($P > 0.05$) ADG and DMI throughout the entire (1 – 286 d) feeding period. However, during the first 112 days on feed, steers supplemented with EO+HYD had a greater ($P \leq 0.05$) gain to feed ratio (G/F) than cattle fed the control diet, but were not different from cattle supplemented with MON ($P > 0.05$). There were no treatment effects ($P > 0.05$) from 112 to 286 d or the overall G/F ratio.

The greater results of G/F for EO+HYD vs. CON calves during the initial 112-d feeding period was also reflected by enhanced efficiency of dietary energy utilization (Table 2). Steers supplemented with EO+HYD had greater ($P \leq 0.05$) estimated NEm and NEg based on cattle growth performance than cattle fed the control diet. The effects of MON supplementation on the efficiency of energy utilization were intermediate ($P > 0.05$) to that of CON and EO+HYD treatments. The overall observed vs. expected dietary NEm and NEg were greater for MON and EO+HYD than for CON. Observed NE for steers fed the Control diet was in close agreement with expected (OBS/EXP NEm and NEg were 0.99), whereas estimated dietary NE for steers supplemented with MON or EO+HYD exceeded expectations throughout the feeding period.

Treatment effects on carcass characteristics are shown in Table 3. Calf-fed Holstein steers supplemented with MON had greater fat thickness as measured over the longissimus than EO+HYD supplemented steers, and both were intermediate ($P \geq 0.05$) to cattle fed the CON diet. There were no other treatment effects ($P > 0.05$) on KPH, longissimus area, marbling score, and retail yield. The health status of cattle in the current study, as well as liver abscess and liver scars at slaughter, were similar ($P > 0.05$) across treatments (Table 3).

Monthly average THI and maximum and minimum temperature during the 286-d feeding period are presented in Figure 1. During July and August of 2021, the average THI exceeded 80, an ambient condition classified as "danger" Brown-Brandl et al. (26). Feeding EO+HYD to calf-fed Holstein steers under these ambient conditions decreased ($P < 0.05$) mean rumenal temperature compared to cattle fed CON or MON diet (Figure 2). There were no major treatment effects on cattle respiration rate (Figure 3). However, it is worth pointing out that regardless of treatment, cattle respiration rate was above "danger" designation (26) during July and August, and were highly correlated with THI measurements.

Discussion

Consistent with the current study (Table 2), Meyer et al. (8) reported that crossbred yearling beef steers fed grain-based diets for 115 days and supplemented with a similar EO had similar overall ADG to the control non-supplmented and MON supplemented diet. However, different than the current experiemnt these authores observed that compared with non-supplemented control and EO supplemented cattle, cattle supplemented with MON decreased DMI, but greater G/F compared to non-supplemtend cattle (8). Meschiatti et al. (9) compared the effects of supplementing EO in a grain-based diet fed to Nellore bulls, and observed that supplemental MON decreased DMI but did not appreciably affect ADG or G/F when compared to diets supplemented with only an EO blend (9). In a similar study, Gouvea et al. (10) observed a similar increase in DMI for cattle supplemented with a blend of EO compared to cattle supplemented with MON, though cattle supplemented with a blend of EO were also supplemented with amylase. Moreover, Toseti et al. (27) observed similar G/F in Nellore bulls fed grain-based diets supplemented with EO or MON. However, authors reported that whereas bulls fed EO had similar DMI, both ADG and final BW were greater for bulls supplemented with EO than for bulls supplemented with MON (27).

Although, results comparing MON vs. EO have been inconsistent. In agreement with the current study (Table 2), Meschiatti et al. (9) and Gouvea et al. (10) observed that cattle fed EO or MON had similar estimated dietary energy utilization, but greater than non-supplemented cattle. Conversely to the current study, Mendoza-Cortéz et al. (28) reported that Zebu-British bulls fed a grain-based diet in high ambient temperature supplemented with EO+HYD had greater efficiency of dietary energy utilization of the diet compared with bulls supplemented

TABLE 2 Influence of feeding an eubiotic blend of essential oils plus 25-hydroxy-vit-D3 on growth-performance of calf-fed Holstein steers.

	Dietary treatments[1]			SEM
	CON	MON, 30 mg/kg	EO+HYD, 200 mg/kg	
Weight, kg				
Initial	153.8	155.7	156.3	3.3
112 d	326.4	331.2	333.8	5.4
286 d	583.5	595.9	599.4	7.8
Average daily gain (kg)				
1–112 d	1.55	1.58	1.59	0.031
112–286 d	1.47	1.51	1.52	0.035
1–286 d	1.50	1.54	1.55	0.021
Dry matter intake (kg/d)				
1–112 d	6.66	6.62	6.57	0.137
112-286 d	9.23	9.22	9.30	0.213
1-286 d	8.15	8.13	8.16	0.155
Gain to feed ratio				
1–112 d[2]	0.233[b]	0.239[ab]	0.244[a]	0.003
112-286 d	0.159	0.164	0.163	0.003
1-286 d	0.184	0.190	0.190	0.002
Net energy for maintenance (NEm), Mcal/kg				
1–112 d[2]	2.02[b]	2.07[ab]	2.10[a]	0.021
112-286 d	2.23	2.29	2.29	0.023
1-286 d[2]	2.19[b]	2.26[a]	2.26[a]	0.020
Net energy for gain (NEg), Mcal/kg				
1–112 d[2]	1.36[b]	1.40[ab]	1.43[a]	0.019
112-286 d	1.55	1.60	1.59	0.020
1-286 d[2]	1.51[b]	1.57[a]	1.58[a]	0.017
Observed/expected NEm				
1–112 d[2]	0.91[b]	0.94[ab]	0.95[a]	0.009
112-286 d	1.01	1.04	1.04	0.010
1-286 d[2]	0.99[b]	1.02[a]	1.02[a]	0.009
Observed/Expected NEg				
1–112 d[2]	0.89[b]	0.92[ab]	0.94[a]	0.012
112-286 d	1.01	1.05	1.05	0.013
1-286 d[2]	0.99[b]	1.03[a]	1.03[a]	0.012

[1] Treatments: CON: control no antibiotic; MON, monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) and Crina® + (D.S.M. Nutritional Products) is a eubiotic blend composed of thymol, eugenol, limonene, and vanillin combined with HyD® (25-hydroxyvitamin D3) supplemented to provide 200 mg/kg DM Crina® (dosage 0.1g/kg D.M.) plus HyD® (dosage 0.1mg/kg D.M.).
[2] Means in a row with different superscripts differ ($P \leq 0.05$).

with MON. Authors attributed the potential bennefícts of supplementing EO+HYD to a greater DMI intake observed in cattle receiving EO+HYD compared to cattle in the MON (28). Moreover, the greater ADG observed by Mendoza-Cortéz et al. (28), could be attributed to potential effect that supplementing HYD would have on net protein retention (lean tissue growth), previous reported in the literature (14). However, in contrast with the present study, none previously mentioned studies had a negative (non-supplemented) control diet.

Consistent with similar studies, there was no effect of MON or EO on major carcass characteristcs (Table 3). In the current study dressing percentage, KPH, longissiumus area, marbling score, or yield grade, though the studies did not include a control diet with no feed additive (8–10). Meschiatti et al. (9) did not observe any difference between EO and MON treatments on the specific carcass characteristics listed, though, conversely to this study, it was reported that cattle had an increased HCW when supplemented with EO + amylase compared to MON.

TABLE 3 Influence of feeding an eubiotic blend of essential oils plus 25-hydroxy-vit-D3 on carcass characteristics and health score of calf-fed Holstein steers.

	Dietary treatment[1]			SEM
	CON	MON, 30 mg/kg	EO+HYD +, 200 mg/kg	
Hot carcass weight, kg	360.3	362.9	370.9	6.85
Dressing percentage	61.7	60.9	61.9	0.50
Kidney, pelvic and heart fat,[3] %	3.43	3.41	3.37	0.07
Fat thickness[2], cm	0.76[ab]	0.85[a]	0.68[b]	0.06
Longissimus area,[3] cm[2]	79.4	79.9	80.1	1.37
Marbling score[4]	5.40	5.88	5.71	0.26
Calculated yield grade	2.99	3.07	2.97	0.15
Pinkeye, %	13.33	7.50	6.67	5.33
Morbidity, %	6.67	10.00	6.67	6.02
Liver abscess, %	0.03	0.03	0.10	0.05
Liver abscess scars, %	20.0	17.5	30.0	6.80

[1] Treatments: CON: control no antibiotic; MON, monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN) and Crina® + (DSM. Nutritional Products) is a eubiotic blend composed of thymol, eugenol, limonene, and vanillin combined with HyD® (25-hydroxyvitamin D3) supplemented to provide 200 mg/kg DM Crina® (dosage 0.1g/kg D.M.) plus HyD® (dosage 0.1mg/kg D.M.).
[2] Means in a row with different superscripts differ ($P \leq 0.05$).
[3] Kidney, pelvic and heart fat fat as a percentage of carcass weight.
[4] Coded: minimum slight, 3.0, minimum small, 4.0, minimum modest, 5.0, minimum moderate, 6.0, and so on.

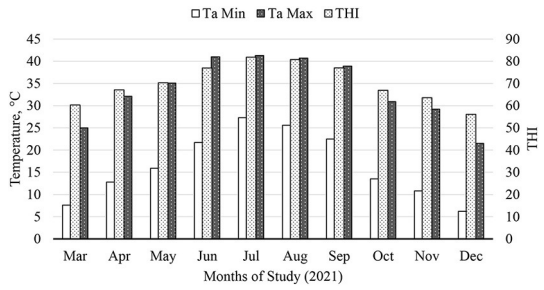


FIGURE 1
Temperature-humidity index (THI) during the course of the 286-d feeding period: THI = (0.8 × Ta) + [(H/100) × (Ta − 14.4)] + 46.4, where Ta is air temperature (°C) and H is relative humidity (Thom, 1959; NOAA, 1976); Min, minimum; Max, maximum.

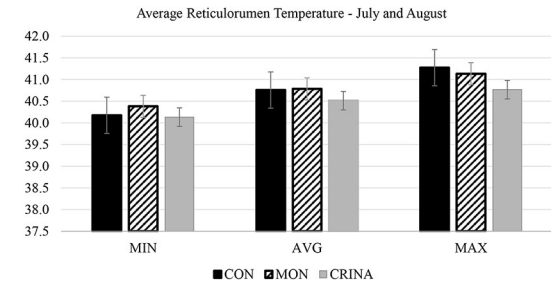


FIGURE 2
Average reticulorumen temperature during July and August.

In contrast to similar studies, the current study reported an increase in fat thickness over the longissimus area for cattle supplemented with EO + HyD compared to MON while the control treatment was intermediary. As previously mentioned in the current manuscript, previous research has reported that cattle supplemented with HYD could have greater lean tissue growth due to greater net protein retention (14), this could be the reason for decrease in fat thickness observed in the current study. However, there were no differences among treatments in other major carcass characteristcs. Therefore, authors in the current experiment recommend that more research needs to be conducted to elucidate the effects of EO+HYD on the carcass composition of cattle.

Converse to the current study where authors did not observed treatment effects in percentage of liver abscess or liver abscess scars (Table 3). Meyer et al. (8) reported that cattle supplemented with MON + Tylosin and EO + Tylosin decrease, and cattle supplemented EO alone tended to decrease liver abscess incidence compared to the control (6.5, 8.6, 16.6, and 27.2%, respectively). Although previous research has reported that calf-fed Holstein steers have a greater incidence of liver abscess than native beef breed (29), studies conducted at our laboratory (19, 30) have reported lower incidences (<10%) of liver abscess in calf-fed Holstein (more characteristic of the southwest desert region in the

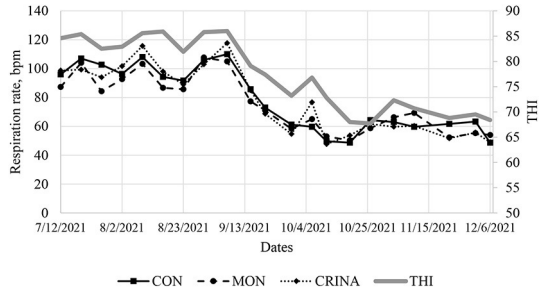


FIGURE 3
Weekly cattle respiration rate (1,100 h) and temperature-humidity index from July through December.

United States) than previous research. Although, previous research has reported that the majority of respiratory diseases in the feedlot occurred within the weeks days after cattle arrival (31), which may impact cattle growth performance. Therefore, potentially explanning the greater G/F efficiency observed in the first 112 days on feed for cattle supplemented with EO+HYD and MON, compared to non-supplemented cattle. There were no effect of cattle supplementation on pikeye incidence or cattle morbidity in the current study. Moreover, consistent with the present study (Figure 2), Silva et al. (32) also observed that lactating dairy cows supplemented with a blend of EO had reduced frequency of high rectal temperature and increased blood oxygenation. However, more research needs to be conducted to illustrate the mechanism of this potential benefit of EO and EO+HYD to cattle health and confort when animals are raised under high-ambient temperatures.

Conclusion

Supplementation of calf-fed Holstein steers with MON or EO+HYD increases the overall efficiency of dietary net energy utilization for maintenance and gain (3 and 4%, respectively) without major effects on carcass characteristics or liver abscess incidence when fed to calf-fed Holstein steers for over 285 days. Moreover, cattle supplemented with EO+HYD decreased mean reticulorumen temperature when experiencing extremely high ambient temperature.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All procedures involving animal care and management were in accordance with and approved by the University of California, Davis, Animal Use and Care Committee (protocol # 18811 and 18812).

Author contributions

All authors were involved in study design, data collection, data analysis, manuscript preparation, and approved the submitted version.

Funding

This project was supported through the University of California Agricultural Experiment Station with Hatch funding from the USDA National Institute of Food and Agriculture (CA-D-ASC-6578-H), California Department of Food and Agriculture, and financial contributions from DSM, Inc.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Samuelson KL, Hubbert ME, Galyean ML, Loest CA. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. *J Anim Sci.* (2016) 94:2648–63. doi: 10.2527/jas.2016-0282

2. Goodrich RD, Garrett JE, Gast DR, Kirick MA, Larson DA, Meiske JC. Influence of monensin on the performance of cattle. *J Anim Sci.* (1984) 58:1484–98. doi: 10.2527/jas1984.5861484x

3. Russell JB, Strobel JH. Effects of additives on in vitro ruminal fermentation: a comparison of monensin and bacitracin, another gram-positive antibiotic. *J Anim Sci.* (1988) 66:552–8. doi: 10.2527/jas1988.662552x

4. Zinn RA, Plascencia A, Barajas R. performance and digestive function Interaction of forage level and monensin in diets for feedlot cattle on growth. *J Anim Sci.* (1994) 72:2209–15. doi: 10.2527/1994.7292209x

5. Zinn RA. Influence of lasalocid and monensin plus Tylosin on comparative feeding value of steam-flaked versus dry-rolled corn diets for feedlot cattle. *J Anim Sci.* (1987) 65:256–66. doi: 10.2527/jas1987.651256x

6. European Commission. Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on Additives for Use in Animal Nutrition. *Off J Eur Union.* (2003) L:L268/29–43.

7. Cobellis G, Trabalza-Marinucci M, Yu ZT. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: a review. *Sci Total Environ.* (2016) 545:556–68. doi: 10.1016/j.scitotenv.2015.12.103

8. Meyer NF, Erickson GE, Klopfenstein TJ, Greenquist MA, Luebbe MK, Williams P, et al. Effect of essential oils, Tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. *J Anim Sci.* (2009) 87:2346–54. doi: 10.2527/jas.2008-1493

9. Meschiatti MAP, Gouvêa VN, Pellarin LA, Batalha CDA , Biehl MV, Acedo TS, et al. Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle. *J Anim Sci.* (2019) 97:456–71. doi: 10.1093/jas/sky415

10. Gouvea VN, Meschiatti MAP, Moraes JMM, Batalha CDA, Dorea JRR, Acedo TS, et al. Effects of alternative feed additives and flint maize grain particle size on growth performance, carcass traits and nutrient digestibility of finishing beef cattle. *J Agr Sci.* (2019) 1–13. doi: 10.1017/S0021859619000728

11. Patra AK. Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. *Asian J Anim Vet Adv.* (2011) 6:416–28. doi: 10.3923/ajava.2011.416.428

12. Khiaosa-Ard R, Zebeli Q. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. *J Anim Sci.* (2013) 91:1819–30. doi: 10.2527/jas.2012-5691

13. Gouvêa VN, Vasconcellos GS, Acedo TS, Tamassia LF. The 25-hydroxyvitamin D3 supplementation improves animal performance of Nellore cattle grazed in tropical grass. *J Anim Sci.* (2019) 97:161. doi: 10.1093/jas/skz258.331

14. Carvalho VV, Perdigão A. Supplementation of 25-hydroxyvitamin-D3 and increased vitamin E as a strategy to increase carcass weight of feedlot beef cattle. *J Anim Sci.* (2019) 97:440. doi: 10.1093/jas/skz258.871

15. Acedo TS, Gouvêa VN, Vasconcellos GSFM, Arrigoni M, Martins CL, Millen DD, et al. Effect of 25-hydroxyvitamin-D3 on feedlot cattle. *J Anim Sci.* (2019) 96:447–8. doi: 10.1093/jas/sky404.977

16. Moore MC, Gray GD, Hale DS, Kerth CR, Griffin DB, Savell JW, et al. National Beef Quality Audit-−2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. *J Anim Sci.* (2012) 90:5143–5151. doi: 10.2527/jas.2012-5550

17. Boykin CA, Eastwood LC, Harris MK, Hale DS, Kerth CR, Griffin DB, et al. National Beef Quality Audit-2016: In-plant survey of carcass characteristics related to quality, quantity, and value of fed steers and heifers. *J Anim Sci.* (2017) 95:2993–3002. doi: 10.2527/jas2017.1543

18. Carvalho PHV, Latack BC, Buenabad L, Zinn RA. Influences of implant hormone strategy on growth performance of calf-fed Holstein steers fed in excess of 300 days. *Applied Animal Science.* (2021) 37:199–206. doi: 10.15232/aas.2020-02123

19. Latack BC, Buenabad L, Carvalho PH, Montano MF, Zinn RA. Influences of timing of liver abscess control and early metabolizable amino acid supplementation on feedlot growth-performance and digestive function of calf-fed Holstein steers. *Applied Animal Sci.* (2021) 37:533–42. doi: 10.15232/aas.2021-02184

20. NASEM. *Nutrient Requirements of Beef Cattle 8th ed*. Washington, DC: Natl. Acad. Press (2016)

21. USDA. *United States Standards for Grading of Carcass Beef*. Washington, DC: Agricultural Marketing Service, USDA (1997).

22. NASEM. *Nutrient Requirements of Beef Cattle. 6th ed*. Washington, DC: Natl. Acad. Press (1984).

23. Zinn RA, Shen Y. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. *J Anim Sci.* (1998) 76:1280–9. doi: 10.2527/1998.7651280x

24. Thom EC. The discomfort index. *Weatherwise.* (1959) 12:57–61. doi: 10.1080/00431672.1959.9926960

25. NOAA (National Oceanic and Atmospheric Administration). *Livestock Hot Weather Stress. Regional Operations Manual Letter C-31–76.* Central Region, Kansas City, MO: US Dept Commerce, Natl Weather Serv (1976).

26. Brown-Brandl TM, Eigenberg RA, Nienaber JA, Hahn GL. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, Part 1: Analyses of indicators. *Biosyst Eng.* (2005) 90:451–62. doi: 10.1016/j.biosystemseng.2004.12.006

27. Toseti LB, Goulart RS, Gouvêa VN, Acedo TS, Vasconcellos GS, Pires AV, et al. Effects of a blend of essential oils and exogenous α-amylase in diets containing different roughage sources for finishing beef cattle. *Anim Feed Sci Technol.* (2020) 269:114643. doi: 10.1016/j.anifeedsci.2020.114643

28. Mendoza-Cortéz DA, Ramos-Méndez JL, Arteaga-Wences Y, Félix-Bernal A, Estrada-Angulo A, Castro-Pérez BI, et al. Influence of a supplemental blend of essential oils plus 25-hydroxy-vitamin-D3 on feedlot cattle performance during the early-growing phase under conditions of high-ambient temperature. *Indian J Anim Res.* (2022) 1:6. doi: 10.18805/IJAR.BF-1520

29. Amachawadi RG, Nagaraja TG. Liver abscesses in cattle: a review of incidence in holsteins and of bacteriology and vaccine approaches to control in feedlot cattle. *J Anim Sci.* (2016) 94:1620–32. doi: 10.2527/jas.2015-0261

30. Carvalho PHV, Latack BC, Flores R, Montano MF, Zinn RA. Interaction of early metabolizable protein supplementation and virginiamycin on feedlot growth performance and carcass characteristics of calf-fed Holstein steers. *Transl Anim Sci.* (2022) 6:1–6. doi: 10.1093/tas/txab228

31. Hollis LC. *Investigating Feedlot Respiratory Disease Outbreaks, Chapter 128 - Food Animal Practice (Fifth Edition)*,ed. W.B. Saunders (2009). p. 673–6.

32. Silva RB, Pereira MN, Araujo RC, Silva WR, Pereira RAN, A. blend of essential oils improved feed efficiency and affected ruminal and systemic variables of dairy cows. *Transl Anim Sci.* (2019) 4:182–93. doi: 10.1093/tas/txz183

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 24 January 2023
DOI 10.3389/fvets.2023.1067434

OPEN ACCESS

EDITED BY
Arda Yildirim,
Gaziosmanpaşa University, Türkiye

REVIEWED BY
Carolina Tobias Marino,
Empresa Brazileira de Pesquisa Agropecuária (EMBRAPA), Brazil
Eman Elwakeel,
Alexandria University, Egypt

*CORRESPONDENCE
Danilo D. Millen
✉ danilo.millen@unesp.br

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 11 October 2022
ACCEPTED 05 January 2023
PUBLISHED 24 January 2023

CITATION
Rigueiro ALN, Pereira MCS, Silvestre AM, Pinto ACJ, Felizari LD, Dias EFF, Demartini BL, Estevam DD, Dellaqua JVT, Souza KLR, Silva LAF, Nunes ABPC, Souza JM and Millen DD (2023) Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on feedlot performance, feeding behavior, carcass, rumen, and cecum morphometrics characteristics of Nellore cattle. *Front. Vet. Sci.* 10:1067434. doi: 10.3389/fvets.2023.1067434

Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on feedlot performance, feeding behavior, carcass, rumen, and cecum morphometrics characteristics of Nellore cattle

André L. N. Rigueiro[1], Murilo C. S. Pereira[1], Antonio M. Silvestre[1], Ana Carolina J. Pinto[1], Luana D. Felizari[1], Evandro F. F. Dias[2], Breno L. Demartini[1], Daniela D. Estevam[1], João V. T. Dellaqua[1], Katia L. R. Souza[1], Leandro A. F. Silva[2], Ana B. P. C. Nunes[2], Johnny M. Souza[2] and Danilo D. Millen[2]*

[1]Department of Breeding and Animal Nutrition, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, Brazil, [2]Department of Animal Production, College of Agricultural and Technological Sciences, São Paulo State University (UNESP), Dracena, Brazil

Feed additives such as monensin (MON) and virginiamycin (VM) are widely used in feedlots diets to maximize rumen fermentation. However, the knowledge about the effects of MON and VM combinations in specifics feedlot periods and the benefits of this association are still unclear. This study aimed to evaluate the effects of withdrawal of MON when associated with VM during the adaptation and finishing periods on feedlot performance of Nellore cattle. The experiment was designed as a completely randomized block replicated six times (four animals/pen) in which 120 Nellore bulls (378.4 ± 24.4 kg) were allocated in 30 pens and fed for 112 days according to the following treatments: (T1) MON during the entire feeding period; (T2) VM during the entire feeding period; (T3) MON+VM during the adaptation period and only VM during the finishing period 1 and 2; (T4) MON+VM during the entire feeding period; (T5) MON+VM during the adaptation and finishing period 1 and only VM during the finishing period 2. After 112 days on feed, no treatment effect was observed for DMI ($P \geq 0.12$). However, bulls fed T5 had greater ($P = 0.05$) final BW and ADG when compared to T1, T2, and T4. Cattle from T3 and T5 groups presented heavier HCW ($P = 0.05$) than that fed T1, T2, and T4. Nellore bulls fed T1 and T5 had lower ($P < 0.01$) DMI variation than those receiving T2. The withdrawal of MON when associated with VM during the final third of the feedlot period improved overall final BW, ADG, and HCW when compared to bulls fed either MON or VM, but did not positively impact feedlot performance when compared to cattle that had MON withdrawn at the end of the adaptation period.

KEYWORDS

antibiotic, ionophore, papillae, rumen, Zebu

1. Introduction

Feed additives such as ionophores are widely used in North American and Brazilian feedlots (1, 2). In a meta-analysis conducted by Duffield et al. (3), sodium monensin (MON) decreased dry matter intake (DMI) by 3.1% and improved the gain-to-feed (G:F) ratio by 6.4% in feedlot cattle. Also, it has been reported by the Brazilian feedlot cattle nutritionists that the use of

antibiotics associated with ionophores as a primary feed additive has become a common practice (2). The most common antibiotic that is associated with ionophores is virginiamycin (VM). Salinas-Chavira et al. (4) and Salinas-Chavira et al. (5) reported that VM improved G:F ratio by 3.83 and 4.20%, respectively when VM was fed to feedlot Holstein cattle. Both additives improved G:F ratio for feedlot cattle when fed as sole feed additives, however, only MON has been shown to decrease DMI (3).

There are few studies that evaluated the effect of MON and VM combinations for cattle, and the benefits of this association are still unclear. Erasmus et al. (6) reported a complementary effect between MON and VM when both were included in the diets of early lactation cows. However, Lemos et al. (7) did not observe any evidence that the combination of MON and VM for the entire feeding period improves feedlot performance or causes a positive impact on carcass characteristics of Zebu cattle. However, although the studies cited above have evaluated the combination between MON and VM in feedlot diets, none of them reported the combination of two feed additives in specifics periods. In the meantime, Rigueiro et al. (8) reported positive effects using different combinations of MON and VM in feedlot Nellore cattle. It was recommended by the authors that Nellore yearling bulls should be fed with diets containing MON and VM only during the adaptation period, and VM during the finishing period to improve overall feedlot performance. In a subsequent study, Rigueiro et al. (9) reported again that the use of MON and VM associated for the entire feeding period did not promote any positive effect on feedlot performance when compared to cattle fed only MON or VM. Furthermore, Rigueiro et al. (8, 9) observed that cattle fed only VM did not decrease DMI in the last 28 days on feed.

Typically, the DMI of feedlot cattle decreases in the final third of the feeding period, and this is one of the challenges feedlot cattle nutritionists have to face to keep cattle performance. One of the main factors related to DMI decrease during this period is the increase in leptin concentrations (10). According to Foote et al. (11), leptin was negatively associated with DMI. Leptin is a hormone produced by the adipocytes, and as an animal grows and approaches mature body size, fat deposition occurs as a normal part of growth (12). For this reason, nutritional strategies to increase DMI during the final third of the feedlot period have become a new research area.

Therefore, we aimed to test the hypothesis that withdrawing MON combined with a higher energy diet during the final third of the feedlot period increases DMI, and as a consequence improves feedlot performance and carcass traits of Nellore cattle. Thus, this study was designed to evaluate the effects of withdrawing MON when associated with VM during the adaptation and finishing periods on feedlot performance, feeding behavior, carcass, rumen, and cecum morphometrics characteristics of Nellore cattle.

2. Material and methods

All the procedures involving the use of animals in this study were in accordance with the guidelines established by the São Paulo State University Ethical Committee for Animal Research (protocol number CEUA 154/2016).

2.1. Animals and treatments

The trial was conducted at the São Paulo State University feedlot, Dracena campus, Brazil. One hundred and twenty 22-mo-old yearling Nellore bulls (378.44 ± 24.43 kg) were allocated in 30 pens (1.5 m of linear bunk space and 18 m^2 of pen space per animal; $n =$ 4 animals per pen) and fed for 112 days, according to the treatments: (1) MON during the entire feeding period (T1); (2) VM during the entire feeding period (T2); (3) MON + VM during the adaptation period and only VM during the finishing period 1 and 2 (T3); (4) MON + VM during the entire feeding period (T4); (5) MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2 (T5). Doses were based on Rigueiro et al. (8) when either MON (30 mg/kg of DM) or VM (25 mg/kg of DM) were fed as sole feed additives in the diet.

2.2. Feeding and management description

At the beginning of the study, all yearling bulls were dewormed (Ivermax, Dispec do Brasil, Maringá, BR). Cattle were fed *ad libitum* 3 times per day at 800 h (35% of total ration), 1,100 h (20% of total ration), and 1,600 h (45% of total ration), targeting 3 to 5% refusal with free-choice water access to a water trough. The experimental diets were formulated according to the Large Ruminant Nutrition System (LRNS; (13)) and are shown in Table 1. The step-up adaptation program consisted of *ad libitum* intake and lasted 14 days, where 3 adaptation diets containing 66, 72, and 78% concentrate were fed for 5, 4, and 5 days, respectively. The finishing period program also consisted of *ad libitum* intake and lasted 98 days, where 2 finishing diets containing 84%, and 88% concentrate were fed for 58, and 40 days, respectively.

Samples of the feed ingredients offered were analyzed for DM weekly and dietary DM was adjusted on a weekly basis according to changes in feed ingredient DM and water was added to the experimental diets to equalize the DM content by approximately 70%. Feed ingredient samples were dried in a forced-air oven for DM determination [(14); method 930.15]. Subsequently, samples were ground using a hammer mill to pass through a 1-mm screen (MA340, Marconi equipamentos para laboratórios Ltda, Piracicaba, BR) and were analyzed for ash [(14); method 942.05], crude protein [(15); method 990.02], and neutral detergent fiber (16).

2.3. Feedlot performance and carcass traits

One day before the start of the study, and every 28 days, all yearling bulls were withheld from feed for 16 h for the body weight (BW) assessment. Consequently, ADG and G:F ratio were calculated at the end of the experiment. The DMI was calculated daily by weighing the ration offered and refusal before the next morning delivery and expressed in kilograms and as a percentage of BW. The DMI variation was calculated as the difference in intake between two consecutive days throughout the study (17). Daily DMI variation was expressed as a percentage of variation. In order to estimate the net energy for maintenance (NEm) and net energy for gain (NEg), it was used the methods described by Lofgreen and Garrett (18), NRC (19), and Zinn and Shen (20).

TABLE 1 Feed ingredients and chemical composition of high-concentrate diets fed to Nellore yearling bulls ($n = 30$) during adaptation and finishing periods.

Item	Percent of concentrate				
	66	72	78	84	88
Days on feed, *n*	5	4	5	58	40
Ingredients, % of DM[a]					
Cynodon dactylon hay	20.00	14.00	4.00	2.00	2.00
Sugarcane bagasse	14.00	14.00	18.00	14.00	10.00
Corn grain fine grind	46.00	54.00	62.00	70.00	76.70
Soybean meal	17.30	15.10	12.90	10.70	8.00
Supplement[b]	1.40	1.40	1.50	1.50	1.50
Urea	0.40	0.60	0.70	0.90	0.90
Limestone	0.90	0.90	0.90	0.90	0.90
Nutrient content, % of DM[c]					
Dry matter, %	74.00	74.00	73.00	73.00	74.00
Total digestible nutrients	72.00	72.00	75.00	78.00	80.00
Crude protein	15.20	15.00	14.60	14.50	14.00
Neutral detergent fiber	34.30	30.50	26.80	23.00	19.20
Non-fiber carbohydrates	43.00	48.00	52.00	57.00	61.00
peNDF[d]	26.00	22.00	18.00	14.00	10.00
Net energy for gain, Mcal/kg	1.09	1.09	1.15	1.25	1.26
Ca	0.60	0.58	0.56	0.54	0.52
P	0.40	0.41	0.42	0.42	0.42

[a]DM: dry matter. [b]Supplement contained: Ca: 18.23%; P: 4.05%, Mg: 0.77%, K: 0.05%, Na: 8.22%, Cl: 12.65%, S: 1.60%, Co: 27.50 ppm, Cu: 757.17 ppm, Fe: 2,498 ppm, I: 37.29 ppm, Mn: 740 ppm, Se: 6.20 ppm, Zn: 1,790 ppm. Monensin (Bovensin 200; Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil) was added at 2,000 mg/kg of supplement and Virginiamycin (V-Max 2; Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil) was added at 1,666 mg/kg of supplement and offered to yearling bulls in the treatments. [c]Estimated by equations according to Large Ruminant Nutrition System (LRNS; (13)). [d]peNDF: physically effective neutral detergent fiber.

Final BW was obtained at the feedlot prior to transportation. Cattle were transported 150 km (~3 h) to a commercial abattoir. Hot carcass weight (HCW) was obtained after a kidney, pelvic, and heart fat removal. Dressing percentage was calculated by dividing HCW by the final BW. The 12 th rib fat thickness, Biceps femoris fat thickness, longissimus muscle (LM) area, and marbling were measured *via* ultrasound at the beginning and at the end of the experimental period following the method described by Perkins et al. (21). The 12th rib fat thickness daily gain, Biceps femoris fat daily gain, and LM area daily gain were calculated as the difference between the two measurements divided by days on feed. Images were collected using an Aloka SSD-1100 Flexus RTU unit (Aloka Co. Ltd., Tokyo, Japan) with a 17.2 cm, 3.5 MHz probe.

2.4. Feeding behavior and particle sorting

All yearling bulls were submitted to visual observations to evaluate feeding behavior, every 5 min, over two periods of 24 h. The visual observations were performed on days 61 (finishing period 1) and 96 (finishing period 2) according to Robles et al. (22). Feeding behavior data were recorded for each animal as follows: time spent resting, ruminating, and eating (expressed in minutes), and the number of meals per day. A meal was considered the non-interrupted time cattle stayed in the feed bunk eating the ration. Meal length in minutes was calculated by dividing time spent eating by the number of meals per day. The DMI per meal in kilograms was calculated by dividing DMI by the number of meals per day.

In addition, data on time spent eating and ruminating were used to calculate the eating rate of DM (time spent eating/DMI) and rumination rate of DM (time spent ruminating/DMI), both expressed in minutes per kilogram of DM, according to Pereira et al. (23). Samples of diets and refusals were collected for chemical analysis of NDF (16) to determine the intake of NDF on the day of feeding behavior. Eating rate of NDF was calculated by dividing the time spent eating by NDF intake. Rumination rate of NDF was determined by dividing the time spent ruminating by NDF intake. Both eating rate and rumination rate were expressed in minutes per kilogram of NDF, according to Pereira et al. (23).

Samples of diets and refusals were also collected on days 61 and 62 (finishing period 1), and 96 and 97 (finishing period 2) of the study, respectively, for the determination of particle-size distribution using the Penn State Particle Separator with aperture sizes of 19, 8, and 1.18 mm, and a pan according to Heinrichs and Kononoff (24). The particle size distribution was determined using representative 1-L samples. Physical effectiveness factor was determined as the proportion of particles retained on 3 sieves (25).

Samples of diets and orts were also collected for the determination of particle size distribution, which was performed by sieving using the Penn State Particle Size Separator and reported on an as-fed basis as described by Heinrichs and Kononoff (24). Particle sorting was determined as follows: n intake / n predicted intake, in which n = particle fraction retained on screens of 19 mm (long), 8 mm (medium), and 1.18 mm (short) and a pan (fine). Particle sorting values equal to 1 indicate no sorting. Those <1 indicate selective refusal (sorting against), and those >1 indicate preferential consumption (sorting for), according to Leonardi and Armentano (26).

2.5. Liver abscess, rumen and cecum morphometrics

Liver abscesses were classified according to incidence according to Brink et al. (27). Rumenitis evaluation was recorded after cattle evisceration, and all entire washed rumens were scored. Rumen epithelium was classified according to the incidence of lesions (rumenitis) and abnormalities (e.g., papillae clumped) as described by Bigham and McManus (28) using a scale of 0 (no lesions and abnormalities noted) to 10 (severe ulcerative lesions). All rumens were scored by 2 trained individuals, who were blinded to the treatments, and the final data represent the average of the 2 scores.

Also, a 1-cm^2 fragment of each rumen was collected from the dorsal cranial sac and placed into a PBS solution for morphometric measurements according to Resende Júnior et al. (29). Manually, the number of papillae per square centimeter of rumen wall (NOP) was determined; 12 papillae were randomly collected from each fragment and scanned, and the mean papillae area (MPA) was

determined using an image analysis system (Image Tool, version 2.01 alpha 4, UTHSCSA Dental Diagnostic Science, San Antonio, TX). The rumen wall absorptive surface area (ASA) in cm^2 was calculated as follows: 1 + (NOP × MPA) – (NOP × 0.002), where 1 represents the 1 cm^2 fragment collected and 0.002 is the estimated basal area of papillae in square centimeters. The papillae area, expressed as a percentage of ASA, was calculated as follows: (NOP × MPA)/ASA × 100.

Likewise, a 1-cm^2 fragment of each rumen was collected from the ventral cranial sac for histological assessment. Histological sections were stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned (30). Histological measurements, such as papillae height, papillae width, papillae surface area, and keratinized layer thickness were performed on 4 papillae per animal using a computer-aided light microscope image analysis. The same 1-cm^2 fragment collected from the ventral cranial sac was also used for the evaluation of cell proliferation of rumen papillae according to the immunohistochemistry method adapted from Pereira et al. (31). The slides were incubated with primary inoculum (PCNA-PC10, Dako, Glostrup, Denmark), and diluted in PBS for positive control. For the negative control, only PBS was added to the IgG Murine Anti-Mouse Isotope Control (Sigma, Saint Louis, MO, EUA) in a dark moist refrigerated chamber (4°C) overnight with a 1:500 dilution, and utilizing 70 μL per sample (for both controls). The slides were then mounted for analyses using a Leica Qwin Image Analyzer within a Leica electron light microscope. Four papillae of each animal were randomly chosen (final data represented the average of the 4 papillae) to determine the number of cell nuclei, as well as the number of proliferating cell nuclei. The cell proliferation index was expressed as a percentage and was calculated as follows: number of proliferating nuclei cells/number of cell nuclei × 100.

Cecum lesions evaluation was performed after cattle evisceration, and all washed cecum were scored. Cecum epithelium was classified according to the presence of cecal wall inflammation, lesions, and petechiae using a scale of 0 (no lesions noted) to 10 (severe lesions), according to Pereira et al. (32). All cecum were scored by 2 trained individuals, who were blinded to the treatments, and the final data represented the average of the 2 scores. In addition, a 1 cm^2 fragment was collected from the center of the cecum epithelium for histological assessment and preserved in buffered paraformaldehyde 4% solution until future histological analyses (33). For the histological analysis of cecum epithelium, tissue samples were dehydrated and embedded in paraffin wax, sectioned at 8 μm, and stained with hematoxylin and eosin. Histological measurements, such as crypt depth and goblet cells, were determined in 10% of the total number of crypts per animal, using a Leica Qwin Image Analyzer within a Leica electron light microscope.

2.6. Statistical analysis

The experimental design was a completely randomized block and the initial BW was utilized as a criterion for block formation, and the block was included in the model as a random effect. Pens were considered experimental unit for this study ($n = 30$; 4 bulls per pen), and each treatment was replicated 6 times (block 1: 338.42 kg; block 2: 358.90 kg; block 3: 371.70 kg; block 4: 386.50; block 5: 395.60 kg; block 6: 412.50 kg; SEM: 9.96). Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Tukey test to compare means. Tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and heterogeneity of treatment variances (GROUP option of SAS) were performed before analyzing the data. Results were considered significant at $P \leq 0.05$ level.

3. Results

3.1. Feedlot performance and carcass traits

Regarding the performance data, the initial BW was not affected by treatments ($P = 0.99$; Table 2). For the first 28 days on feed, there were no effects of treatments for final BW ($P = 0.10$), and DMI ($P \geq 0.35$), expressed both in kg and as a percentage of BW. However, Nellore bulls fed T5 had greater ($P \leq 0.03$) ADG and improved G:F ratio, when compared to animals receiving T2, T3, and T4. Cattle fed T2 had greater ($P = 0.02$) DMI variation than animals from other treatments. After 56 days on feed, no significant treatments effects were observed for final BW ($P = 0.11$), and DMI ($P \geq 0.49$), expressed both in kg and as a percentage of BW. However, cattle fed T5 had greater ($P = 0.05$) ADG and improved G:F ratio when compared to animals receiving T1, T2, and T4. Cattle fed T1 had lower ($P = 0.04$) DMI variation than those receiving T2 and T4.

After 72 days on feed, no significant treatments effects were observed for DMI ($P \geq 0.29$; Table 2), expressed both in kg and as a percentage of BW. However, cattle fed T5 had greater ($P = 0.04$) final BW when compared to animals receiving T1, T2, and T4. Nellore bulls fed T5 presented grater ($P = 0.02$) ADG than those receiving T2. Cattle fed T5 improved ($P < 0.01$) G:F ratio when compared to animals from other treatments. Nellore bulls fed T1 and T5 had lower ($P = 0.01$) DMI variation than those receiving T2 and T4.

After 84 days on feed, no significant treatments effects were observed for DMI ($P \geq 0.30$; Table 2), expressed both in kg and as a percentage of BW. However, Nellore bulls fed T5 had greater ($P < 0.05$) final BW and ADG when compared to animals receiving T1, T2, and T4. Cattle fed T5 improved ($P = 0.03$) G:F ratio when compared to animals receiving T1, T2, and T4. Nellore bulls fed T1 and T5 had lower ($P < 0.01$) DMI variation than animals receiving T2 and T4.

Overall, after 112 days on feed, no significant treatments effects were observed for DMI ($P \geq 0.12$; Table 2), expressed both in kg and as a percentage of BW, NEm ($P = 0.11$) and NEg ($P = 0.11$). However, bulls fed T5 had greater ($P = 0.05$) final BW and improved G:F when compared to animals receiving T2. Cattle fed T5 had greater ($P = 0.05$) ADG when compared to animals receiving T1, T2, and T4. Nellore bulls fed T1 and T5 had lower ($P < 0.01$) DMI variation than animals from other treatments.

There was no significant treatment effect ($P > 0.05$) for most of the carcass characteristics variables evaluated (Table 3). However, cattle fed T3 and T5 had heavier ($P = 0.05$) HCW when compared to other treatments. Nellore bulls fed T3, T4, and T5 increased ($P < 0.01$) final 12th rib fat and 12th rib fat daily gain when compared to other treatments. Cattle fed T3 increased ($P < 0.01$) final BF fat thickness when compared to animals receiving T1 and T2. Moreover, cattle fed T1 reduced ($P < 0.01$) BF fat daily gain when compared to others treatments.

TABLE 2 Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on feedlot performance of Nellore yearling bulls ($n = 30$) consuming high-concentrate diets.

Item[2]	Period	Treatments[1]					s.e.m.[3]	P-value
	Adaptation:	MON	VM	MONVM	MONVM	MONVM		
	Finishing 1:	MON	VM	VM	MONVM	MONVM		
	Finishing 2:	MON	VM	VM	MONVM	VM		
		(T1)	(T2)	(T3)	(T4)	(T5)		
Initial BW, kg		378.23	378.33	378.60	378.57	378.48	9.96	0.99
0–28 days								
Final BW, kg		411.44	402.68	409.52	409.57	417.60	10.75	0.10
ADG, kg		1.18^{ab}	0.87^{c}	1.10^{bc}	1.11^{bc}	1.40^{a}	0.11	0.03
Daily DMI, kg		8.62	8.58	8.72	8.29	8.58	0.35	0.59
Daily DMI, % of BW		2.06	2.10	2.13	2.06	2.09	0.03	0.47
G:F ratio, kg/kg		0.140^{ab}	0.102^{c}	0.126^{b}	0.131^{b}	0.160^{a}	0.011	0.01
DMI variation, %		7.29^{b}	9.88^{a}	7.43^{b}	7.71^{b}	7.11^{b}	0.62	0.02
0–56 days								
Final BW, kg		448.84	444.56	455.20	448.79	459.64	12.17	0.11
ADG, kg		1.26^{bc}	1.18^{c}	1.36^{ab}	1.25^{bc}	1.45^{a}	0.07	0.05
Daily DMI, kg		9.07	9.19	9.36	9.00	9.40	0.30	0.51
Daily DMI, % of BW		2.02	2.07	2.06	2.00	2.04	0.03	0.49
G:F ratio, kg/kg		0.139^{bc}	0.128^{c}	0.146^{ab}	0.139^{bc}	0.155^{a}	0.006	0.03
DMI variation, %		5.96^{c}	8.25^{a}	7.04^{abc}	7.56^{ab}	6.10^{bc}	0.57	0.04
0–72 days								
Final BW, kg		472.72^{bc}	468.24^{c}	479.84^{ab}	473.00^{bc}	485.80^{a}	11.50	0.04
ADG, kg		1.31^{bc}	1.24^{c}	1.40^{ab}	1.30^{bc}	1.49^{a}	0.05	0.02
Daily DMI, kg		9.31	9.46	9.67	9.19	9.62	0.30	0.39
Daily DMI, % of BW		1.97	2.02	2.02	1.94	1.98	0.03	0.29
G:F ratio, kg/kg		0.141^{bc}	0.132^{c}	0.145^{b}	0143^{b}	0.156^{a}	0.005	<0.01
DMI variation, %		5.48^{b}	7.57^{a}	6.67^{ab}	7.39^{a}	5.92^{b}	0.46	0.01
0–84 days								
Final BW, kg		487.40^{bc}	485.36^{c}	496.96^{ab}	488.31^{bc}	501.28^{a}	12.23	0.05
ADG, kg		1.29^{bc}	1.27^{c}	1.40^{ab}	1.30^{bc}	1.46^{a}	0.05	0.02

(Continued)

10.3389/fvets.2023.1067434

TABLE 2 (Continued)

Item[2]	Period	Treatments[1]					s.e.m.[3]	P-value
	Adaptation:	MON	VM	MONVM	MONVM	MONVM		
	Finishing 1:	MON	VM	VM	MONVM	MONVM		
	Finishing 2:	MON	VM	VM	MONVM	VM		
		(T1)	(T2)	(T3)	(T4)	(T5)		
Daily DMI, kg		9.40	9.55	9.79	9.24	9.71	0.30	0.31
Daily DMI, % of BW		1.92	1.97	1.97	1.89	1.94	0.03	0.30
G:F ratio, kg/kg		0.139^{bc}	0.133^{c}	0.144^{ab}	0.142^{bc}	0.152^{a}	0.005	0.03
DMI variation, %		5.49^{b}	7.55^{a}	6.61^{ab}	7.23^{a}	5.73^{b}	0.44	<0.01
0–112 days								
Final BW, kg		533.67^{bc}	529.25^{c}	540.79^{ab}	531.18^{bc}	548.25^{a}	13.43	0.05
ADG, kg		1.39^{b}	1.34^{b}	1.45^{ab}	1.36^{b}	1.51^{a}	0.05	0.05
Daily DMI, kg		9.50	9.69	9.97	9.27	9.94	0.31	0.14
Daily DMI, % of BW		1.78	1.83	1.83	1.74	1.81	0.03	0.12
G:F ratio, kg/kg		0.146^{ab}	0.140^{b}	0.145^{ab}	0.147^{ab}	0.153^{a}	0.004	0.05
DMI variation, %		5.26^{b}	7.13^{a}	6.93^{a}	6.74^{a}	5.46^{b}	0.41	<0.01
NEm (Mcal/kg of DM)		2.03	1.96	2.00	2.04	2.08	0.03	0.11
NEg (Mcal/kg of DM)		1.37	1.31	1.34	1.39	1.41	0.03	0.11

[1]T1 (MON during the entire feeding period); T2 (VM during the entire feeding period); T3 (MON + VM during the adaptation period and only VM during the finishing period 1 and 2); T4 (MON + VM during the entire feeding period); T5 (MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2). [2]BW, body weight; G: F, gain-to-feed ratio; AGD, average daily gain; DMI, dry matter intake; NEm, energy for maintenance; NEg, net energy for gain. [3]s.e.m.: standard error of the mean, referent to $n = 6$ pens per treatment. Values within a row with different lower case letters differ ($P < 0.05$).

10.3389/fvets.2023.1067434

TABLE 3 Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on carcass characteristics of Nellore yearling bulls ($n = 30$) consuming high-concentrate diets.

Hot carcass weight, kg		289.48[b]	289.02[b]	295,06[a]	289.12[b]	296,71[a]	7.97	0.05
Dressing percentage		54.25	54.61	54.58	54.39	54.09	0.31	0.47
Initial 12[th] rib fat, mm		2.29	2.31	2.40	2.40	2.42	0.07	0.50
Final 12[th] rib fat, mm		5.03[b]	5.26[b]	5.80[a]	5.94[a]	5.75[a]	0.20	<0.01
12[th] rib fat daily gain, mm		0.024[b]	0.025[b]	0.030[a]	0.031[a]	0.029[a]	0.001	<0.01
Initial BF[2] fat thickness, mm		4.16	4.12	4.09	4.01	4.15	0.10	0.84
Final BF fat thickness, mm		7.55[c]	8.36[b]	9.00[a]	8.50[ab]	8.57[ab]	0.24	<0.01
BF fat daily gain, mm		0.030[c]	0.037[b]	0.043[a]	0.040[ab]	0.039[ab]	0.002	<0.01
Initial LM[3] area, cm^2		63.19	61.04	60.73	62.99	59.89	1.84	0.14
Final LM area, cm^2		79.81	77.72	77.92	77.39	78.65	1.93	0.86
LM area daily gain, cm^2		0.150	0.150	0.150	0.140	0.163	0.01	0.79
Initial marbling, %		2.06	2.01	2.14	2.27	2.19	0.12	0.48
Final marbling, %		2.82	2.70	2.73	2.92	2.86	0.08	0.23

[1]T1 (MON during the entire feeding period); T2 (VM during the entire feeding period); T3 (MON + VM during the adaptation period and only VM during the finishing period 1 and 2); T4 (MON + VM during the entire feeding period); T5 (MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2). [2]BF, *Biceps femoris* muscle; LM, Longissimus muscle. [3]s.e.m., standard error of the mean, referent to $n = 6$ pens per treatment. Values within a row with different superscripts differ ($P < 0.05$).

10.3389/fvets.2023.1067434

TABLE 4 Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on feeding behavior and particle sorting at day 61 (finishing period 1) of Nellore yearling bulls (n = 30) consuming high-concentrate diets.

Item[2]	Period	Treatments[1]					s.e.m.[3]	P-value
	Adaptation:	MON	VM	MONVM	MONVM	MONVM		
	Finishing 1:	MON	VM	VM	MONVM	MONVM		
	Finishing 2:	MON	VM	VM	MONVM	VM		
		(T1)	(T2)	(T3)	(T4)	(T5)		
Feeding behavior								
Time spent resting, min		994.17^{b}	1002.08^{b}	985.00^{b}	1051.74^{a}	980.63^{b}	19.63	0.05
Time spent ruminating, min		292.50	274.17	272.71	220.14	289.37	21.87	0.16
Time spent eating, min		153.33	163.75	182.29	168.13	170.00	11.68	0.53
Meals per day, n		10.54	11.96	13.58	12.55	11.75	1.13	0.43
Meal length, min		14.91	14.04	13.62	13.69	15.12	1.05	0.70
DMI per meal, kg		0.96	0.88	0.76	0.83	0.92	0.09	0.55
DMI, kg		9.88	9.81	10.04	9.69	9.98	0.39	0.96
ER of DM[3], min/kg of DM		15.72	17.06	18.17	17.74	17.05	1.48	0.91
RR of DM, min/kg of DM		30.11	28.04	27.00	23.20	29.15	2.32	0.26
NDF intake, kg		1.80	1.54	1.93	1.72	1.85	0.21	0.53
ER of NDF, min/kg of NDF		87.62	112.04	96.70	113.87	106.30	17.76	0.38
RR of NDF, min/kg of NDF		179.26	190.06	148.77	137.61	157.27	18.30	0.20
Particle sorting[4]								
Long		0.91^{b}	0.97^{ab}	1.04^{a}	0.92^{b}	1.01^{a}	0.06	0.05
Medium		0.98	0.95	0.99	1.06	1.03	0.03	0.15
Short		1.00	1.01	1.01	1.01	1.01	0.01	0.25
Fine		1.00^{a}	1.00^{a}	0.99^{ab}	0.97^{b}	0.97^{b}	0.01	0.05

[1]T1 (MON during the entire feeding period); T2 (VM during the entire feeding period); T3 (MON + VM during the adaptation period and only VM during the finishing period 1 and 2); T4 (MON + VM during the entire feeding period); T5 (MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2). [2]DMI, dry matter intake; ER, eating rate; DM, dry matter; RR, rumination rate; NDF, neutral detergent fiber; peNDF, physically effective neutral detergent fiber. [3]s.e.m., standard error of the mean, referent to n = 6 pens per treatment. Values within a row with different lower case letters differ ($P < 0.05$). [4]Particle fraction retained on screens of 19 mm (long), 8 mm (medium), 1.18 mm (short) and a pan (fine).

10.3389/fvets.2023.1067434

TABLE 5 Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on feeding behavior and particle sorting at day 96 (finishing period 2) of Nellore yearling bulls (n = 30) consuming high-concentrate diets.

Feeding behavior								
Time spent resting, min		1,068.67[a]	1,030.96[b]	1,015.62[b]	1,054.74[ab]	1,050.50[ab]	20.27	0.01
Time spent ruminating, min		210.13	247.84	245.00	219.38	225.88	13.47	0.19
Time spent eating, min		154.38	154.38	179.38	172.71	171.46	10.06	0.30
Meals per day, *n*		10.21	9.92	11.42	9.81	10.42	0.98	0.49
Meal length, min		16.52	15.88	15.91	18.34	17.10	1.69	0.83
DMI per meal, kg		1.06	1.08	0.93	1.04	1.07	0.12	0.87
DMI, kg		9.94[ab]	10.15[a]	10.51[a]	9.26[b]	10.72[a]	0.48	0.04
ER of DM[3], min/kg of DM		15.78	15.61	17.18	19.11	16.06	1.42	0.39
RR of DM, min/kg of DM		21.51	24.57	23.52	23.87	21.15	1.44	0.26
NDF intake, kg		1.82	2.09	2.13	1.84	2.05	0.16	0.31
ER of NDF, min/kg of NDF		93.11	77.38	87.26	97.62	86.83	10.69	0.65
RR of NDF, min/kg of NDF		126.21	121.28	120.40	121.56	115.03	13.08	0.98
Particle sorting[4]								
Long		1.06	1.08	1.06	1.01	1.03	0.02	0.26
Medium		1.02	1.03	1.03	1.01	1.03	0.01	0.85
Short		1.01	1.01	1.00	1.00	1.00	0.002	0.48
Fine		0.98	0.98	0.99	0.99	0.99	0.01	0.44

[1]T1 (MON during the entire feeding period); T2 (VM during the entire feeding period); T3 (MON + VM during the adaptation period and only VM during the finishing period 1 and 2); T4 (MON + VM during the entire feeding period); T5 (MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2). [2]DMI, dry matter intake; ER, eating rate; DM, dry matter; RR, rumination rate; NDF, neutral detergent fiber; peNDF, physically effective neutral detergent fiber. [3]s.e.m., standard error of the mean, referent to $n = 6$ pens per treatment. Values within a row with different lower case letters differ ($P < 0.05$). [4]Particle fraction retained on screens of 19 mm (long), 8 mm (medium), 1.18 mm (short) and a pan (fine).

10.3389/fvets.2023.1067434

TABLE 6 Withdrawal of sodium monensin when associated with virginiamycin during adaptation and finishing periods on rumen and cecum morphometrics of Nellore yearling bulls ($n = 30$) consuming high-concentrate diets.

Item	Period	Treatments[1]					s.e.m.[3]	P-value
	Adaptation:	MON	VM	MONVM	MONVM	MONVM		
	Finishing 1:	MON	VM	VM	MONVM	MONVM		
	Finishing 2:	MON	VM	VM	MONVM	VM		
		(T1)	(T2)	(T3)	(T4)	(T5)		
Rumen measurements								
Rumenitis score		1.28^{ab}	0.99^{b}	1.08^{b}	1.44^{a}	1.01^{b}	0.12	0.05
Macroscopic variables								
Number of papillae, *n*		75.24	79.48	79.56	72.74	81.35	6.53	0.84
Mean papillae area, cm^2		0.54^{a}	0.45^{b}	0.42^{b}	0.57^{a}	0.43^{b}	0.05	<0.01
ASA[2], cm^2/cm^2 of rumen wall		38.10	34.47	34.19	41.49	35.05	2.93	0.12
Papillae area, % of ASA		97.31	97.15	96.57	97.57	97.19	0.32	0.26
Microscopic variables								
Papillae height, mm		4.40	4.27	4.30	4.50	4.53	0.28	0.89
Papillae width, mm		0.46	0.40	0.44	0.43	0.44	0.02	0.08
Papillae surface area, mm^2		1.83	1.59	1.80	1.77	1.84	0.12	0.42
Keratinized layer thickness, μm		12.33	11.86	11.70	11.60	11.91	0.40	0.78
Mitotic index, %		2.49	2.22	2.43	2.56	2.30	0.15	0.52
Mitotic index, *n*		49.71	44.33	48.67	51.13	46.08	3.15	0.52
Cecum variables								
Cecum score		2.08	2.21	2.29	1.99	1.79	0.42	0.92
Crypt depth, μm		170.63	154.23	150.65	162.77	162.77	11.20	0.42
Goblet cells, *n*		34.22	37.47	34.84	34.18	34.15	2.00	0.73

[1]T1 (MON during the entire feeding period); T2 (VM during the entire feeding period); T3 (MON + VM during the adaptation period and only VM during the finishing period 1 and 2); T4 (MON + VM during the entire feeding period); T5 (MON + VM during the adaptation and finishing period 1 and only VM during the finishing period 2). [2]ASA, absorptive surface area. [3]s.e.m., standard error of the mean, referent to $n = 6$ pens per treatment. Values within a row with different lower case letters differ ($P < 0.05$).

10.3389/fvets.2023.1067434

3.2. Feeding behavior and particle sorting

No significant treatment effect was observed ($P > 0.05$) for most of the feeding behavior and particle sorting variables evaluated after 61 days on feed (Table 4). However, Nellore bulls fed T4 spent more time resting ($P = 0.05$) when compared to other treatments. Animals fed T3 and T5 sorted more intensively for long particles when compared to animals receiving T1 and T4. In addition, cattle fed T4 and T5 sorted ($P = 0.05$) against fine particles when compared to animals receiving T1 and T2.

Also, no significant treatment effects were observed ($P > 0.05$) for most of the feeding behavior and particle sorting variables evaluated after 96 days on feed (Table 5). However, Nellore bulls fed T1 spent more time resting ($P = 0.01$) when compared to animals receiving T2 and T3. Cattle fed T2, T3, and T5 had greater ($P = 0.04$) DMI when compared to animals receiving T4.

3.3. Liver abscess, rumen and cecum morphometrics

No liver abscess was found in the cattle evaluated in this study. No treatment effect was observed ($P > 0.05$) for any of the microscopic and cecum variables evaluated (Table 6). However, Nellore bulls fed T4 presented higher rumenitis scores ($P = 0.05$) when compared to animals receiving T2, T3, and T5. No treatment effect ($P > 0.05$) was observed for any of the macroscopic variables evaluated, except for mean papillae area (MPA), where cattle fed T1 and T4 had larger ($P < 0.01$) MPA when compared to other treatments.

4. Discussion

The effect of combinations between MON and VM for cattle are unclear. Nuñez et al. (34) and Lemos et al. (7) tested combination of MON, lasalocid, VM, and flavomycin, but no positive feedlot performance effects were reported by the authors. Although the studies cited above have evaluated the combination between MON and VM in feedlot diets, none of them reported the combination of two feed additives in specifics periods. In this context, the present study was part of a larger research performed by this research group assessing the effect of different combinations of MON and VM in specifics feedlot periods. Rigueiro et al. (8) investigated different combinations of MON and VM during adaptation and finishing periods of feedlot Nellore cattle. The authors concluded that Nellore yearling bulls should be fed high-concentrate diets containing MON and VM during the adaptation period, and only VM during the finishing period to improve overall feedlot performance. Same authors also reported that cattle fed only VM during the finishing period had most of the increased performance in the last 40 days of the study. Consequently, it was hypothesized that the withdrawal of MON, when associated with VM, combined with a higher energy diet during the final third of the feedlot period would increase DMI, in order to improve feedlot performance and carcass traits.

In this context, DMI is an important indicator to evaluate how well cattle are either accepting or adapted to the diets (35), and the faster cattle reach a DMI of 2% of BW, more adapted they are to the diets. In the present study, there were no effects of treatments on DMI, expressed both in kg and as a percentage of BW, in the first 28 days on feed. Based on results described by Rigueiro et al. (8), where Nellore bulls fed VM as the sole feed additive during adaptation reached a DMI of 2% of the initial BW in 4.3 days on average, whereas those fed MON needed 20.7 days to reach a similar intake, another study of this research group was developed by Rigueiro et al. (9) where it was hypothesized that the adaptation period could be shortened to 9 days or even 6 days when VM (25 mg/kg DM) is used in finishing diets of Nellore cattle as the sole feed additive. The authors reported that, during the first 28 days on feed, the DMI decreased linearly as the adaptation was shortened for the cattle fed VM as a sole feed additive, where cattle fed VM for 14 days presented a greater DMI, expressed as % of BW, than animals fed either MON or MON+VM for 14 days.

The different combinations between MON and VM during adaptation and finishing periods in the current study did not affect the DMI expressed both in kg and as a percentage of BW overall. However, the MON effect on reducing DMI has been reported in the literature (3, 36, 37). In relation to the VM effect on DMI, Erasmus et al. (6), Lemos et al. (7) and Salinas-Chavira et al. (5) did not report a decrease in DMI when VM was fed to feedlot cattle. In the present study, Nellore bulls fed T5 did not consume MON during the final third of feedlot period (last 40 days), and therefore, DMI was not increased when compared to cattle fed MON + VM during the entire feeding period, which did not confirm one the hypothesis of the present study. On the other hand, Rigueiro et al. (8) observed an increase in DMI when MON was withdrawn during the finishing period. However, the finishing period lasted 71 days, 31 more days on feed when compared with the current study. Despite to the time of exposure to the treatment, the withdrawal of MON when associated with VM during the last 40 days of the feedlot period improved the animal performance overall when compared to bulls fed either MON or VM.

Although withdrawal of MON during the final third of the feedlot period does not affect DMI, cattle fed T5 consumed more 0.44 and 0.25 kg of DM per day when compared to cattle fed only MON and VM during the entire feeding period, respectively, which may have contributed to an increase HCW in 7.23 and 7.69 kg when compared to cattle fed only MON and VM, respectively. Nellore bulls fed only MON during the entire feeding period presented lower fat deposition. Goodrich et al. (36) also observed negative effects of MON on dressing percentage and 12th rib fat thickness. According to the authors, standard deviations for percentage change in carcass characteristics indicate that these effects of MON are highly variable. In a meta-analysis, Duffield et al. (3) did not report negative effects of feeding MON on carcass characteristics. However, the slower 12th rib fat daily gain and Biceps femoris fat daily gain observed in the current study, which led to thinner final 12th rib fat and final Biceps femoris fat thickness, may be associated with the decreased acetate:propionate ratio (38), which may negatively impact the lipid metabolism. It is noteworthy to mention that based on the results reported by Rigueiro et al. (9), the shortening of the adaptation period from 14 to 9 or 6 days did not negatively impact the feedlot performance overall, where there were no effects of treatments on final body weight, ADG and HCW. However, the authors reported that cattle fed VM for 9 days had more meals per day and less DMI intake per meal, due to the rumen acidification, resulting in a linear decrease in the 12th rib fat and BF fat daily gain.

Associated with the performance assay performed by Rigueiro et al. (9), Squizatti et al. (39) performed a rumen metabolism assay,

and the authors reported that as the adaptation length decreased for animals consuming only VM, the rumen degradability of DM, NDF and starch decreased. Associated with that, it was observed higher proportions of protozoa for animals receiving VM adapted for 6 or even 9 days, justifying the reduced rate of ruminal degradation since protozoa predate bacteria, resulting in a reduction of colonization rate of feed and, consequently, reducing DMI as shown by Rigueiro et al. (9). In addition, both authors concluded that feedlot cattle fed VM as a sole feed additive should not be adapted to high-concentrate diets in less than 14 days, since it compromises DMI, carcass fat deposition and disrupts feeding behavior patterns.

In this context, analyzing the feeding behavior allows the adjustment of dietary management in order to achieve better production performance in beef cattle (40). The particle sorting affects the individual nutrient intake, since there are indications that this sorting of the diet is associated with an increased risk of metabolic diseases (41). In the present study, Nellore bulls fed T4 at day 61 sorted against long and fine particles, which led to spend more time resting. In addition, cattle fed T3 and T5 sorted in favor of long particle and against fine particles at day 61, which lead to a numerical increase in NDF intake and results in less time resting, which may be a response to control rumen acidification. Similarly, Rigueiro et al. (9) reported that the cattle adapted with VM for 9 days sorted for medium and against fine diet particles during both the adaptation and finishing periods and consumed significantly more NDF during adaptation.

It is recognized that reducing the particle size of fiber decreases chewing activity, saliva flow, and rumen pH, increasing the risk of subacute ruminal acidosis, as well as increasing resting time and reducing sorting behaviors and eating time (42). Consequently, increasing the consumption of large particles led to increased intake of physically effective fiber, which was positively associated with fiber digestion and chewing time, preventing subclinical ruminal acidosis (43). In relation to rumen measurements, rumenitis scores reported in this study were very low, the average score was less than 2, using a scale of 0 (no lesions noted) to 10 (severe ulcerative), according to Bigham and McManus (28). Also, cattle fed T1 and T5 presented larger MPA, which may have contributed to a larger development of rumen epithelium, which allows a faster SCFA clearance; however, larger MPA in the current study did not influence ASA. The rumen wall absorptive surface area (ASA) is the morphometric variable most correlated to the speed of SCFA absorption, playing an important role to increase the ruminal pH to adequate levels (44). Rigueiro et al. (9) observed that the shortening of the adaptation period for cattle fed only VM did not negatively impact rumenitis score; however, cattle receiving VM by 14 days presented a higher incidence of rumen lesions when compared to those fed MON+VM by 14 days (0.85 vs. 0.38, respectively). In addition, the authors reported that cattle fed virginiamycin for 9 days had lower rumen development in the number of papillae, mean papillae area, ASA and papillae area expressed as % of ASA. Moreover, there is evidence that VM supplementation results in increased propionate synthesis and reduced acetate and butyrate concentration, as well as reduced lactate production and increased ruminal pH (45). In this context, decreasing the ruminal acetate/propionate ratio may have contributed to a development of rumen epithelium, increasing the SCFA absorption. Squizatti et al. (46) observed a quadratic effect for adaptation length when only VM for mean pH, duration of pH below 5.2 and 6.2, where cattle consuming VM adapted for 9 days had higher mean pH and shorter period of pH below 5.2 and 6.2 compared to animals adapted in 6 days. However, it is important to note that these results do not guarantee that adaptation length can be reduced since it was observed lower DMI for cattle adapted for 9 days, as already described by Rigueiro et al. (9), a fact associated with inadequate adaptation. On other hand, the authors reported that animals consuming only VM and adapted for 14 days had higher maximum pH and acetate:proprionate ratio, as well as lower ox-redox potential than cattle receiving MON+VM for 14 days.

4. Conclusion

In conclusion, the withdrawal of MON when associated with VM during the last 40 days of the feedlot period did not increase DMI; however, this withdrawal of MON combined with a higher energy diet during the final third of the feedlot period improved overall final BW, ADG and HCW of Nellore cattle when compared to bulls fed either MON or VM, but did not positively impact feedlot performance and carcass characteristics when compared to cattle that had MON withdrawn by the end of the adaptation period. According to the results, Nellore cattle should be fed high-concentrate diets containing MON and VM during adaptation, and only VM during the finishing period to improve overall feedlot performance.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by São Paulo State University Ethical Committee for Animal Research (Protocol No.: CEUA 154/2016).

Author contributions

AR and DM: conceived and designed study, collected and complied, and analyzed data. MP, AS, AP, LF, ED, BD, DE, JD, KS, LS, and AN: investigation. AR, JS, and DM: provided intellectual input and drafted and edited manuscript. All authors contributed to the article and approved the submitted version.

Funding

This project was financially supported by FAPESP, The São Paulo Research Foundation (Grant Nos.: 2017/17896-8) and Phibro Animal Health; Scholarship for the first author was provided by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasília, DF, Brazil; Finance Code 001). The funding company (Phibro) had no influence on designing the experiment and analyzing the data.

Acknowledgments

We wish to acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Phibro Animal Health, and FAPESP (São Paulo Research Foundation, São Paulo, Brazil).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Samuelson KL, Hubbert ME, Galyean ML, Löest CA. Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. *J Anim Sci.* (2016) 94:2648–63. doi: 10.2527/jas.2016-0282

2. Silvestre AM, Millen DD. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *R Bras Zootec.* (2021) 50:e20200189. doi: 10.37496/rbz5020200189

3. Duffield TF, Merrill JK, Bagg RN. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *J Anim Sci.* (2012) 90:4583–92. doi: 10.2527/jas.2011-5018

4. Salinas-Chavira J, Lenin J, Ponce E, Sanchez U, Torrentera N, Zinn RA. Comparative effects of virginiamycin supplementation on characteristics of growth-performance, dietary energetics, and digestion of calf-fed Holstein steers. *J Anim Sci.* (2009) 87:4101–8. doi: 10.2527/jas.2009-1959

5. Salinas-Chavira J, Barreras A, Plascencia A, Montano MF, Navarrete JD, Torrentera N, et al. Influence of protein nutrition and virginiamycin supplementation on feedlot growth performance and digestive function of calf-fed Holstein steers. *J Anim Sci.* (2016) 94:4276–86. doi: 10.2527/jas.2016-0576

6. Erasmus LJ, Muya C, Erasmus S, Coertze RF, Catton DG. Effect of virginiamycin and monensin supplementation on performance of multiparous Holstein cows. *Livest Sci.* (2008) 119:107–15. doi: 10.1016/j.livsci.2008.03.005

7. Lemos BJM, Castro FGF, Santos LS, Mendonça BPC, Couto VRM, Fernandes JJR. Monensin, virginiamycin, and flavomycin in a no-roughage finishing diet fed to zebu cattle. *J Anim Sci.* (2016) 94:4307–14. doi: 10.2527/jas.2016-0504

8. Rigueiro ALN, Pereira MCS, Squizatti MM, Ferreira MM, Donde SC, Luiz FP. Different combinations of sodium monensin and virginiamycin during feedlot finishing of Nellore cattle. *Anim Prod Sci.* (2020) 60:1061–72. doi: 10.1071/AN18657

9. Rigueiro ALN, Squizatti MM, Silvestre AM, Pinto ACJP, Estevam DD, Felizari LD, et al. The potential of shortening the adaptation of Nellore cattle to high-concentrate diets using only Virginiamycin as sole feed additive. *Front Veter Sci.* (2021) 8:692–705. doi: 10.3389/fvets.2021.692705

10. Ingvartsen KL, Boisclair YR. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. *Domest Animal Endocrinol.* (2001) 21:215–50. doi: 10.1016/S0739-7240(02)00119-4

11. Foote AP, Hales KE, Kuehn LA, Keisler DH, King DA, Shackelford SD, et al. Relationship of leptin concentrations with feed intake, growth, and efficiency in finishing beef steers. *J Animal Sci.* (2015) 93:4401–7. doi: 10.2527/jas.2015-9339

12. Owens FN, Dubeski P, Hanson CF. Factors that alter the growth and development of ruminants. *J Anim Sci.* (1993) 71:3138–50. doi: 10.2527/1993.71113138x

13. Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE. The Cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. *Anim Feed Sci Technol.* (2004) 112:29–78. doi: 10.1016/j.anifeedsci.2003.10.006

14. Association of Official Analytical Chemist (1990). *Official Methods of Analysis of AOAC*. 15th ed, Washington, DC: Association of Official Analytical Chemist.

15. Association of Official Analytical Chemists (1997). *Official Methods of Analysis of AOAC*. 16th ed. Arlington, VA: Association of Official Analytical Chemists.

16. Van Soest PJ, Robertson JB, Lewis AB. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2

17. Bevans DW, Beauchemin KA, Schwartzkopf-Genswein KS, McKinnon JJ, McAllister TA. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. *J Anim Sci.* (2005) 83:1116–32. doi: 10.2527/2005.8351116x

18. Lofgreen GP, Garret WN, A. system for expressing net energy requirements and feed values for growing and finishing beef cattle. *J Anim Sci.* (1968) 27:793–806. doi: 10.2527/jas1968.273793x

19. National Research Council (NRC). *Nutrient Requirements of Beef Cattle*. 6th ed. Washington, DC: National Academy Press (1984).

20. Zinn RA, Shen Y. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. *J Anim Sci.* (1998) 76:1280–9. doi: 10.2527/1998.7651280x

21. Perkins TL, Green RD, Hamlin KE. Evaluation of ultrasonic estimates of carcass fat thickness and longissimus muscle area in beef cattle. *J Anim Sci.* (1992) 70:1002–10. doi: 10.2527/1992.7041002x

22. Robles V, González LA, Ferret A, Manteca X, Calsamiglia S. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high concentrate diets. *J Anim Sci.* (2007) 85:2538–47. doi: 10.2527/jas.2006-739

23. Pereira MC, Cruz GD, Arrigoni MD, Rigueiro AL, Silva J, Carrara TV, et al. Relationships of feedlot performance, feeding behavior, rumen morphometrics, and carcass characteristics of Nellore cattle differing in phenotypic residual feed intake. *J Animal Sci.* (2016) 94:4287–96. doi: 10.2527/jas.2016-0579

24. Heinrichs J, Kononoff P. In evaluating particle size of forages and TMRs using the Penn State Particle Size Separator. *Anim Sci.* (1996) 42:1–14.

25. Kononoff PJ, Heinrichs AJ, Buckmaster DR. Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. *J Dairy Sci.* (2003) 86:1858–63. doi: 10.3168/jds.S0022-0302(03)73773-4

26. Leonardi C, Armentano LE. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *J Dairy Sci.* (2003) 86:557–64. doi: 10.3168/jds.S0022-0302(03)73634-0

27. Brink DR, Lowry SR, Stock RA, Parrott JC. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. *J Anim Sci.* (1990) 68:1201–7. doi: 10.2527/1990.6851201x

28. Bigham ML, McManus WR. Whole wheat grain feeding of lambs. V Effects of roughage and wheat grain mixtures. *J Agric Res.* (1975) 26:1053–62. doi: 10.1071/AR9751053

29. Resende Júnior JC, Alonso LS, Pereira MN. Effect of the feeding pattern on rumen wall morphology of cows and sheep. *Braz J Vet Res Anim Sci*. (2006) 43:526–36. doi: 10.11606/issn.1678-4456.bjvras.2006.26469

30. Odongo NE, AlZaha lO, Lindinger MI, Duffield TF, Valdes EV, Terrel SP, et al. Effects of mild heat stress and grain challenge on acid-base balance and rumen tissue histology in lambs. *J Anim Sci.* (2006) 84:447–55. doi: 10.2527/2006.842447x

31. Pereira FTV, Braga FC, Burioli KC, Kfoury JR, Oliveira LJ, Papa PC, et al. Transplacental transfer of iron in the Water Buffalo (Bubalus bubalis): uteroferrin and erythrophagocytosis. *Reprod Domest Animals.* (2010) 45:907–14. doi: 10.1111/j.1439-0531.2009.01462.x

32. Pereira MCS, Dellaqua JVT, Sousa OA, Santi PF, Felizari LD, Reis BQ. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of Nellore cattle submitted to strategic diets prior the adaptation period. *Livest Sci.* (2020) 234:103985. doi: 10.1016/j.livsci.2020.103985

33. Devant M, Penner GB, Marti S, Quintana B, Fábregas F, Bach A, et al. Behavior and inflammation of the rumen and cecum in Holstein bulls fed high-concentrate diets with different concentrate presentation forms with or without straw supplementation. *J Anim Sci.* (2016) 94:3902–17. doi: 10.2527/jas.2016-0594

34. Nuñez AJC, Caetano M, Berndt A, Demarchi JJAA, Leme PR, Lanna DPD. Combined use of ionophore and virginiamycin for finishing Nellore steers fed high concentrate diets. *Sci Agric.* (2013) 70:229–36. doi: 10.1590/S0103-90162013000400002

35. Brown MS, Ponce CH, Pulikanti R. Adaptation of beef cattle to high-concentrate diets: performance and ruminal metabolism. *J Anim Sci.* (2006) 84:25–33. doi: 10.2527/2006.8413_supplE25x

36. Goodrich RD, Garrett JE, Gast DR, Kirick MA, Larson DA, Meiske JC. Influence of monensin on the performance of cattle. *J Anim Sci.* (1984) 58:1484–98. doi: 10.2527/jas1984.5861484x

37. Tedeschi LO, Fox DG, Tylutki TP. Potential environmental benefits of ionophores in ruminant diets. *J Environ Qual.* (2003) 32:1591–602. doi: 10.2134/jeq2003.1591

38. Ellis JL, Dijkstra J, Bannink A, Kebreab E, Hook SE, Archibeque, et al. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. *J Animal Sci.* (2012) 90:2717–26. doi: 10.2527/jas.2011-3966

39. Squizatti MM, Rigueiro ALN, Soares CHG, Nunes ABPC, Demartini BL, Felizari LD, et al. Reduction of adaptation to high-energy diets using virginiamycin: protozoa and rumen degradability. In: Chizzotti, ML, editor *6th EAAP International Symposium on Energy and Protein Metabolism and Nutrition,* Belo Horizonte: Wageningen Academic Publishers. (2019) 138:257–257. doi: 10.3920/978-90-8686-891-9_62

40. Santos BP, Santana Júnior HA, Araújo MJ, Oliveira AP, Santana EOC, Mendes FBL. Feeding behavior of categories of feedlot finished beef cattle. *Acta Scientiar Animal Sci.* (2018) 40:1–5. doi: 10.4025/actascianimsci.v40i0.35528

41. Jurkovich V, Konyves L, Bakony M. Association between feed sorting and the prevalence of metabolic disorders in Hungarian large-scale dairy herds. *J Dairy Res.* (2019) 86:162–4. doi: 10.1017/S002202991900027X

42. Kronqvist C, Petters F, Robertsson U, Lindberg M. Evaluation of production parameters, feed sorting behaviour and social interactions in dairy cows: Comparison of two total mixed rations with different particle size and water content. *Livest Sci.* (2021) 251:104662. doi: 10.1016/j.livsci.2021.104662

43. Yang WZ, Beauchemin KA. Physically effective fiber: method of determination and effects on chewing, ruminal acidosis, and digestion by dairy cows. *J Dairy Sci.* (2006) 89:2618–33. doi: 10.3168/jds.S0022-0302(06)72339-6

44. Melo LQ, Costa SF, Lopes F, Guerreiro MC, Armentano LE, Pereira MN. Rumen morphometrics and the effect of digesta pH and volume on volatile fatty acid absorption. *J Anim Sci.* (2013) 91:1775–83. doi: 10.2527/jas.2011-4999

45. Lanna, DPD, Medeiros, SR. Uso de aditivos na bovinocultura de corte. In: Santos FAP, Moura JC, Faria VP, eds. *Requisitos de qualidade na bovinocultura de corte*, Piracicaba: FEALQ (2007) p. 297–324.

46. Squizatti MM, Rigueiro ALN, Soares CHG, Assumpcao AHP, Dias EFF, Lirian K, et al. Reduction of adaptation to high-energy diets using virginiamycin: effects on ruminal fermentation. In: Chizzotti, ML, editor *6th EAAP International Symposium on Energy and Protein Metabolism and Nutrition*, Belo Horizonte: Wageningen Academic Publishers. (2019) vol. 138, p. 255–255. doi: 10.3920/978-90-8686-891-9_61

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 24 February 2023
DOI 10.3389/fvets.2023.1039323

OPEN ACCESS

EDITED BY
Bangyuan Wu,
China West Normal University, China

REVIEWED BY
Ilias Giannenas,
Aristotle University of Thessaloniki, Greece
Zachary Smith,
South Dakota State University, United States
Jon Schoonmaker,
Purdue University, United States

*CORRESPONDENCE
Richard A. Zinn
✉ razinn@ucdavis.edu

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 08 September 2022
ACCEPTED 07 February 2023
PUBLISHED 24 February 2023

CITATION
Carvalho PHV, Latack BC, Ferraz Junior MVC, Flores R, Sanchez-Cruz G, Montaño MF and Zinn RA (2023) The effects of NutraGen supplement on cattle growth performance, energetic efficiency, carcass characteristics, and characteristics of digestion in calf-fed Holstein steers. *Front. Vet. Sci.* 10:1039323. doi: 10.3389/fvets.2023.1039323

The effects of NutraGen supplement on cattle growth performance, energetic efficiency, carcass characteristics, and characteristics of digestion in calf-fed Holstein steers

Pedro H. V. Carvalho[1], Brooke C. Latack[2], Marcos V. C. Ferraz Junior[1,3], Ruben Flores[4], Gerson Sanchez-Cruz[4], Martin F. Montaño[4] and Richard A. Zinn[1]*

[1]Department of Animal Science, University of California, Davis, Davis, CA, United States, [2]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, Holtville, Holtville, CA, United States, [3]Departamento de Zootecnia Instituto de Ciências Sociais, Educação e Zootecnia, Universidade Federal do Amazonas, Parinitns, Brazil, [4]Department of Nutrition and Biotechnology of Ruminants, Instituto de Investigaciones en Ciencias Veterinarias-UABC, Mexicali, Baja California, México

Evaluation of the effects of feeding NutraGen supplement (NutraGen, NTG; Phibro Animal Health, Teaneck, NJ, USA) on growth performance, energetic efficiency, carcass characteristcs, and characteristics of digestion in calf-fed Holstein steers fed a conventional growing-finishing diet. Trial 1 evaluated growth performance, dietary energetics and carcass characteristics. Two hundred Holstein steer calves (134 ± 5 kg) were blocked by initial body weight (BW) and randomly assigned to 40 pens (5 steers/pen). Dietary treatments consisted of a steam-flaked corn-based growing-finishing diet supplemented with 0, 0.2, 0.4, or 0.6% NTG (DM basis). In trial 2, four Holstein steers (170 ±6 kg) with cannulas in the rumen and proximal duodenum were used in a 4 × 4 Latin square experiment to evaluate digestibility and ruminal characteristics using the treatments from trial 1. Compared to non-supplemented cattle, NTG increased BW (2.0%, $P = 0.02$) and tended to increase ADG (3.6%, $P = 0.07$) during the initial 56 d period. However, there were no treatment effects on overall growth performance and efficiency of dietary energy utilization after the first 56 days ($P > 0.10$). Supplementation of NTG increased (linear effect; $P \leq 0.03$) longissimus muscle area and kidney, pelvic, and heart fat. There was no effect ($P \geq 0.05$) of NTG supplementation on other carcass characteristics, liver abscess incidence, or liver abscess scars. Supplementation decreased the molar proportion of ruminal propionate ($P = 0.05$) and tended to increase acetate:propionate molar ratio ($P = 0.09$). However, there was no effect of NTG supplementation on ruminal and total tract diet digestion. NTG increased performance of Holstein steers during the first 56 d on feed in the feedlot. In addition, the steers had an increase in KPH fat and LM area, indicating that the additive induced change in metabolism of the steers.

KEYWORDS

Holstein, feedlot, performance, heat-stress, cattle

Introduction

Recently, there has been an increased concern about the over-routine use of supplemental antibiotics, which has increased the investigation of potential non-antibiotic alternatives in cattle diets (1). NutraGen (NTG; Phibro Animal Health, Teaneck, NJ, USA) contains similar properties to OmniGen (OMG; Phibro Animal Health, Teaneck, NJ, USA), which is used in the dairy industry (2). Previous research has reported that the use of this blend in dairy cows' diets includes improved metabolic response to a glucose tolerance test (2), modulation of inflammatory responses (3), increased milk production (4), and enhanced immunomodulatory response to heat stress (5, 6). The latter may be particularly relevant for enhancing growth performance responses in feedlot cattle during extreme ambient temperatures. The NTG is a blend of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewer's yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate, and folic acid.

Although some of these compounds have been studied in the beef industry individually, to the authors' knowledge, the mechanism of action of NTG and OMG on heat stress is still unknown. Wang et al. (7) observed that OMG supplementation increased the expression of neutrophil associated genes in peri-parturient dairy cows, such as interleukin-1b converting enzyme, interleukin-4 receptor, interleukin-8 receptor and thymopoeitin-α (7). Feeding OMG for 2 weeks increased mammary inflammatory response and antigen presentation to mammary infections caused by a single strain of mastitis pathogens (Streptococcus uberis, Escherichia coli, and Staphylococcus aureus) in a mouse model of infection, suggesting OMG could decrease the incidence of infectious diseases in ruminants (8). These reports together indicated that OMG might reduce the incidence of infectious diseases in ruminants. However, no information has been published on the effects of supplemental NTG on cattle growth performance and digestive function when cattle are fed a common steam-flaked corn-based diet.

Moreover, there is no information on the potential benefits of feeding NTG as a heat load mitigator for calf-fed Holstein steers in the southern desert region of the United States. A region characterized by its extreme heat conditions during the summer months. Therefore, the objective of the current study was to evaluate the effects of NTG supplementation on growth performance, energetic efficiency, carcass characteristics, and digestive function in calf-fed Holstein steers.

Materials and methods

Procedures for animal care and management were conducted under a protocol (#20548) approved by the University of California, Animal Use and Care Advisory Committee.

Trial 1

Cattle management and treatments

Two hundred Holstein steer calves (130.9 $\pm$ 4 kg) were utilized to evaluate the influence of NTG supplementation on growth performance, dietary energetics, and carcass characteristics. The trial was initiated on January 14, 2020, and completed following a 322-d feeding period (December 1, 2020). Calves were purchased from a commercial calf ranch (CalfTech, Tulare, CA). Upon arrival at the University of California Desert Research and Extension Center (Holtville, CA), calves were vaccinated for IBR, BVD, PI3, and BRSV (Bovi-shield® Gold One Shot, Zoetis Animal Health, New York, NY), clostridials (Ultrabac® 7, Zoetis Animal Health, New York, NY), treated against internal and external parasites (Dectomax, Zoetis Animal Health, New York, NY), injected with 1,500 IU vitamin E (as d-alpha-tocopherol) 500,000 IU vitamin A (as retinyl-palmitate) and 50,000 I.U. vitamin D3 (Vital E-AD, Stuart Products, Bedford, TX), and 300 mg tulathromycin (Draxxin, Zoetis Animal Health, New York, NY). Calves were blocked by initial shrunk (off truck) weight and randomly assigned within weight groupings to 40 pens (5 steers/pen, 10 pens/treatment). On day 28, calves received the Ultrabac® 7 booster vaccination and were vaccinated with Endovac-Beef (Endovac Animal Health, Columbia, MO). On d 56 calves received the Endovac-Beef booster vaccination. Pens were 62 m^2 with 25 m^2 overhead shade, automatic waterers, and 2.4 m fence-line feed bunks. Steers were allowed *ad libitum* access to feed and water. Fresh feed was provided daily.

Dietary treatments are shown in Table 1, consisting of a steam-flaked corn-based diet supplemented with (DM basis): (1) no feed additive; (2) 0.2% of NTG; (3) 0.4% of NTG; (4) 0.6% of NTG. Nutragen doses were based on a previous study on dairy cows, and no feed additive was included in the diet. Diets were prepared weekly and stored in plywood boxes in front of each pen. On d 112 and 224, all steers were reinjected subcutaneously with 500,000 IU vitamin A (Vital E-A + D, Stuart Products, Bedford, TX) and implanted with Revalor-S (Intervet, Millsboro, DE). The health status of cattle was checked daily. Cattle were monitored daily by trained personnel for signs of illness or pinkeye. Cattle with signs of illness were pulled out, classified as morbid, and treated with an antimicrobial if the rectal temperature was $\geq$ 39.5°C. Antimicrobial treatments were conducted following a veterinarian's recommendation. A post-treatment interval of 3 days was implemented after the first and second treatments. If cattle remained morbid after the third treatment and the prognosis of a full recovery was unlikely, cattle were removed from the study (two animals died during the experiment for causes unrelated to dietary treatments).

Steer full body weight (BW) was recorded every 28 days until the end of the experiment to monitor live weight changes. Steers were not denied feed or water before weighing. In determining ADG, interim and final weights were reduced by 4% to account for digestive tract fill (9). From March 9, 2020, to the end of the experiment, SmaX-tec intraruminal boluses (SmaX-tec animal care technology®, Graz, Austria) were orally inserted into the rumen (1 steer per pen) to monitor the ruminal temperature. Continuous real-time temperature data was retrieved using a monitoring device located in the proximity of cattle pens.

10.3389/fvets.2023.1039323

TABLE 1 Composition of experimental diets (DM basis).

Item	NutraGen[b] level, %			
	0	0.2	0.4	0.6
Ingredient composition, %				
DM				
Sudangrass hay	8.00	8.00	8.00	8.00
Alfalfa hay	4.00	4.00	4.00	4.00
Tallow	2.50	2.50	2.50	2.50
Molasses, cane	4.00	4.00	4.00	4.00
Distillers Grains w/solubles	10.00	10.00	10.00	10.00
Steam flaked corn	68.12	67.92	67.72	67.52
Urea	1.15	1.15	1.15	1.15
Limestone	1.68	1.68	1.68	1.68
Dicalcium phosphate	0.10	0.10	0.10	0.10
Magnesium oxide	0.15	0.15	0.15	0.15
TM Salt[a]	0.30	0.30	0.30	0.30
NutraGen[b]	0.00	0.20	0.40	0.60
Nutrient composition, DM basis[c]				
Dry matter, %	87.9	87.9	87.9	87.9
NEm, Mcal/kg	2.21	2.20	2.20	2.20
NEg, Mcal/kg	1.53	1.52	1.52	1.52
Crude protein, %	14.3	14.3	14.3	14.3
Rumen DIP, %	62.7	62.7	62.7	62.7
Rumen UIP, %	37.3	37.3	37.3	37.3
Ether extract, %	6.70	6.70	6.70	6.70
Ash, %	5.76	5.76	5.76	5.76
Nonstructural CHO, %	58.0	58.0	58.0	58.0
NDF, %	17.7	17.7	17.7	17.7
Calcium, %	0.80	0.80	0.80	0.80
Phosphorus, %	0.35	0.35	0.35	0.35
Potassium, %	0.77	0.77	0.77	0.77
Magnesium, %	0.28	0.28	0.28	0.28
Sulfur, %	0.19	0.19	0.19	0.19

[a]Trace mineral salt contained: CoSO4, 0.068%; CuSO4, 1.04%; FeSO4, 3.57%; ZnO, 0.75%; MnSO4, 1.07%; KI, 0.052%; and NaCl, 93.4%.

[b]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.

[b]Based on tabular values for individual feed ingredients (33).

Carcass measurements

Hot carcass weights (HCW) and liver abscess incidence [based on size and number, scaled as 0, A–, A, and A+; (10)], as well as liver scarring measures, were obtained at the time of slaughter. After carcasses were chilled for 24 h, the following measurements were obtained: Longissimus muscle (LM) area (cm^2) by direct grid reading of the muscle at the 12th rib; subcutaneous fat (cm) over the LM at the 12th rib taken at a location 3/4 the lateral length from the chine bone end (adjusted for unusual fat distribution); kidney, pelvic and heart fat (KPH) as a percentage of HCW; marbling score [(11); using 3.0 as minimum slight, 4.0 as minimum small, 5.0 as minimum modest, 6.0 as minimum moderate, etc.], and preliminary as well as estimated retail yield of boneless, closely trimmed retail cuts from the round, loin, rib and chuck as a percentage of HCW [Yield, % = 52.56–1.95 × subcutaneous fat−1.06 × KPH + 0.106 × LM area−0.018 × HCW; (12)].

Estimation of dietary net energy

Daily energy gain (EG; Mcal/d) was calculated by the equation: $EG = ADG^{1.097}\ 0.0557W^{0.75}$, where W is the mean shrunk B.W. [kg; (13)] Maintenance energy (EM) was calculated by the equation: $EM = 0.086W^{\ 0.75}$. Dietary NEg was derived from NEm by the equation: NEg = 0.877 NEm−0.41 (14). Dry matter intake is related to energy requirements and dietary NEm according to the equation: DMI = (EM/NEm) + (EG/(0.877NEm−0.41). From this relationship, dietary NE can be resolved by means of the quadratic formula: $x = (-b - \sqrt{b2 - 4ac})/ 2c$, where: x = NEm, a = – 0.42 EM, b = 0.887 EM + 0.41 DMI + EG, and c = – 0.887 DMI (15).

Weather measurement and temperature and humidity index estimation

Climatic variables (ambient temperature and relative humidity) were obtained every hour from an on-site weather station (California Irrigation Management Information System; Meloland Station) throughout the experimental period. The temperature humidity index was calculated using the following formula THI = (0.8 × Ta) + [(H/100) × (Ta – 14.4)] + 46.4, where Ta is air temperature (°C) and H is relative humidity (16, 17); Min = minimum; Max = maximum.

Statistical design and analysis

Pens were used as experimental units. The experimental data were analyzed as a randomized complete design experiment according to the following statistical model: Yij = μ + Bi + Tj + Eij, where μ is the common experimental effect, Bi represents the initial weight group effect (df = 6), Tj represents the dietary treatment effect (df = 3), and Eij represents the residual error (df = 18). Treatment effects were tested using the following contrasts: 0 vs. NTG and linear and quadratic polynomials to assess the effect of dosage (Stastix 10, Analytical Software, Tallahassee, FL).

Trial 2

Cattle management and treatments

Four Holstein steers (170 ±6 kg) with cannulas in the rumen and proximal duodenum were used in a 4 × 4 Latin square experiment. Treatments are the same as in Trial 1 (Table 1). A single basal diet was prepared with 0.3% chromic oxide as a digesta marker. Corresponding amounts of NTG (0, 0.2, 0.4, and 0.6%

10.3389/fvets.2023.1039323

TABLE 2 Effects of feeding NutraGen supplement on health, growth performance and dietary net energy utilization.

	NutraGen[a] (%)					*P*-value		
Item	0	0.2	0.4	0.6	SEM	L	Q	0 vs. TMT
Days on test	322	322	322	322				
Pen replicated	10	10	10	10				
Morbidity, %	6.0	10.0	4.0	2.0	3.1	0.20	0.34	0.85
Mortality, %	0.0	2.0	0.0	0.0	1.0	0.66	0.33	0.57
Live weight[2] (kg)								
Initial	129.8	132.3	130.5	130.9	0.71	0.63	0.16	0.10
56 d	196.3	202.0	199.3	199.3	1.4	0.31	0.05	0.02
112 d	281.9	284.8	283.8	283.5	1.9	0.69	0.42	0.37
Final	586.2	587.1	581.4	590.6	5.2	0.75	0.43	0.97
ADG (kg)								
1–56 d	1.19	1.25	1.23	1.22	0.02	0.36	0.12	0.07
56–112 d	1.53	1.48	1.51	1.50	0.02	0.64	0.36	0.25
1–112 d	1.36	1.36	1.37	1.36	0.02	0.83	0.79	0.78
112–322 d	1.45	1.44	1.42	1.46	0.02	0.84	0.23	0.06
1–322 d	1.42	1.41	1.40	1.43	0.02	0.79	0.32	0.84
DMI (kg/d)								
1–56 d	4.82	4.96	4.90	4.91	0.07	0.49	0.35	0.20
56–112 d	6.99	7.01	7.03	7.02	0.09	0.85	0.89	0.85
1–112 d	5.91	5.98	5.97	5.96	0.07	0.65	0.59	0.46
112–322 d	8.65	8.45	8.51	8.56	0.11	0.68	0.26	0.27
1–322 d	7.69	7.59	7.62	7.66	0.09	0.84	0.45	0.50
ADG/DMI (kg/kg)								
1–56 d	0.246	0.252	0.251	0.249	0.003	0.64	0.30	0.30
56–112 d	0.219	0.211	0.215	0.214	0.003	0.51	0.27	0.16
1–112 d	0.230	0.228	0.230	0.229	0.003	0.85	0.87	0.72
112–322 d	0.168	0.170	0.167	0.171	0.002	0.55	0.77	0.52
1–322 d	0.184	0.186	0.184	0.187	0.002	0.62	0.84	0.59
Dietary ME (Mcal/kg)								
Maintenance								
1–112 d	1.88	1.88	1.88	1.88	0.02	0.89	0.90	0.96
112–322 d	2.27	2.31	2.27	2.31	0.03	0.47	0.96	0.33
1–322 d	2.19	2.22	2.18	2.22	0.02	0.58	0.91	0.48
Gain								
1–112 d	1.24	1.24	1.24	1.24	0.01	0.89	0.90	0.96
112–322 d	1.58	1.62	1.58	1.62	0.02	0.47	0.96	0.33
1–322 d	1.51	1.53	1.50	1.53	0.02	0.58	0.91	0.48
Observed/ expected dietary NE								
Maintenance								
1–112 d	0.85	0.85	0.85	0.85	0.01	0.89	0.90	0.96
112–322 d	1.03	1.05	1.03	1.05	0.01	0.47	0.96	0.33

(Continued)

TABLE 2 (Continued)

Item	NutraGen[a] (%)					*P*-value		
	0	0.2	0.4	0.6	SEM	L	Q	0 vs. TMT
1–322 d	0.99	1.00	0.99	1.00	0.01	0.58	0.91	0.48
Gain								
1–112 d	0.81	0.81	0.81	0.81	0.01	0.89	0.90	0.96
112–322 d	1.03	1.06	1.03	1.06	0.01	0.47	0.96	0.33
1–322 d	0.99	1.00	0.98	1.00	0.01	0.58	0.91	0.48

[1]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.
[2]Live weight reduced by 4% to account for gut fill.

TABLE 3 Effects of feeding NutraGen supplement on carcass characteristics, liver abscess, and cattle morbidity.

Item	NutraGen[a] (%)					*P*-value		
	0	0.2	0.4	0.6	SEM	L	Q	0 vs. TMT
Carcass weight (kg)	364.0	364.6	361.1	366.8	3.2	0.75	0.43	0.97
Dressing percentage (%)	62.0	62.3	61.9	62.2	0.22	0.81	0.93	0.60
KPH fat[b] (%)	3.10	3.14	3.20	3.31	0.06	0.01	0.53	0.09
Fat thickness (cm)	0.83	0.79	0.73	0.78	0.04	0.32	0.29	0.24
LM area (cm[b])	83.8	86.5	85.5	88.1	1.1	0.03	0.94	0.04
Marbling score[c]	4.35	4.32	4.53	4.41	0.10	0.41	0.71	0.59
Retail yield	51.3	51.7	51.6	51.7	0.17	0.17	0.50	0.12
Yield Grade	2.82	2.66	2.64	2.63	0.09	0.13	0.39	0.09
Abscessed liver (%)	12.0	14.0	6.0	10.0	5.0	0.54	0.84	0.73
Liver abscess scars (%)	30.0	22.0	22.0	32.0	6.2	0.83	0.16	0.52
Morbidity (%)	6.0	10.0	4.0	2.0	3.1	0.20	0.34	0.85

[a]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.
[b]KPH fat as a percentage of carcass weight.
[c]Coded: minimum slight = 3.0, minimum small = 4.0, minimum modest = 5.0, minimum moderate = 6.0, and so on.

of diet DM) were top-dressed on the basal diet at the time of feeding. Dry matter intake was restricted to 2.2% of live weight to avoid feed refusals. Diets were fed at 0,800 and 2,000 daily. Experimental periods consisted of a 17-d diet adjustment period followed by a 4-d collection period. During the collection period, duodenal and fecal samples were obtained from all steers twice daily: d 1, 0750, and 1,350; d 2, 0,900, and 1,500; d 3, 1,050, and 1,650; and d 4, 1,200 and 1,800. Individual samples consisted of approximately 700 ml duodenal chyme and 200 g (wet basis) fecal material. Additionally, on d4 of the collection period at 1,200 h, 100 mL of ruminal fluid was obtained from each steer *via* the ruminal cannula. Ruminal fluid pH was determined on freshly collected samples. Ruminal fluid was then strained through 4 layers of cheesecloth. Freshly prepared 25% (wt/vol) *m*-phosphoric acid (2 mL) was added to 8 mL of the strained ruminal fluid, centrifuged (17,000 × g for 10 min) and supernatant fluid stored at −20° C for VFA analysis [direct injection gas chromatography, using HP 5890A gas chromatograph, Hewlett Packard, Palo Alto, CA; DB-FFAP column, J&W Instruments, New Brighton, MN; WSFA-2 VFA standards, Supelco Analytical, Bellefonte, PA; 3-methyvaleric acid internal standard, TCI America, Portland, OR; (18)]. Duodenal and fecal samples from each steer within each collection period were composited for analysis. Upon completion of the experiment, ruminal fluid was obtained *via* the ruminal cannula from all steers and composited for the isolation of ruminal bacteria by differential centrifugation (19). Feed, duodenal fluid, and fecal samples were subjected to the following analysis: DM [oven drying at 105°C until no further weight loss; method 930.15; (20)]; ash [method 942.05; (20)], Kjeldahl N [method 984.13; (20)]; aNDFom (21), corrected for NDF-ash, incorporating heat stable α-amylase (Ankom FAA, Ankom Technology, Macedon, NY) at 1 mL per 100 mL of NDF solution]; chromic oxide (22); and starch (23). Duodenal samples were also analyzed for ammonia N (method 941.04, 20) and purines (24). Duodenal flow and fecal excretion of DM were determined based on marker ratio using chromic oxide. Microbial organic matter (MOM) and nitrogen (MN) leaving the abomasum were estimated using purines as microbial markers (24). Organic matter (OM) fermented in the rumen is considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and MOM reaching the duodenum. Feed N escape

TABLE 4 Effects of feeding NutraGen supplement on ruminal temperature during summer months.

Item	NutraGen[a] (%)					*P*-value	
	0	0.2	0.4	0.6	SEM	L	Q
June ruminal temp, °C							
Min temp	39.3	39.2	39.3	39.3	0.05	0.92	0.53
Ave temp	40.0	39.9	40.1	39.9	0.06	0.93	0.95
Max temp	40.7	40.5	40.8	40.6	0.07	0.95	0.92
July ruminal temp, °C							
Min temp	39.4	39.3	39.4	39.4	0.07	0.49	0.57
Ave temp	40.3	40.1	40.4	40.2	0.07	0.74	0.89
Max temp	40.9	40.9	41.2	40.9	0.08	0.64	0.59
August ruminal temp, °C							
Min temp	39.5	39.4	39.5	39.5	0.06	0.77	0.45
Ave temp	40.4	40.2	40.4	40.3	0.06	0.80	0.87
Max temp	41.1	40.9	41.2	41.0	0.07	0.99	0.77
September ruminal temp, °C							
Min temp	38.9	38.9	39.1	39.0	0.05	0.18	0.66
Ave temp	39.8	39.8	39.9	39.8	0.07	0.41	0.61
Max temp	40.4	40.4	40.6	40.5	0.09	0.35	0.60

[a]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.

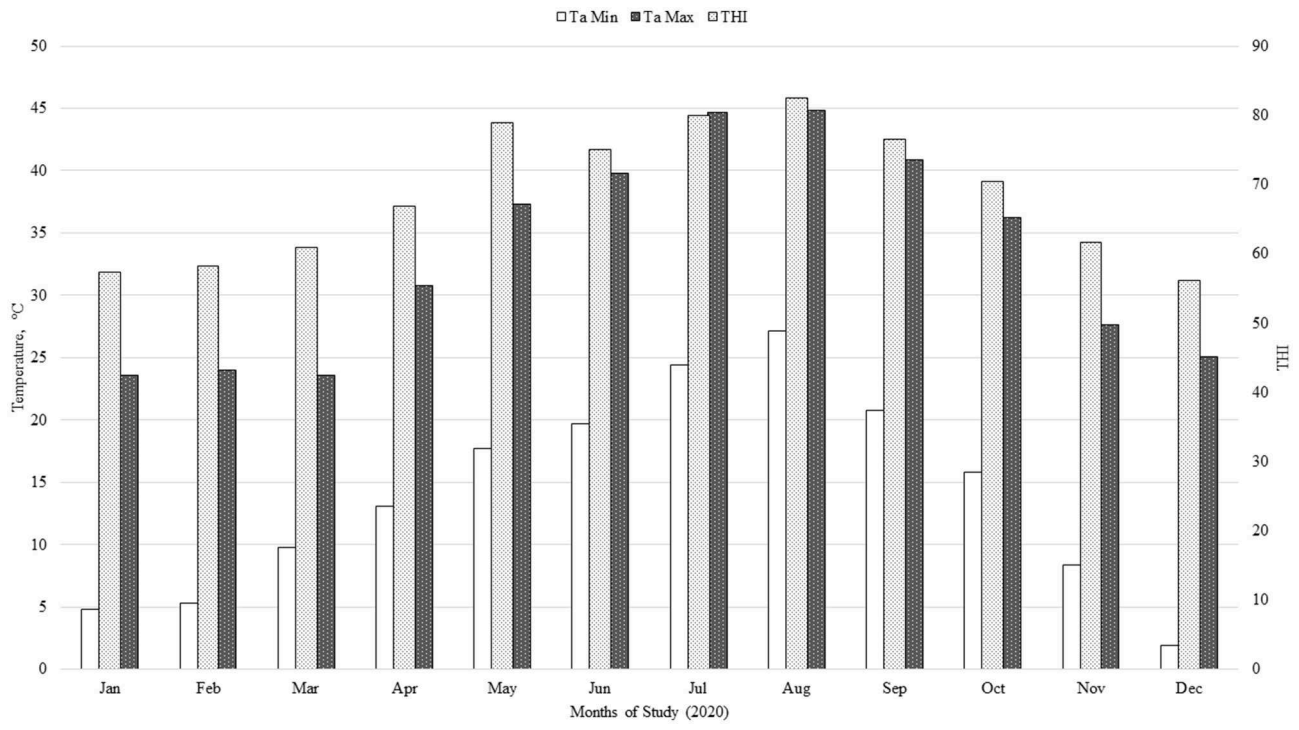


FIGURE 1
Temperature-humidity index (THI) during the 286-d feeding period: THI = (0.8 × Ta) + [(H/100) × (Ta − 14.4)] + 46.4, where Ta is air temperature (°C), and H is relative humidity (16, 17); Min = minimum; Max = maximum.

10.3389/fvets.2023.1039323

TABLE 5 Effects of feeding NutraGen supplement on characteristics of ruminal and total tract digestion.

Item	NutraGen[a] (%)					*P*-value		
	0	0.2	0.4	0.6	SEM	L	Q	0 vs. TMT
Steer replication	4	4	4	4				
Intake, g/d								
DM	3,672	3,672	3,672	3,672				
OM	3,464	3,464	3,464	3,464				
NDF	554.2	554.2	554.2	554.2				
N	80.4	80.4	80.4	80.4				
Starch	1,706	1,706	1,706	1,706				
Flow to the duodenum, g/d								
OM	1,737	1,711	1,708	1,680	40.4	0.38	0.98	0.46
NDF	269.0	238.6	283.3	255.9	22.6	0.96	0.94	0.72
Starch	268.7	272.6	265.6	216.0	30.5	0.27	0.41	0.64
Microbial N	45.0	45.6	44.0	49.1	3.3	0.49	0.52	0.76
Nonammonia N	84.6	84.7	83.9	85.4	1.1	0.73	0.56	0.94
Feed N	30.4	29.9	30.7	27.1	2.7	0.48	0.58	0.73
Ruminal								
OM	62.9	63.8	63.4	65.7	0.02	0.34	0.71	0.50
NDF	51.5	57.0	48.9	53.8	0.04	0.96	0.94	0.72
Starch	84.2	84.0	84.4	87.3	0.02	0.27	0.41	0.64
Feed N	62.2	62.8	61.8	66.3	0.03	0.48	0.58	0.73
Microbial efficiency[2]	20.7	20.5	20.0	21.6	1.2	0.69	0.48	0.99
Protein efficiency[3]	1.05	1.05	1.04	1.06	0.01	0.73	0.56	0.94
Fecal excretion, g/d								
DM	803.2	784.6	796.9	805.7	22.4	0.85	0.57	0.78
OM	672.1	656.4	655.3	662.1	23.9	0.78	0.65	0.63
NDF	275.5	265.2	254.7	263.0	14.2	0.50	0.52	0.42
Starch	10.6	10.5	8.49	8.46	1.8	0.34	0.99	0.51
N	23.4	22.8	23.2	23.5	0.61	0.82	0.51	0.74
Total tract digestion, %								
DM	78.1	78.6	78.3	78.1	0.61	0.85	0.57	0.78
OM	80.6	81.1	81.1	80.9	0.01	0.78	0.65	0.62
NDF	50.3	52.2	54.0	54.2	0.03	0.50	0.52	0.42
Starch	99.4	99.4	99.5	99.5	0.001	0.34	0.99	0.51
N	70.9	71.7	71.1	70.8	0.01	0.82	0.51	0.74

[a]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.

[b]Duodenal microbial N, g kg^{-1} OM fermented in the rumen.

[c]Duodenal non-ammonia N, g g^{-1} N intake.

to the small intestine is considered equal to total N leaving the abomasum minus ammonia-N, MN, and endogenous N [0.195 × $BW^{0.75}$; (25)]. Methane production was estimated based on the theoretical fermentation balance for observed molar distribution of VFA and OM fermented in the rumen (26).

Statistical design and analysis

The trial was analyzed as a balanced 4 × 4 Latin square according to the following statistical model: $Y_{ijk} = \mu + A_i + P_j + T_k + E_{ijk}$, where Y_{ijk} is the response variable, μ is the common experimental effect, A_i is the steer effect, P_j is the period effect, T_k is

10.3389/fvets.2023.1039323

TABLE 6 Effects of feeding NutraGen supplement on ruminal pH and VFA concentrations.

Item	NutraGen[a] (%)					*P*-value		
	0	0.2	0.4	0.6	SEM	L	Q	0 vs. TMT
Ruminal pH	5.91	5.95	6.06	6.10	0.12	0.26	0.98	0.41
Total VFA, mM	72.1	74.4	71.9	75.9	8.6	0.82	0.92	0.85
Ruminal VFA, mol/100 mol								
Acetate	49.4	48.9	51.3	52.3	2.0	0.26	0.72	0.55
Propionate	32.5	28.1	27.2	26.1	1.9	0.06	0.43	0.05
Isobutyrate	0.69	0.38	0.45	1.01	0.11	0.08	<0.01	0.54
Butyrate	11.9	13.9	14.3	14.9	1.2	0.13	0.58	0.12
Isovalerate	1.62	2.10	2.32	1.95	0.32	0.42	0.23	0.22
Valerate	3.90	6.56	4.39	3.63	1.9	0.75	0.42	0.69
Acetate/propionate	1.59	1.88	1.98	2.04	0.16	0.09	0.52	0.09
Methane/mol glucose[b]	0.43	0.46	0.48	0.50	0.02	0.05	0.71	0.07

[a]NutraGen (Phibro Animal Health, Ridgefield Park, NJ) is a proprietary formulation containing a mixture of silicon dioxide, calcium aluminosilicate, sodium aluminosilicate, dehydrated brewers yeast, mineral oil, calcium carbonate, rice hulls, niacin, biotin, d-calcium pantothenate, choline chloride, thiamine mononitrate, pyridoxine hydrochloride, riboflavin-5-phosphate and folic acid.
[b]Methane, mol/mol glucose equivalent fermented.

the treatment effect, and E_{ijk} is the residual error. Treatment main effects were assessed by means of orthogonal polynomials.

Results

In the present study, NTG was supplemented at 0, 0.2, 0.4, and 0.6% of diet DM. During the first 56 days of the study, steers consumed 0, 59, 119, and 178 mg per kg of live BW per day, respectively. However, the observed intake from 112 to the end of the experiment averaged 0, 39, 79, and 117 mg per kg of live BW per day, respectively. Treatment effects on health, growth performance, and estimated dietary NE are shown in Table 2. Morbidity and mortality were low and not affected ($P > 0.20$) by dietary treatments, averaging 5.5 and 0.5%, respectively. Supplementation of NTG increased (quadratic effect; $P = 0.05$) shrunk live weight on d 56 of the trial, with cattle supplemented with 0.2% NTG being the heaviest. Moreover, NTG supplementation tended to increase ($P = 0.07$) ADG from d 1–56 and decrease ($P = 0.06$) ADG from d 112–322. There was no effect of NTG on growth at any other time and no effect on DMI, feed efficiency, or efficiency of energy utilization ($P > 0.10$) (Table 2).

Treatment effects on carcass characteristics, liver abscess incidence, liver scars measures, and morbidity are shown in Table 3. Dietary supplementation of NTG increased LM area (linear effect; $P = 0.03$) and KPH fat (linear effect; $P = 0.01$). However, there was no effect ($P \geq 0.12$) of NTG supplementation on other carcass characteristics, liver abscess incidence, or liver abscess scars.

Treatment effects on minimum, average, and maximum ruminal temperature during the summer months (June, July, August, and September) are presented in Table 4. There was no effect ($P \geq 0.18$) of NTG supplementation on minimum, average, or maximum ruminal temperature during summer months when cattle were under high ambient temperature conditions (Figure 1).

Treatment effects on ruminal and total tract digestion and ruminal pH and VFA are shown in Tables 5, 6, respectively. There was no effect ($P \geq 0.12$) of NTG supplementation on ruminal and total tract digestion. The supplementation of NTG decreased ($P = 0.05$) propionate concentration in the rumen by 16.5% compared to the non-supplemented diet; therefore, the ruminal acetate/propionate ratio tended ($P = 0.09$) to increase linearly with NTG supplementation. There was a quadratic effect ($P < 0.01$) on isobutyrate concentration in the rumen, with isobutyrate minimal concentration being observed when cattle were supplemented with 0.2% of NTG in the diet. Moreover, cattle supplemented with NTG had a linear increase ($P = 0.05$) in the methane/mol glucose ratio compared to non-supplemented cattle. There was no effect ($P \geq 0.10$) of NTG supplementation on ruminal pH and other VFA concentrations.

Discussion

Although this product had not been previously tested in cattle under the conditions of the present study, we hypothesized that NTG supplementation might enhance growth performance, dry matter intake, and apparent digestibility of nutrients, ruminal parameters, and carcass characteristics of calf-fed Holstein steers. According to the manufacturer, NTG combines natural components with immunostimulant function, especially under stressful circumstances. Previous research reported that most respiratory diseases in the feedlot occurred within the first 30 days after arrival (27). Therefore, the greater growth performance observed in calves supplemented with NTG during the first weeks of the experiment may be associated with a greater immune response to challenges being faced during the receiving period, as has been suggested in previous studies (1–4, 6). However, in agreement with the current study, Colombo et al. (6) did not observe an overall effect on growth performance when crossbred

yearling cattle were fed a similar immunomodulatory supplement in the feedlot. Sanchez et al. (2) observed an increase in the final BW of beef heifers supplemented with OMG, a similar product used in the current experiment. The positive effects of OMG (mainly in dairy studies; 2–4, 6) have been attributed to the enhanced immune system, growth performance responses to OMG may largely depend on the stress conditions that animals are experiencing. For comparison, no prior studies evaluate growth performance responses to NTG supplementation of calf-fed Holstein steers.

In the present study, the dosage of NTG was supplied in the percentage of DMI (0, 0.2, 0.4, and 0.6% of DM), representing an increase in the dosage consumed according to BW. However, the intake in the first 56 days (0, 59, 119, and 178 mg of NTG per kg of live BW per day) was greater compared from 112 to the end of the experiment (0, 39, 79, and 117 mg of NTG per kg of live BW per day). However, the effect of NTG on growth performance at the beginning of the experiment was not dose-dependent. Sanchez et al. (2) and Moriel et al. (28) reported an enhancement in the metabolism of beef cattle supplemented with 100 and 88 mg of OMG per kg of BW, respectively.

Brown-Brandl et al. (29) stated that under ambient conditions where THI is $\geq$ 78, cattle are experiencing heat stress and "danger" conditions. In the present study, cattle were experiencing THI greater than 70 for almost half of their time on feed. Colombo et al. (6) fed 111 mg of OMG per kg of live BW per day and observed that this supplementation ameliorated hyperthermia in finishing beef cattle exposed to heat stress conditions. Although supplemental OMG has been shown to decrease the negative effect of heat-stress conditions on the performance of heifers and steers in feedlot and pasture (2, 6, 28), these extreme heat conditions faced by steers in the present study may have reduced the potential benefits of feeding an immunomodulatory supplement (NTG). The weather conditions that calf-fed Holstein steers were exposed to in this study were unfavorable from a growth performance standpoint (Figure 1). Nevertheless, no death loss occurred during the period of elevated THI. Historically, very low or no mortality during the early growing phase of calf-fed Holstein has been observed at the Research Center used in this study.

The percentage of KPH fat and LM area increased linearly with increasing levels of NTG supplementation, indicating that the metabolic effect that effects of NTG on carcass characteristics were dose-dependent. The acetate and glucose are used in ruminant metabolism as substrates for fatty acid synthesis. Sanchez et al. (2) observed that heifers supplemented with OMG were more sensitive to changes in blood glucose. Moreover, Buntyn et al. (30) observed an increase in blood glucose in steers fed OMG without affecting the growth performance of feedlot steers or carcass characteristics. These findings indicate that OMG (or NTG) supplementation may affect the storage and redistribution of energy deposits: expressed by changes in KPH fat and LM area. However, research is needed to provide more information on how NTG supplementation may have brought about the observed changes.

Very little information is available that evaluates the effects of NTG on ruminal parameters digestibility. Prior research in dairy cows supplemented with OMG reported no effects of supplementation on ruminal pH and VFA concentrations (31). In the current study, NTG decreased ruminal propionate molar proportion, leading to increased estimated methane energy loss. According to these results appears that NTG may affect microorganisms that enhance methanogens. Nevertheless, components of NTG, such as dehydrated brewer's yeast, have been observed to increase ruminal propionate molar concentration in dairy cattle (32). However, as previously stated, to the authors' knowledge, there are no prior studies evaluating the effects of NTG (or OMG) on diet digestibility or ruminal kinetics in cattle fed a grain-based feedlot finishing diet.

Conclusion

Supplementing calf-fed Holstein steers with NTG increased the live weight of steers during the initial 56 days on feed. However, despite decreasing propionate concentration, treatment effects were not appreciable on overall cattle growth performance. Calf-fed Holstein steers supplemented with NTG had a greater percentage of KPH fat and LM area. However, NTG supplementation had no major effect on diet digestibility and ruminal temperature.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Procedures for animal care and management were conducted under protocol (#20548) approved by the University of California, Animal Use and Care Advisory Committee.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This project was supported through the University of California Agricultural Experiment Station with Hatch funding from the USDA National Institute of Food and Agriculture (CA-D-ASC-6578-H).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Broadway P, Carroll J, Callaway T. Alternative antimicrobial supplements that positively impact animal health and food safety. *Agri Food Anal Bacteriol.* (2014) 4:109–21.

2. Sanchez NCB, Carroll JA, Broadway PR, Schell TH, Puntenney SB, McLean DJ. Supplementation of OmniGen-AF improves the metabolic response to a glucose tolerance test in beef heifers. *Transl Anim Sci.* (2019) 3:1521–9. doi: 10.1093/tas/txz130

3. Wu Z, Alugongo GM, Xiao J, Li J, Yu Y, Li Y, et al. Effects of an immunomodulatory feed additive on body weight, production parameters, blood metabolites, and health in multiparous transition Holstein cows. *J Anim Sci.* (2019) 90:167–77. doi: 10.1111/asj.13066

4. Brandão AP, Cooke RF, Correa FN, Piccolo MB, Gennari R, Leiva T, Vasconcelos JLM. Physiologic, health, and production responses of dairy cows supplemented with an immunomodulatory feed ingredient during the transition period. *J Dairy Sci.* (2016) 99:5562–5572. doi: 10.3168/jds.2015-10621

5. Leiva T, Cooke RF, Brandao AP, Schubach KM, Batista LFD, Miranda MF, et al. Supplementing an immunomodulatory feed ingredient to modulate thermoregulation, physiological, and production responses in lactating dairy cows under heat stress conditions. *J Dairy Sci.* (2017) 100:4829–38. doi: 10.3168/jds.2016-12258

6. Colombo EA, Cooke RF, Millican AA, Schubach KM, Scatolin GN, Rett B, et al. Supplementing an immunomodulatory feed ingredient to improve thermoregulation and performance of finishing beef cattle under heat stress conditions. *J Anim Sci.* (2019) 97:4085–92. doi: 10.1093/jas/skz266

7. Wang YQ, Puntenney SB, Burton JL, Forsberg NE. Use of gene profiling to evaluate the effects of a feed additive on immune function in periparturient dairy cattle. *J Anim Physiol Anim Nutr.* (2009) 93:66–75. doi: 10.1111/j.1439-0396.2007.00780.x

8. Rowson AD, Wang YQ, Aalseth E, Forsberg NE, Puntenney SB. Effects of an immunomodulatory feed additive on the development of mastitis in a mouse infection model using four bovine-origin isolates. *Animal.* (2011) 5:220–9. doi: 10.1017/S1751731110001850

9. NASEM. *Nutrient requirements of beef cattle 8[[sp]]th[[/sp]].* Washington, DC: Natl Acad Press (2016).

10. Brown H, Bing RF, Grueter HP, McAskill JW, Cooley CO, Rathmacher RP. Tylosin and Chlortetracycline for the prevention of liver abscesses, improved weight gains, and feed efficiency in feedlot cattle. *J Anim Sci.* (1975) 40:207–13. doi: 10.2527/jas1975.402207x

11. USDA. United States Standards for Grading of Carcass Beef. Washington, DC: Agricultural Marketing Service, USDA (1997).

12. Murphey CE, Hallett DK, Tyler WE, Pierce JC. *Estimating Yields of Retail Cuts from Beef Carcasses.* Presented at the 62nd Meet Am Soc Anim Prod. Chicago, IL (1960).

13. NASEM. Nutrient Requirements of Beef Cattle (6[[sp]]th[[/sp]] Ed.). Washington, DC: National Academy Press (1984)

14. Zinn RA. Influence of lasalocid and monensin plus Tylosin on comparative feeding value of steam-flaked versus dry-rolled corn diets for feedlot cattle. *J Anim Sci.* (1987) 65:256–66. doi: 10.2527/jas1987.651256x

15. Zinn RA. Shen Y. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feed lot calves. *J Anim Sci.* (1998) 76:1280–9. doi: 10.2527/1998.7651280x

16. Thom EC. The discomfort index. *Weatherwise.* (1959) 12:57–61. doi: 10.1080/00431672.1959.9926960

17. NOAA (National Oceanic and Atmospheric Administration). Livestock Hot Weather Stress. Regional Operations Manual Letter C-31–76. Central Region, Kansas City, MO (1976) US Dept Commerce, Natl Weather Serv.

18. Zinn RA. Crude protein and amino acid requirements of growing-finishing Holstein steers gaining 1.43 kilograms per day. *J Anim Sci.* (1988) 66:1755–63. doi: 10.2527/jas1988.6671755x

19. Bergen WG, Purser DB, Cline JH. Effect of ration on the nutritive quality of rumen microbial protein. *J Anim Sci.* (1968) 27:1497–501. doi: 10.2527/jas1968.2751497x

20. AOAC. *Official Methods of Analysis of AOAC International. 17th ed.* Gaithersburg, MD: Assoc Off Anal Chem (2000).

21. Van Soest PJ, Robertson B, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* (1991) 74:3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

22. Hill FW, Anderson DL. Comparison of metabolizables energy and productive energy determinations with growing chicks. *J Nutr.* (1958) 64:587–603. doi: 10.1093/jn/64.4.587

23. Zinn RA. Influence of flake density on the comparative feeding value of steam-flaked corn for feedlot cattle. *J Anim Sci.* (1990) 68:767–75. doi: 10.2527/1990.683767x

24. Zinn RA, Owens FN. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can J Anim Sci.* (1986) 66:157–66. doi: 10.4141/cjas86-017

25. Ørskov ER, MacLeod NA, Kyle DJ. Flow of nitrogen from the rumen and abomasum in cattle and sheep given protein-free nutrients by intragastric infusion. *Br J Nutr.* (1986) 56:241–8. doi: 10.1079/BJN19860103

26. Wolin, MJ. A theoretical rumen fermentation balance. *J Dairy Sci.* (1960) 43:1452–1459. doi: 10.3168/jds.S0022-0302(60)90348-9

27. Hollis LC. Investigating feedlot respiratory disease outbreaks, chapter 128 - food animal practice (Fifth Edition), W.B. Saunders (2009). p. 673–76. doi: 10.1016/B978-141603591-6.10128-9

28. Moriel P, Palmer EA, Oliveira RA, Vedovatto M, Izquierdo VS, Silva HM, et al. Stair-step strategy and immunomodulatory feed ingredient supplementation for grazing heat-stressed Bos indicus-influenced beef heifers. *J Anim Sci.* (2022) 100:1–13. doi: 10.1093/jas/skac107

29. Brown-Brandl TM, Eigenberg RA, Nienaber JA, Hahn GL. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, Part 1: analyses of indicators. *Biosyst Eng.* (2005) 90:451–462. doi: 10.1016/j.biosystemseng.2004.12.006

30. Buntyn JO, Sieren SE, Curtis JBB, Dirk B, Galen EE, Sanchez NCB, et al. Effects of Feeding OmniGen- AF® on Immune Function, Performance, and Carcass Characteristics during the Feeding Period. Nebraska Beef Cattle Reports 864 (2016).

31. Mezzetti M, Minuti A, Piccioli-Cappelli F, Gabai G, Trevisi E. Administration of an immune stimulant during the transition period improved lipid metabolism and rumination without affecting inflammatory status. *Animals.* (2019) 28:619. doi: 10.3390/ani9090619

32. Baker LM, Kraft J, Karnezos TP, Greenwood SL. Review: the effects of dietary yeast and yeast-derived extracts on rumen microbiota and their function. *Animal Feed Sci Technol.* (2022) 294:115476. doi: 10.1016/j.anifeedsci.2022.115476

33. NASEM. *Nutrient requirements of beef cattle 7[[sp]]th[[/sp]] rev. ed.* Washington, DC: Natl Acad Press (2000).

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 06 March 2023
DOI 10.3389/fvets.2023.1090097

OPEN ACCESS

EDITED BY
Anil Kumar Puniya,
National Dairy Research Institute (ICAR), India

REVIEWED BY
Anusorn Cherdthong,
Khon Kaen University, Thailand
Kendall C. Swanson,
North Dakota State University, United States

*CORRESPONDENCE
Danilo D. Millen
✉ danilo.millen@unesp.br

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 04 November 2022
ACCEPTED 14 February 2023
PUBLISHED 06 March 2023

CITATION
Silva TIS, Souza JM, Acedo TS, Carvalho VV, Perdigão A, Silva LAF, Silvestre AM, Niehues MB, Schleifer WF, Casali DM, Martins CL, Arrigoni MDB and Millen DD (2023) Feedlot performance, rumen and cecum morphometrics of Nellore cattle fed increasing levels of diet starch containing a blend of essential oils and amylase or monensin. *Front. Vet. Sci.* 10:1090097. doi: 10.3389/fvets.2023.1090097

Feedlot performance, rumen and cecum morphometrics of Nellore cattle fed increasing levels of diet starch containing a blend of essential oils and amylase or monensin

Thaiano I. S. Silva[1], Johnny M. Souza[1], Tiago S. Acedo[2], Victor V. Carvalho[2], Alexandre Perdigão[2], Leandro A. F. Silva[1], Antonio M. Silvestre[3], Maria Betania Niehues[1], Werner F. Schleifer[1], Daniel M. Casali[1], Cyntia L. Martins[3], Mario D. B. Arrigoni[3] and Danilo D. Millen[1]*

[1]Department of Animal Production, College of Agricultural and Technological Sciences, São Paulo State University (UNESP), Dracena, Brazil, [2]DSM Nutritional Products SA, São Paulo, Brazil, [3]São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu, São Paulo, Brazil

Feed additives used in finishing diets improve energy efficiency in ruminal fermentation, resulting in increased animal performance. However, there is no report evaluating the effect of BEO associated with exogenous α-amylase in response to increased starch content in feedlot diets. Our objective was to evaluate increasing levels of starch in the diet associated with a blend of essential oils plus amylase or sodium Monensin on performance, carcass characteristics, and ruminal and cecal morphometry of feedlot cattle. 210 Nellore bulls were used (initial body weight of 375 ± 13.25), where they were blocked and randomly allocated in 30 pens. The experiment was designed in completely randomized blocks in a 3 × 2 factorial arrangement: three starch levels (25, 35, and 45%), and two additives: a blend of essential oils plus α-amylase (BEO, 90 and 560 mg/kg of DM, respectively) or sodium Monensin (MON, 26 mg/kg DM). The animals were fed once a day at 08:00 *ad libitum* and underwent an adaptation period of 14 days. The diets consisted of sugarcane bagasse, ground corn, soybean hulls, cottonseed, soybean meal, mineral-vitamin core, and additives. The animals fed BEO35 had higher dry matter intake ($P = 0.02$) and daily weight gain ($P = 0.02$). The MON treatment improved feed efficiency ($P = 0.02$). The treatments BEO35 and BEO45 increased hot carcass weight ($P < 0.01$). Animals fed BEO presented greater carcass yield ($P = 0.01$), carcass gain ($P < 0.01$), rib eye area gain ($P = 0.01$), and final rib eye area ($P = 0.02$) when compared to MON. The MON25 treatment improved carcass gain efficiency ($P = 0.01$), final marbling ($P = 0.04$), and final subcutaneous fat thickness ($P < 0.01$). The use of MON reduced the fecal starch% ($P < 0.01$). Cattle-fed BEO increased rumen absorptive surface area ($P = 0.05$) and % ASA papilla area ($P < 0.01$). The MON treatment reduced the cecum lesions score ($P = 0.02$). Therefore, the use of BEO with 35 and 45% starch increases carcass production with similar biological efficiency as MON; and animals consuming MON25 improve feed efficiency and reduce lesions in the rumen and cecum.

KEYWORDS

additives, concentrate, efficiency, fermentation, non-fiber carbohydrates

1. Introduction

The increase in performance associated with lowering the age at slaughter is often achieved through the use of high-concentrate feeds to meet energy requirements and maximize production in feedlot cattle (1, 2). The inclusion of grains in diets, as well as the extent of processing, promotes an increase in starch availability and starch breakdown in the rumen, thereby increasing energy intake and rumen fermentation, resulting in increased microbial protein production and high short-chain fatty acid (SCFA) release (3). However, in response to this increased availability of starch in the rumen, negative effects may also occur, such as a decrease in digestibility of fibrous carbohydrates and metabolic disturbances (4). The use of large amounts of starch in conjunction with proper feed management and feed additives is essential for controlling metabolic disorders such as acidosis and bloat (5).

Feed additives used in finishing diets improve energy efficiency in ruminal fermentation, resulting in increased animal performance. Diets with high starch content promote high production of SCFA in the rumen, which leads to a drop in pH. The use of additives is important for controlling rumen acidification and manipulating the rumen fermentation process to maximize the utilization of dietary nutrients (6). Among them, monensin (MON) is widely used in feedlot diets to prevent diseases and metabolic disorders, as well as to improve feed efficiency and animal performance (2, 7). However, the European Union have banned the use of ionophores as they are classified as antibiotics and growth promoters (European Union Regulation, 1831/2003/EC), considering the risk of these products is increasing bacterial resistance to antibiotics and, consequently, possible risks to human health (8).

In this context, this has instigated a search for natural alternatives to ionophores, such as essential oils and their blends (BEO), which act as growth promoters when added to animal feed diets (9). BEO are plant-derived compounds such as thymol, with known antimicrobial, anti-inflammatory, antioxidant, and coccidiostatic properties, that can modulate rumen fermentation to improve nutrient utilization in ruminants (10, 11). The BEO has been reported to make the rumen more energy efficient by increasing the molar ratio of propionate and decreasing the acetate:propionate ratio in the rumen (11), thereby improving nutrient utilization and animal performance (12, 13). BEO can also decrease amino acid deamination and ammonia production in the rumen, improve protein digestibility and promote greater nitrogen retention (11, 14, 15). In this context, some studies have been conducted to evaluate the efficacy of essential oils fed to cattle on animal performance and meat quality (16–18). However, the animal performance was similar in animals that did not receive additives, MON, or BEO. In addition, BEO functionality depends on the source and composition of BEO, as well as the extraction method and dosages used (19, 20). In this context, BEO is an additive with the potential to be utilized in association with monensin or other additives.

It has been suggested that exogenous amylase improves feed efficiency by increasing nutrient utilization, resulting in improved animal performance (18). Some studies reported increased milk production, improved conversion of feed to milk, and increased starch digestion in the rumen (21–23) in response to the administration of exogenous amylase to lactating dairy cows. However, there are few studies investigating the effect of exogenous amylase in finishing cattle (18, 24, 25). Meschiatti et al. (18) reported synergism between BEO and exogenous amylase in a feedlot finishing diet, where the combination of both additives resulted in an increase in DMI, ADG, BW, and hot carcass weight compared to monensin. In addition, BEO in combination with exogenous amylase was reported to reduce liver abscesses and fecal starch in cattle fed high-starch diet (18, 26).

However, based on the aforementioned information, there is no report evaluating the effect of BEO associated with exogenous α-amylase in response to increased starch content in a feedlot diet. It was hypothesized that BEO should be an alternative to MON in finishing feedlot diets, as well as that BEO associated with exogenous enzymes would further improve nutrient utilization and animal performance in high-starch diets, reducing fecal starch and cecum lesions score. Therefore, the objective of this study was to evaluate the increasing starch content in the diet associated with a blend of essential oils plus amylase or sodium monensin on performance, carcass characteristics, and ruminal and cecal morphometry of feedlot cattle.

2. Material and methods

All the procedures involving the use of animals in this study were by the guidelines established by the São Paulo State University Ethical Committee for Animal Research, and approved by Ethical Commission in Use of Animals of the Innovation and Applied Science DSM Nutritional Products SA (number BR190313).

2.1. Animals and treatments

The trial was conducted at the feedlot facility of the Center for Innovation and Applied Science in Ruminants, from DSM Nutritional Products (I&AS Beef Center; Rio Brilhante, Mato Grosso do Sul, Brazil), using 210 22-mo-old yearlings Nellore bulls (375 ± 13.25 kg). Animals were housed in 30 pens, with 120 m^2 of area, a water trough, and a collective bunk (50 cm/animal, 5 m of bunk/pen).

The experimental design was performed in completely randomized blocks and the initial BW was utilized as a criterion for block formation, in a 3 × 2 factorial arrangement, in which were evaluated: 3 levels of starch (25, 35, and 45%) and 2 feed additives (BEO + α-amylase or sodium monensin), totaling 6 treatments: (1) MON25: 25% of starch + monensin; (2) BEO25: 25% of starch + essential oil and α-amylase; (3) MON35: 35% of starch + monensin; (4) BEO35: 35% of starch + essential oil and α-amylase; (5) MON45: 45% of starch + monensin; (6) BEO45: 45% of starch + essential oil and α-amylase. Each treatment had 5 replicates (pen as an experimental unit), with 7 animals/pen (total of 30 pens).

The sodium monensin (Rumensin, Elanco, Greenfield, Indiana, USA) was included in the diet at a dose of 26 ppm. The blend of essential oil (Crina ® Ruminants DSM Nutritional Products Ltd.,

Basel, Switzerland) which contains thymol, eugenol, limonene, and vanillin (14), and the exogenous enzyme α-amylase (Ronozyme Rumistar; DSM Nutritional Products Ltd., Basel, Switzerland) were added to the diet at a dose of 90 and 560 mg/kg of DM, respectively. The doses of feed additives used in this study were to the company's recommendations. It's noteworthy to mention that MON has been largely used around the world, and since results have been very consistent over decades, it was adopted as a positive control in this study.

2.2. Feeding and management description

At the beginning of the study, all yearling bulls were weighed, dewormed, and vaccinated (tetanus, bovine viral diarrhea virus, 7-way *Clostridium* sp.; Cattlemaster and Bovishield, Pfizer Animal Health, New York, NY). The animals were submitted to a pre-adaptation period of 10 days to standardize their ruminal population and adapt to the facilities and management. Cattle were fed *ad libitum* once a day at 08:00 h, targeting 3–5% refusal, with free-choice water access to a water trough.

The experimental diets were formulated according to the LRNS (Large Ruminant Nutrition System, Table 1), meeting the nutritional requirements, with daily weight gains between 1.5 and 1.7 kg/day/animal. The experimental diets were composed of sugarcane bagasse, ground corn, soybean hulls, cottonseed, soybean meal, mineral-vitamin core, urea, and additives. The step-up adaptation program diet consisted of *ad libitum* intake and lasted 14 days, whereas 2 adaptation diets containing 65 and 75% concentrate were fed for 7 days each (Table 1). The finishing period program also consisted of *ad libitum* intake and lasted 89 days, where the finishing diet contained 85% concentrate. The different starch content of the diets (25, 35, and 45%) was obtained by increasing the proportion of Corn grain fine grind. During the experimental period, weekly samples were taken of the rations for the chemical analysis of dry matter (DM), crude protein (CP), ether extract (EE), and mineral matter (MM) according to AOAC (27), and neutral detergent fiber (NDF) according to Van Soest et al. (28).

The duration of the experimental period was 99 days, which included a pre-adaptation period of 10 days (day −10 to day 0), a step-up adaptation program diet of 14 days (day 0 to day 14), and a finishing period program of 89 days (day 14 to day 89).

2.3. Feedlot performance and carcass traits

At the beginning of the experimental period (day 0), and 30 and 89 days of the study, the feed was withheld from bulls for 16 h before every body weight (BW) assessment. Consequently, average daily gain (ADG), and feed efficiency (gain-to-feed, G/F) were calculated at the end of the experiment. The DMI was calculated for each pen daily, by weighing the ration offered and refused before the next morning delivery and expressed in kilograms and as a percentage of BW. To estimate the net energy for maintenance and net energy for gain, the methods described by Lofgreen and Garrett (29), NRC (30), and Zinn and Shen (31) were used. The values obtained by the equations of net energy for gain were related to the average values estimated by the LRNS, proportional to each diet (25, 35, and 45% starch) and additives evaluated (32).

At the end of the adaptation period, one animal from each block ($n = 6$) was randomly chosen to be slaughtered as a reference to the evaluation of the gain composition in the function of the initial BW, and determination of the equation of adjustment of the BW in the function of the carcass weight (model equation reference: HCW = 12.2581 + 0.4837*initial BW). The remaining Nellore yearling bulls were harvested at the end of the study. The final BW was obtained at the feedlot before truck loading, and the cattle were then transported to a commercial abattoir. The hot carcass weight (HCW) was obtained after kidney, pelvic, and heart fat removal. The dressing percentage was calculated by dividing HCW by the final BW (33).

2.4. Feeding behavior and particle sorting

All yearling bulls were submitted to visual observations to evaluate feeding behavior, every 5 min, over one period of 24 h (day 71), according to Johnson and Combs (34). Feeding behavior data were recorded for each animal as follows: time spent resting, ruminating, and eating (expressed in minutes), and the number of meals per day. Meal length (expressed in minutes) was calculated by dividing the time spent eating by the number of meals per day. The DMI per meal in kilograms was calculated by dividing DMI by the number of meals per day. In addition, data on time spent eating and ruminating were used to calculate the eating rate of DM (time spent eating / DMI) and rumination rate of DM (time spent ruminating / DMI), both expressed in minutes per kilogram of DM, according to Pereira et al. (35).

During the observation period, samples of diets and orts were collected after 24 h for future analyses of DM [AOAC, (27)] and neutral detergent fiber [NDF; (28)], to determine DMI and NDF intake on the day of the feeding behavior evaluations. Moreover, the eating rate (DM and NDF), as well as the rumination rate (DM and NDF), time spent eating per meal, and dry matter intake per meal was calculated as described by Carvalho et al. (36).

On the day of observation of feeding behavior (day 71), samples of diets and orts were also collected from all pens for the determination of particle size distribution, which was performed by sieving using the Penn State Particle Size Separator (Nasco, Fort Atkinson, WI, EUA) and reported on an as-fed basis as described by Heinrichs and Kononoff (37). Particle sorting was determined as follows: n intake/n predicted intake, in which n = particle fraction retained on screens of 19 (long), 8 (medium), and 1.18 mm (short) and a pan (fine). Particle sorting values equal to 1 indicate no sorting. Those <1 indicate selective refusal (sorting against), and those >1 indicate preferential consumption sorting for Leonardi and Armentano (38).

2.5. Rumen and cecum morphometrics

Rumenitis evaluation was performed after bull evisceration, and all rumens were scored after washing. Rumen epithelium was classified according to the incidence of lesions (rumenitis and

TABLE 1 Feed ingredients and chemical composition of diets containing levels of starch fed to Nellore yearling bulls supplemented with essential oils and amylase or sodium monensin.

Starch, %[a]	25%			35%			45%		
Concentrate, %[b]	65%	75%	85%	65%	75%	85%	65%	75%	85%
Ingredients, % of DM[c]									
Sugarcane bagasse	35	25	15	35	25	15	35	25	15
Corn grain fine grind	30	33	36	30	40	50	30	47	64
Soybean meal	9	5,5	2	9	6,5	4	9	7,5	6
Cottonseed	6	8	10	6	8	10	6	8	10
Soybean hull	15	23,5	32	15	15,5	16	15	7,5	0
Mineral Supplement	5	5	5	5	5	5	5	5	5
Nutrient content, % of DM[d]									
Crude protein	14,6	14,7	14,6	14,6	14,7	14,6	14,6	14,5	14,5
Total digestible nutrients	66	68	69	66	69	73	66	72	77
DIP	51	51	50	51	51	52	51	52	53
Neutral detergent fiber	43,7	42,4	41,2	43,7	38,2	33	43,7	31,6	25,2
peNDF[d]	36	30	25	36	29	23	36	28	22
Ca	0,77	0,75	0,73	0,77	0,75	0,73	0,77	0,76	0,75
P	0,31	0,28	0,25	0,31	0,31	0,31	0,31	0,36	0,37
Starch	20,95	23,08	25,46	20,95	28,40	35,50	20,95	37,28	45,80
ME, Mcal/kg DM[e]	2,40	2,44	2,48	2,40	2,51	2,63	2,40	2,62	2,77

[a] Level of starch (25, 35, and 45%); [b] Percent of concentrate of step-up adaptation diets and finishing diet: Adaptation 1 = 65% concentrate, 0–7 days; Adaptation 2 = 75% concentrate, 7–14 days; Finishing diet = 85% concentrate, 14–89 days; [c] DM, dry matter; [d] Estimated by equations according to Large Ruminant Nutrition System (LRNS; 20). [e] Metabolizable energy. DIP, degradable intake protein.

hyperkeratosis) as described by Bigham and McManus (39) based on a scale of 0 (no lesions) to 10 (severe ulcerative lesions).

A small fragment (1 cm^2) of each rumen was collected from the dorsal cranial sac, and placed into a phosphate-buffered saline solution for future morphometric measurements according to Resende Júnior et al. (40). The number of papillae per square centimeter of rumen wall (NOP) was determined manually, where 12 papillae were randomly collected from each fragment (1 cm^2) and scanned, and the mean papillae area (MPA) was determined using an image analysis system (Image Tool, version 2.01-4; UTHSCSA Dental Diagnostic Science, San Antonio, TX, USA). The rumen wall absorptive surface area (ASA) was calculated according to Daniel et al. (41).

For histological analyzes, a method adapted from Odongo et al. (42) was used. A 1 cm^2 fragment of each rumen was collected from the ventral cranial sac, stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned. Histological measurements, such as papillae height, papillae width, papillae surface area, and keratinized layer thickness were performed in four papillae per bull using a computer-aided light microscope image analysis. Measurements were performed per animal, in 10% of the total papillae per cm^2, which were chosen randomly. The final value for each variable was the average of measured papillae (Figure 1).

The same 1-cm^2 fragment collected from the ventral cranial sac was also used for the evaluation of cell proliferation of rumen papillae according to the immunohistochemistry method adapted from Pereira et al. (43). To determine the mitotic index, the nuclei of 2,000 cells of the basal layer of the ruminal epithelium were marked, and among these, the number of mitotic figures was counted. Thus, the proportion of cells in mitosis in the basal layer of the ruminal epithelium was determined.

Cecum epithelium was classified according to the presence of cecal wall lesions and abnormalities, according to the method adapted from Pereira et al. (44). Histological measurements, such as crypt depth and goblet cells, were determined in 10% of the total number of crypts per animal, using a Leica Qwin Image Analyzer within a Leica electron light microscope.

2.6. Shear force analysis and meat color

For meat quality analysis, 2.54-cm thick steak samples of *Longissimus dorsi* (LM) were obtained between the 12th and 13th ribs of animals (3 animals/pen). The samples were identified, vacuum packaged, and frozen at −20°C for further analysis. The analyzes were performed at the Food Products of Animal Origin Laboratory (Economy, Sociology and Technology Department—School of Agriculture UNESP—Botucatu, Brazil).

For the shear force analysis, the samples were thawed, for 24 h at 2°C, and processed according to the American Meat Science Association (AMSA) (45) and Wheeler et al. (46) using a Warner—Bratzler shear force device. The shear force of each sample was considered as the average of 6–8 repetitions (cylinders).

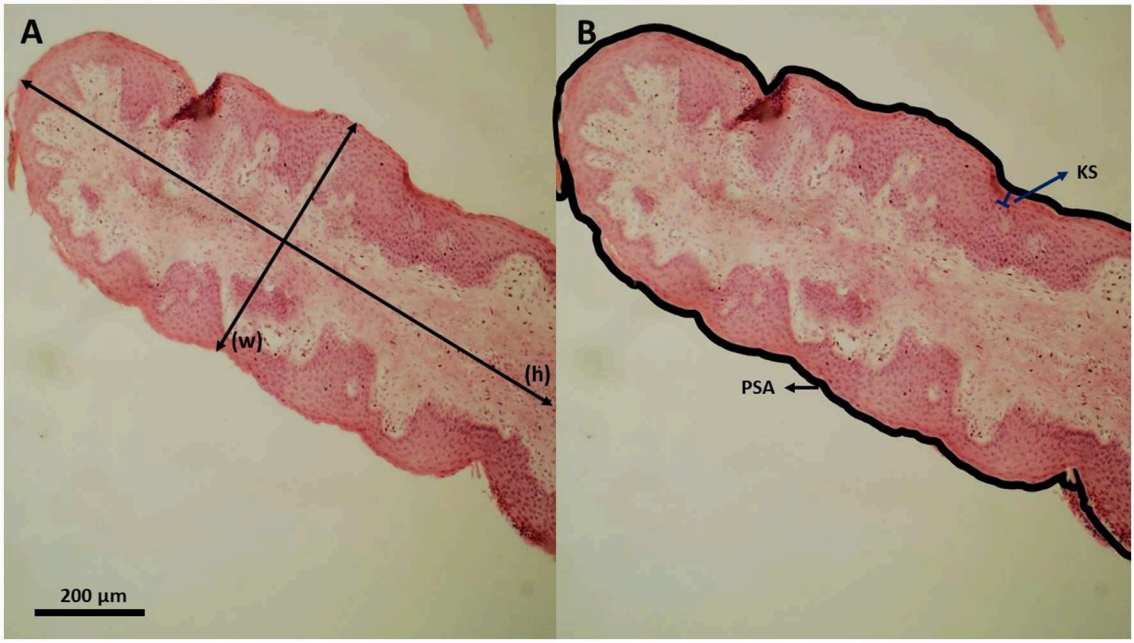


FIGURE 1
Histological section of a cattle ruminal papilla; **(A)** Papillae height (h) and papillae width (w; measured perpendicular to papilla length). **(B)** Papillae surface area (PSA; thick outer line) and stratum corneum thickness (KS; keratinized surface).

The analysis of cooking losses was performed following the methodology described by Honikel (47).

The color was determined by the CIE Lab system (48). Color measurement was performed in three distinct points of the sample, utilizing a spectrophotometer, model CM2500d (Konica Minolta Brazil, São Paulo, Brazil) with D65 illuminating standard, observation angle of 10°, and 30 mm shutter opening. The samples were left exposed to the environment for 20 min for color determination, where L* is the chrome associated with luminosity (L* = 0 black; 100 white), a* is the chrome that varies from green (–) to red (+), and b* is the chrome that ranges from blue (–) to yellow (+). Color values were considered the average of the three readings.

For the evaluation of the proximate composition, samples of *Longissimus dorsi* were used, and thawed the day before the analysis in a refrigerator at 2–5°C. Moisture was evaluated according to AOAC (method 39.1.02) (49). The total nitrogen was quantified using the Kjeldahl method according to AOAC (method 39.1.19) (49). Total protein was calculated as a function of total nitrogen, using the conversion index of 6.25. The ethereal extract was determined according to AOAC (method 39.1.05) (49). Ash was determined according to AOAC (method 39.1.09) (49).

2.7. Statistical analysis

The experimental design was performed in completely randomized blocks, and the initial BW was utilized as a criterion for block formation, in a 3 × 2 factorial arrangement of treatments (3 levels of starch and 2 feed additives). Pens were considered the experimental unit for this study ($n = 30$), and each treatment was replicated five times. Data were analyzed using the MIXED procedure of SAS (SAS, 2003) and the Tukey test to compare means. Tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and heterogeneity of treatment variances (GROUP option of SAS) were performed before analyzing the data. The model also included fixed effects of treatments and their interaction. The block was considered a random effect in the model. Results were considered significant at $P \leq 0.05$ level.

3. Results

3.1. Feedlot performance and carcass traits

The results of feedlot performance are presented in Table 2. A significant interaction between starch level and additives was observed for BW ($P < 0.01$) and ADG ($P = 0.01$) on the first 30 days of the study, where the cattle fed BEO45 had greater values when compared with MON in the same levels of starch. Similarly, there is a significant interaction between starch level and additives for DMI ($P < 0.01$) and DMI expressed as %BW ($P = 0.01$) on the first 30 days of the study; and for final BW ($P = 0.04$), ADG ($P = 0.02$), DMI ($P = 0.02$) and DMI expressed as %BW ($P = 0.05$) at the end of the experimental period, where the cattle fed BEO35 had greater values when compared with MON35 and MON45. No effects of treatments were observed ($P > 0.05$) for G/F in the first 30 days of the study. However, at the end of the study, there was a significant linear decrease in G/F ($P = 0.05$) in response to increasing levels of starch (0.151, 0.149, and 0.145, for

TABLE 2 Levels of starch associated with a blend of essential oils or sodium monensin on feedlot performance of Nellore yearling bulls.

Treatments[a]	BEO			MON				*P*-value[c]		
Level of starch, %	25%	35%	45%	25%	35%	45%	SEM[b]	Additive	Starch	Additive∗Starch
Initial BW, kg[d]	375.99	376.02	378.61	374.98	375.14	373.94	5.61	0.17	0.90	0.53
BW 30 days, kg	421.33^{b}	428.57^{a}	431.86^{a}	422.02^{ab}	418.64^{b}	417.39^{b}	4.62	0.05	0.47	<0.01
Final BW, kg	510.35^{a}	516.72^{a}	510.27^{a}	506.45^{ab}	497.23^{bc}	487.47^{c}	5.68	<0.01	0.04	0.04
ADG 30 days, kg	1.46^{b}	1.70^{a}	1.72^{a}	1.52^{b}	1.40^{b}	1.40^{b}	0.07	<0.01	0.49	0.01
ADG, kg/days	1.51^{ab}	1.58^{a}	1.48^{b}	1.48^{b}	1.37^{c}	1.28^{d}	0.04	<0.01	<0.01	0.02
DMI 30 days, kg/days	9.30^{b}	9.92^{a}	9.73^{a}	9.22^{b}	8.92^{c}	8.77^{c}	0.10	<0.01	0.16	<0.01
DMI, kg/days	10.37^{ab}	10.68^{a}	10.29^{b}	9.54^{c}	9.16^{d}	8.76^{e}	0.11	<0.01	<0.01	0.02
DMI, % of BW 30 days, kg/days	2.33^{c}	2.47^{a}	2.40^{b}	2.31^{c}	2.25^{d}	2.22^{d}	0.12	<0.01	0.08	0.01
DMI, % of BW	2.34^{ab}	2.39^{a}	2.32^{b}	2.16^{c}	2.10^{cd}	2.03^{d}	0.02	<0.01	<0.01 (L)	0.05
G/F ratio 30 days, kg/kg	0.157	0.171	0.177	0.165	0.158	0.160	0.008	0.19	0.58	0.15
G/F, kg/kg	0.146	0.148	0.144	0.155	0.150	0.146	0.004	0.02	0.05 (L)	0.19
Fecal starch, %	4.94	10.73	9.76	3.47	6.86	7.38	0.64	0.05	<0.0001 (Q)	0.39

[a]BEO: blend of essential oil (Crina ® Ruminants and exogenous enzyme α-amylase Ronozyme RumiStar^{TM}); MON: monensin; [b]SEM = Standard error mean; [c]L = linear effect; Q = quadratic effect; [d]BW, body weight; G: F, gain-to-feed ratio; ADG, average daily gain; DMI, dry matter intake. Values within a row with different superscripts differ ($P < 0.05$).

25, 35, and 45% starch, respectively). Moreover, cattle-fed MON improved G:F (0.146 vs. 0.150, for BEO and MON, respectively, $P = 0.02$). Regarding fecal starch, there was a significant ($P < 0.01$) quadratic effect in response to increasing levels of starch, where cattle fed BEO had greater fecal starch content when compared with MON (8.48 vs. 5.90, for BEO and MON, respectively, $P = 0.05$; Table 2).

The results of carcass traits are presented in Table 3. A significant interaction between starch level and additives was observed for final HCW ($P = 0.01$) and HCW gain ($P = 0.01$), where the cattle fed BEO25 and BEO35 had greater values when compared with MON35 and MON45. However, a significant interaction between starch level and additives was observed for final carcass gain efficiency ($P = 0,01$), biological efficiency ($P = 0,01$), final marbling ($P = 0,04$), final subcutaneous fat thickness ($P = 0,01$) and P8 rump fat thickness gain ($P = 0,01$), where the cattle fed MON25 had greater values when compared to BEO25 and BEO45. Moreover, there was a significant linear decrease in dressing percentage ($P = 0.01$), carcass gains ($P = 0.05$), final P8 rump fat thickness ($P = 0.01$), rib-eye area gain ($P = 0.03$) and P8 rump fat thickness gain ($P = 0.01$) in response to increasing levels of starch. In addition, cattle fed BEO had higher dressing percentage (55.38 vs. 54.56 %, for BEO and MON, respectively, $P = 0.01$), carcass gain (66.78 vs. 64.33 %, for BEO and MON, respectively, $P = 0.01$), rib-eye area (67.72 vs. 65.47 cm^2, for BEO and MON, respectively, $P = 0.02$) and rib-eye area gain (0.18 vs. 0.16 cm^2/days for BEO and MON, respectively, $P = 0.01$) when compared with MON.

The results of meat color and chemical composition are presented in Table 4. There was a significant quadratic effect for cooking losses ($P < 0.01$) and moisture ($P = 0.02$) in response to increasing levels of starch, where the highest value for cooking loss and the lowest value for moisture was obtained on a 35% starch diet. No effects of treatments were observed ($P > 0.05$) for chroma L, chroma A, chroma B, pH, marbling, shear force, ash, and protein (Table 4).

3.2. Feeding behavior and particle sorting

The results on feeding behavior are summarized in Table 5. A significant interaction between starch level and additives was observed for feed efficiency ($P = 0.04$), time spent resting ($P = 0.02$), and meal length ($P = 0.02$). Regarding feed efficiency, the highest value was observed in cattle fed MON45, followed by MON35 and BEO45. For time spent resting, the highest values were observed for BEO35 and MON45. In addition, for meal length, the lowest values were observed for BEO45 and MON45. Regarding particle sorting, no significant treatment effect was observed ($P > 0.05$) for most of the feeding behavior and particle sorting variables evaluated. However, animals fed CR35 sorted ($P = 0.02$) more intensively for medium diet particles when compared to MON35.

There was a significant quadratic effect for DMI ($P < 0.01$) in response to increasing levels of starch, where the highest value was obtained on 35% starch (11.01, 11.37, and 10.66 kg/days for 25, 35, and 45% starch, respectively). Moreover, there was a significant linear increase in time spent eating ($P < 0.01$), meals per day ($P < 0.01$), eating rate of NDF ($P < 0.01$), rumination rate of NDF ($P < 0.01$), in response to increasing levels of starch. There was a significant linear decrease in DMI per meal ($P < 0.01$) and eating rate of NDF ($P < 0.01$) in response to increasing levels of starch.

Besides that, DMI (11.65 vs. 10.37 kg/days, for BEO and MON, respectively; $P < 0.01$), time spent ruminating (244.86 vs. 219.86 min, for BEO and MON, respectively; $P = 0.02$) and NDF

TABLE 3 Levels of starch associated with *blend of* essential oils or sodium monensin on carcass characteristics of Nellore yearling bulls.

Treatments[a]	BEO			MON				*P*-value[c]		
Level of starch, %	25%	35%	45%	25%	35%	45%	SEM[b]	Additive	Starch	Additive∗Starch
Initial Hot carcass weight, kg	194.12	194.14	195.39	193.64	193.71	193.13	2.71	0.17	0.90	0.53
Final Hot carcass weight, kg	282.76^{ab}	287.42^{a}	281.38^{b}	279.90^{b}	270.91^{c}	263.00^{d}	3.91	<0.01	<0.01	0.01
Hot carcass weight gain, kg	1.00^{ab}	1.06^{a}	0.98^{b}	0.98^{b}	0.88^{c}	0.79^{d}	0.02	<0.01	<0.01	0.01
Carcass gain efficiency, kg/kg	0.097^{b}	0.099^{ab}	0.095^{bc}	0.102^{a}	0.096^{b}	0.090^{c}	0.001	0.592	0.01	0.01
Dressing, %	55.39	55.62	55.13	55.26	54.48	53.95	0.32	0.01	0.01 (L)	0.07
Carcass gain, %	66.90	67.40	66.03	66.46	64.18	62.34	1.96	0.01	0.05 (L)	0.22
Biological efficiency, kg DM/@	156.8^{b}	152.9^{bc}	160.14^{ab}	148.18^{c}	158.81^{b}	167.51^{a}	2.05	0.51	0.01	0.01
Initial rib-eye area, cm^2	50.99	51.16	51.04	50.85	51.16	50.83	0.62	0.88	0.96	0.99
Final rib-eye area, cm^2	67.91	68.44	66.82	66.52	65.73	64.17	0.73	0.02	0.24	0.79
Initial marbling, %	2.05	2.15	2.22	2.2	1.93	2.26	0.054	0.87	0.07	0.08
Final marbling, %	2.45^{b}	2.61^{ab}	2.64^{ab}	2.72^{a}	2.51^{b}	2.65^{ab}	0.05	0.314	0.47	0.04
Initial sub. fat thickness, mm	2.24	2.25	2.29	2.31	2.27	2.41	0.03	0.03	0.08	0.53
Final sub. fat thickness, mm	3.77^{b}	3.95^{ab}	3.97^{ab}	4.33^{a}	3.81^{b}	3.71^{b}	0.101	0.64	0.28	0.01
Initial P8 rump fat thickness, mm	4.22	4.26	4.15	4.19	4.21	4.34	0.046	0.44	0.76	0.12
Final P8 rump fat thickness, mm	6.64	6.38	6.37	6.89	6.09	6.08	0.167	0.52	0.01 (L)	0.30
Rib-eye area daily gain, cm^2/days	0.19	0.19	0.17	0.17	0.16	0.14	0.01	0.01	0.03 (L)	0.49
Sub. fat thickness daily gain, mm/days	0.017^{bc}	0.019^{ab}	0.019^{ab}	0.022^{a}	0.017^{bc}	0.014^{c}	0.001	0.86	0.13	0.01
P8 rump fat thickness daily gain, mm/days	0.027	0.023	0.024	0.030	0.021	0.019	0.002	0.36	0.01 (L)	0.14

[a]BEO: blend of essential oil (Crina ® Ruminants and exogenous enzyme α-amylase Ronozyme RumiStar^{TM}); MON: monensin; [b]SEM = Standard error mean; [c]L = linear effect; Q = quadratic effect; Values within a row with different superscripts differ ($P < 0.05$).

intake (4.07 vs. 3.73 kg, for BEO and MON, respectively; $P = 0.02$) were affected by the type of additive, where cattle fed BEO obtained the best results. No effects of treatments were observed ($P > 0.05$) for the rumination rate of DM and drinking bouts.

3.3. Rumen and cecum morphometrics

The results on rumen and cecum morphometrics are presented in Table 6. There was a significant quadratic effect for rumenitis score ($P = 0.03$) in response to increasing levels of starch, where the highest value was obtained on 35% starch (0.80, 1.05, and 0.88 for 25, 35, and 45% starch, respectively).

Moreover, animals fed BEO obtained greater absorptive surface area (ASA; 32.78 vs. 29.95 cm^2/cm^2 of rumen wall, for BEO and MON, respectively; $P = 0.05$), papillae area (97.06 vs. 96.74% of ASA, for BEO and MON, respectively; $P = 0.01$) and papillae height (4.20 vs. 3.97, for BEO and MON, respectively; $P = 0.03$) when compared to MON treatment. However, the supplementation with BEO increased the cecum lesions score (2.11 vs. 1.43, for BEO and MON, respectively; $P = 0.02$), crypt/enterocytes ratio (4.72 vs. 4.51, for BEO and MON, respectively; $P = 0.01$) and crypt/goblet ratio (51.75 vs. 47.51, for BEO and MON, respectively; $P = 0.02$).

4. Discussion

Essential oils (EO) and exogenous enzymes may have the potential to replace ionophores, increase BW and ADG and contribute to improved beef production and animal performance (50), but the amount of research information on the potential benefits of these new technologies compared to the most common feed additives for feedlot cattle is limited (18). In this context, the present study answers new questions regarding the use of BEO, evaluating the effect of essential oil associated with exogenous α-amylase in response to increased starch content in a feedlot diet. The EO are aromatic oily liquids extracted from plant material that possess a broad spectrum of antimicrobial activities (11, 51). However, a detailed explanation of how EO affects ruminal fermentation by altering microorganisms has not been established (18, 50).

In the present study, cattle fed BEO35 had the highest final BW and ADG, and DMI compared with treatments at MON; however, the results for feed efficiency were more satisfactory compared with treatments fed BEO regardless of starch content than when MON was used. These results could be related to the change in rumen fermentation pattern in response to essential oil treatment in combination with exogenous α-amylase, which may alter the acetate/propionate ratio in the rumen. However, results regarding DMI in response to supplementation with EO

TABLE 4 Levels of starch associated with blend of essential oils or sodium monensin on *shear force* analysis and meat color of Nellore yearling bulls.

Treatments[a]	BEO			MON				*P*-value[c]		
Level of starch, %	25%	35%	45%	25%	35%	45%	SEM[b]	Additive	Starch	Additive∗Starch
Chroma L	39.16	39.93	39.06	39.15	39.57	38.95	0.43	0.77	0.55	0.96
Chroma A	18.84	19.34	18.52	18.96	19.69	19.04	0.32	0.42	0.28	0.92
Chroma B	8.57	8.98	8.45	8.58	9.13	8.55	0.20	0.73	0.11	0.98
pH, *n*	5.85	5.71	5.80	5.81	5.77	5.77	0.04	0.85	0.10	0.45
Cooking losses, %	27.93	30.23	26.49	27.75	29.44	28.65	0.55	0.60	<0.01 (Q)	0.39
Marbling, %	2.08	2.04	1.96	2.18	1.94	1.98	0.09	0.93	0.18	0.55
Shear force, kgf	3.98	4.26	4.48	4.31	4.34	4.55	0.14	0.25	0.18	0.62
Moisture, %	75.10	74.59	75.36	74.89	75.01	75.45	0.17	0.56	0.02 (Q)	0.31
Ash, %	1.11	1.08	1.11	1.08	1.14	1.12	0.01	0.47	0.56	0.15
Protein, %	17.65	17.57	17.95	17.85	17.81	17.65	0.12	0.74	0.85	0.30

[a]BEO: blend of essential oil (Crina ® Ruminants and exogenous enzyme α-amylase Ronozyme RumiStar^{TM}); MON: monensin; [b]SEM = Standard error mean; [c]L = linear effect; Q = quadratic effect; Values within a row with different superscripts differ ($P < 0.05$).

are inconsistent because several factors may influence the mode of action of this additive, such as the type of feed, breed, sex, and age, as well as the type of essential oil used in the blend (18, 52). Essential oils are plant-derived compounds with known antimicrobial, anti-inflammatory, antioxidant, and coccidiostatic properties. In addition, EO has properties similar to ionophores and contains compounds that have been shown to modulate rumen fermentation by selecting microorganisms in the rumen, thereby improving nutrient utilization in ruminants (53). The BEO might have a flavor effect on feed which may have contributed to increase DMI, as was previously reported (54). In addition, the association of EO with α-amylase, such as BEO, ensures the better breakdown of glycosidic bonds present in starch, which serve as a substrate and consequently increase the abundance of non-fibrous carbohydrate-fermenting bacteria. Salazar et al. (54) reported greater DMI for cattle receiving BEO when compared to MON. Moreover, Tricarico et al. (55) reported an increase in DMI when exogenous α-amylase was used due to a change in the molar ratio of butyrate and propionate, and a decrease in rumen lactate concentration. In this context, previously study reported the negative effects of increasing ruminal propionate on DMI in dairy cows (56). This may justify the increase in DMI in BEO-treated animals, which may have increased the rumen passage rate.

In this context, data on feeding behavior may help in the interpretation of DMI in response to BEO supplementation. However, evaluation of the feeding behavior in feedlot cattle supplemented with BEO is limited in the literature (57, 58). In the present study, the variables examined were significantly affected by starch content. Cattle fed BEO35 sorted for medium diet particles and spent more time feeding NDF and ruminating NDF, which may be a response to control rumen acidity and a possible explanation for the increased DMI of cattle consuming BEO. In addition, increasing the starch content of the diet resulted in more meals per day and lower DM intake per meal while attempting to control rumen acidosis. Moreover, essential oils may decrease protein breakdown in the rumen, which promotes less colonization by bacteria with proteolytic activity (59). This increase in rumination rate in response to BEO treatment may have a positive effect on reducing the size of feed particles and increasing the contact area for bacteria and rumen fermentation. On the other hand, longer rumination times may affect feed passage rate and, thus, DMI.

Regarding monensin, it is well documented that the main effects of ionophores are a reduction in DMI and increased feed efficiency (6, 7, 33). A meta-analysis by Duffield et al. (7) reported that the use of monensin decreased DMI by 3% and improved feed efficiency by 2.5–3.5% in feedlot beef cattle. This higher DMI of BEO-supplemented animals may also be attributed to the essential oils' palatability. Depending on the plants extracted, some essential oils have active ingredients that can improve the palatability of the diet (60). Meschiatti et al. (18) reported higher DMI in Nellore feedlot cattle fed a 55% starch diet associated with the use of a BEO plus exogenous α-amylase compared with a treatment containing only essential oils or monensin. However, Meyer et al. (50) found no difference in DMI in feedlot cattle receiving a blend of essential oils.

In this context, starch-rich diets can increase the risk of rumen acidosis, as well as lesions in the ruminal epithelium, due to the greater rumen fermentation and consequently higher production of SCFA (33). The number of papillae, papillae area, and absorptive surface area (ASA) are important parameters directly related to the absorption of SCFA, preventing the accumulation of these acids in the rumen environment and acidification (17, 61). In the present study, the highest rumenitis score was observed in diets containing 35% starch, regardless of the additive. On the other hand, supplementation with BEO increased the papillae height and papillae area (% of ASA). Due to the higher DMI observed in response to BEO, this treatment increased the production of SCFA and, consequently, resulted in greater papillae development. In addition, the use of essential oil may act as a modulator of rumen fermentation by selecting lactic acid-producing bacteria and protecting the rumen from a possible drop in rumen pH,

TABLE 5 Levels of starch associated with blend of essential oils or sodium monensin on feeding behavior and particle sorting of Nellore yearling bulls.

Treatments[a]	BEO			MON				*P*-value[c]		
Level of starch, %	25%	35%	45%	25%	35%	45%	SEM[b]	Additive	Starch	Additive∗Starch
Feeding behavior										
DMI, kg[d]	11.34	12.23	11.37	10.67	10.50	9.94	0.23	<0.01	<0.01 (Q)	0.09
Feed efficiency of DM,	15.61[b]	15.06[b]	18.43[a]	16.15[b]	19.29[a]	19.60[a]	0.54	<0.01	<0.01	0.04
Time spent eating, min	176.43	183.95	208.57	171.71	202.71	194.14	4.80	0.98	<0.01 (L)	0.07
Time spent ruminating, min	256.00	228.29	250.29	213.86	235.71	210.00	12.13	0.02	0.93	0.11
Time spent resting, min	1,007.57[ab]	1,027.76[a]	981.14[b]	1,054.43[a]	1,001.57[ab]	1,035.86[a]	14.55	0.05	0.32	0.02
Drinking bouts, *n*	3.24	3.94	3.71	3.57	3.54	3.65	3.61	0.85	0.45	0.43
Meals per day, *n*	15.09	16.80	17.29	13.89	16.31	17.23	0.60	0.42	<0.01 (L)	0.76
ER of NDF, min/kg DM[e]	37.92	42.95	65.93	38.92	52.55	68.92	2.34	0.09	<0.01(L)	0.37
RR of DM, min/kg DM	22.75	18.74	21.98	20.19	22.53	21.25	1.16	0.88	0.71	0.06
RR of NDF, min/kg DM[f]	55.13	53.37	78.49	48.69	61.61	74.99	3.92	0.89	<0.01(L)	0.15
Meal length, min	11.79[a]	11.04[ab]	12.25[a]	12.42[a]	12.66[a]	11.31[ab]	0.51	0.21	0.72	0.02
DMI per meal, kg	0.76	0.74	0.67	0.78	0.66	0.58	0.03	0.11	<0.01 (L)	0.35
NDF intake, kg	4.69	4.30	3.21	4.43	3.90	2.87	0.11	0.02	<0.01(L)	0.91
Particle sorting[g]										
Long	1.02	0.92	0.91	1.00	0.87	0.96	0.03	0.92	0.01	0.33
Medium	1.01[a]	0.99[a]	0.94[b]	1.00[a]	0.94[b]	0.97[ab]	0.01	0.40	<0.01	0.02
Short	1.00	1.01	1.02	1.00	1.01	1.01	0.00	0.40	0.01	0.06
Fine	0.99	1.01	1.01	1.00	1.02	1.01	0.0045	0.26	0.03	0.40

[a]BEO: blend of essential oil (Crina ® Ruminants and exogenous enzyme α-amylase Ronozyme RumiStar^{TM}); MON: monensin; [b]SEM = Standard error mean; [c]L = linear effect; Q = quadratic effect; [d]DMI, dry matter intake; [e]ER, eating rate; [f]RR, rumination rate; [g]Particle fraction retained on screens of 19 (long), 8 (medium), 1.18 mm (short) and a pan (fine). Values within a row with different superscripts differ ($P < 0.05$).

due to the high amounts of fermented starch (62). Li et al. (63) reported that the use of a BEO altered rumen fermentation with increased propionate concentration, improved fiber digestibility, and decreased methane production.

However, treatment with BEO also increased the cecum lesions score and decreased the number of goblet cells. Considering the higher DMI observed, it may be that animals fed BEO had a higher rumen passage rate so that a greater amount of starch bypassed the rumen and some of this starch entered the cecum, which may lead to excessive fermentation in the cecum, increasing the production and accumulation of SCFA, and lowering the pH in the cecum. Thus, acidification of the intestinal environment may lead to an increase in lesions in the cecal epithelium and a decrease in the number of goblet cells and enterocytes because the intestinal epithelium is more sensitive to pH changes than the rumen (64). However, in a companion study, Rocha et al. (65) reported that cattle fed BEO plus amylase surprisingly did not have proteins related to inflammatory processes (leukocyte elastase inhibitors) in the cecal tissues, although cecal lesions increased in response to BEO. This could be because feed enzymes remain active in the intestine and can help digest nutrients that escape rumen fermentation, or they may have even had an anti-inflammatory effect, but they cannot control cecal lesions.

Just as SCFA concentrations and rumen pH cause changes in SCFA absorption rate (17, 66), SCFA act as signaling and regulatory molecules for biological processes to protect gut health (67). Alteration of goblet cell differentiation, such as expression of mucin-related genes, is associated with increased acetate and propionate concentrations (68). The intestinal epithelium was mechanically stimulated to secrete mucus, which they attributed to the effect of SCFAs on mucus thickening (69). The use of high-starch diets for cattle can lead to lesions and an inflammatory response in the cecum (65). Studies evaluating cecal morphometric parameters, cecal epithelial health, and the correlation of these parameters with animal performance are limited in the literature (44, 70). It has been previously reported in the literature that diet and infusion of fatty acids affect mitotic index, crypt depth, and intestinal mucosal weight in rats (71–74). In the present study, the higher DMI in animals supplemented with BEO may have contributed to this increase in cecum acidity in response to a greater amount of starch fermentation. On the other hand, the higher DMI could have caused a higher production of SCFA, which would

TABLE 6 Levels of starch associated with blend of essential oils or sodium monensin on rumen and cecum morphometrics of Nellore yearling bulls.

Treatments[a]	BEO			MON				*P*-value[c]		
Level of starch, %	25%	35%	45%	25%	35%	45%	SEM[b]	Additive	Starch	Additive∗starch
Rumenitis score	0.85	1.00	0.95	0.75	1.10	0.80	0.06	0.50	0.03 (Q)	0.36
Macroscopic variables										
Mean papillae area, cm^2	0.52	0.50	0.46	0.48	0.45	0.47	0.02	0.26	0.40	0.40
Number of papillae, *n*	63.98	69.77	70.13	62.33	64.58	64.51	2.63	0.18	0.45	0.84
ASA, cm^2/cm^2 of rumen wall[d]	32.82	35.24	30.29	30.23	29.50	30.13	1.13	0.05	0.46	0.28
Papillae area, % of ASA	97.09	97.07	97.02	96.82	96.64	96.77	0.09	0.01	0.76	0.78
Microscopic variables										
Mitotic index, *n*	159.73	170	155.4	183.93	162.87	165.93	8.50	0.39	0.69	0.49
Mitotic index, %	7.99	8.50	7.77	9.20	8.14	8.30	0.42	0.39	0.69	0.49
Papillae height, mm	4.18	4.17	4.24	4.04	4.04	3.83	0.08	0.03	0.78	0.41
Papillae surface area, mm^3	1.02	0.96	1.01	1.01	0.94	0.92	0.02	0.14	0.22	0.29
Papillae width, mm	0.18	0.16	0.15	0.16	0.16	0.17	0.01	0.78	0.55	0.19
Keratinized layer thickness, μm	7.74	8.29	9.26	7.92	8.04	7.86	0.22	0.09	0.12	0.07
Cecum variables										
Cecum lesions score, *n*	1.61	2.55	2.16	1.30	1.43	1.55	0.21	0.02	0.25	0.47
Crypt depth, μm	75.48	76.57	74.88	74.40	76.06	74.17	1.41	0.65	0.73	0.99
Enterocytes, *n*	16.25	16.05	15.95	16.93	16.57	16.51	0.35	0.18	0.77	0.99
Goblet cells, *n*	1.51	1.48	1.45	1.67	1.65	1.65	0.06	0.06	0.94	0.98
Crypt/Enterocytes	4.66	4.79	4.70	4.43	4.60	4.51	0.06	0.01	0.27	0.97
Crypt/Goblet, *n*	50.99	52.21	52.05	46.77	48.56	47.19	1.28	0.02	0.76	0.94

[a]BEO: blend of essential oil (Crina ® Ruminants and exogenous enzyme α-amylase Ronozyme RumiStar^{TM}); MON: monensin; [b]SEM = Standard error mean; [c]L = linear effect; Q = quadratic effect; [d]ASA: absorptive surface area. Values within a row with different superscripts differ ($P < 0.05$).

develop the cecum and make it less susceptible to inflammatory processes (33, 44). The increase in dietary starch content may promote the down-regulation of enzymes related to carbohydrate degradation, likely caused by damage to the cecal epithelium due to increased responses associated with inflammatory injury (65).

In addition, an increase in fecal starch was observed in animals fed high starch levels, with cattle fed BEO35 having higher fecal starch, suggesting that the cecum was unable to digest the starch from the rumen and absorb SCFA, thereby increasing fecal starch. On the other hand, animals receiving MON had the lowest levels of fecal starch, which may be related to the effectiveness of this additive in influencing rumen fermentation, selection, and stimulating the growth of rumen bacteria, thereby preventing starch losses *via* feces. Ionophore antibiotics have limited effects on the microbiota and intestinal fermentation of ruminants, as the effect of monensin is more likely to be observed in the rumen (65). There are reports that BEO can affect the colonization of starch-rich substrates by rumen bacteria, thereby increasing the availability and utilization of starch in the rumen (75). In the present study, it was observed that BEO did not improve the utilization of dietary starch. Although, as diet starch levels increased, an increase in performance would be expected, as a result of greater diet energy content (Table 1). However, we had no increase in performance as the starch levels increased for BEO, and for MON we had a decrease in performance and intake as starch levels increased. This indicates that we had a non-optimal condition for digestion as starch levels increased, which may have affected animals' digestibility. Meschiatti et al. (18) reported that fecal starch concentration was 25.6% lower and total tract starch digestibility was 5.11% higher ($P = 0.04$) with BEO than with MON.

Thus, the manipulation of rumen fermentation and better feed efficiency, in response to additives use may affect carcass characteristics. In the present study, it was observed that cattle fed BEO35 had the best HCW values. However, the best results in final fat thickness were obtained when cattle were treated with MON25. As mentioned earlier, it was observed that increasing the starch content in the diet resulted in more meals per day and lower DMI per meal while trying to control rumen acidity. As a result, carcass fat deposition was negatively affected in cattle-fed MON because increasing starch content linearly decreased final fat thickness and daily fat gain. Better dressing percentage and carcass gain were observed in cattle fed BEO, which may be explained by the higher DMI observed in this treatment as a function of manipulation of rumen fermentation, and the higher carcass yields may be related to higher production of propionate in the rumen, which is an SCFA precursor of glucose in ruminants (76). Tricarico et al. (55) reported an increase in HCW in feedlot cattle-fed diets supplemented with α-amylase and attributed these results to the improvement in the

molar ratio of acetate and propionate and the decrease in lactate. Several active compounds present in essential oils such as carvacrol, thymol, allicin, and pinene have an antimicrobial activity that can affect gram-positive and gram-negative bacteria (77). Because of their ability to modulate the rumen microbiota, essential oils can directly affect protein degradation and SCFA production (78). Meschiatti et al. (18) reported higher HCW in animals fed BEO on a finishing diet containing 55% starch when compared to MON, but no significant difference in dressing percentage was observed. In the present study, the feedlot cattle fed BEO had better results for the rib-eye area, as well as a greater rib-eye area gain, which can be explained by the higher values of HCW and dressing found in these treatments, where greater carcass deposition is a consequence of a greater muscle tissue production.

The change in rumen fermentation pattern in response to the use of these additives affects the production of SCFA and, consequently, the acetate:propionate ratio. There are reports that glucose is the major contributor to fatty acid biosynthesis in intramuscular adipose tissue (79, 80). Considering that propionate is the main substrate for glucose formation in ruminants, maximizing its production in the rumen environment seems to be essential to improve marbling (76). In this context, using feed additives and increasing the availability of starch for rumen fermentation can increase propionate production in the rumen and positively influence intramuscular fat deposition. In the present study, the effect of feed additives on final marbling and final fat thickness was dependent on starch content, where the MON25 presented the best results. In this context, different starch levels associated with this additive may increase fat deposition, leading to better carcass quality. However, Ornaghi et al. (58) reported no differences in final fat thickness, rib-eye area and final marbling in feedlot cattle fed a diet containing 41% starch supplemented with essential oil. Regarding meat quality, higher cooking loss, and lower moisture were observed for treatments BEO35 and MON35. The other quality variables were not affected by the treatments, and the values found in this study are within what is considered normal in the literature (26). Cooking losses are related to intramuscular fat, which has a positive effect on water retention capacity and prevents fluid loss after cooking (81). Meat maturation time is also related to low water loss, which is due to a slight increase in pH and the change from divalent to monovalent ions (82). In general, the average values found for cooking loss vary between 20 and 28% (8, 83), similar to the values obtained in the present study. Toseti et al. (26) observed no difference in cooking loss, pH, shear force, and chroma A and B in response to treatment with BEO.

5. Conclusion

The BEO increased DMI and enhanced overall animal performance but the effect was dependent on starch content, with no effect on feed efficiency. Animals receiving monensin improved feed efficiency and decreased lesions in the rumen and cecum in finishing feedlot cattle. Therefore, the blend of essential oil evaluated in the current study can be an alternative to replacing MON in finishing feedlot diets. Overall, cattle fed MON25 improved feed efficiency, whereas BEO presented better results on feedlot performance and carcass traits with 35 and 45% starch diets.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by São Paulo State University Ethical Committee for Animal Research, and approved by Ethical Commission in Use of Animals of the Innovation and Applied Science DSM Nutritional Products SA (number BR190313).

Author contributions

TS and DM: conceived and designed the study, collected and complied, and analyzed data. TS, TA, VC, AP, LS, AS, MN, WS, and DC: investigation. CM and MA: provided intellectual input. TS, JS, and DM: provided intellectual input, drafted, and edited manuscript. All authors contributed to the article and approved the submitted version.

Funding

This project was financially supported by DSM (Nutritional Products SA, São Paulo, Brazil); and Scholarship for TS was provided by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasília, DF, Brazil; Finance Code 001).

Acknowledgments

We wish to acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES, Finance Code 001) and DSM (Nutritional Products SA, São Paulo, Brazil).

Conflict of interest

Authors TA, VC, and AP were employed by company DSM Nutritional Products SA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors declare that this study received funding from DSM Nutritional Products SA. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Publisher's note

References

1. Ye H, Liu J, Feng P, Zhu W, Mao S. Grain-rich diets altered the colonic fermentation and mucosa-associated bacterial communities and induced mucosal injuries in goats. *Sci Rep.* (2016) 6:20329. doi: 10.1038/srep 20329

2. Silvestre AM, Millen DD. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia.* (2021) 50:189. doi: 10.37496/rbz5020200189

3. Nocek JE, Tamminga S. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *J Dairy Sci.* (1991) 74:3598–629. doi: 10.3168/jds.S0022-0302(91)78552-4

4. McCarthy MM, Yasui T, Ryan CM, Mechor GD, Overton TR. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. *J Dairy Sci.* (2015) 98:3335–50. doi: 10.3168/jds.2014-8820

5. Cheng KJ, McAllister TA, Popp JD, Hristov AN, Mir Z, Shin HT, et al. review of bloat in feedlot cattle. *J Anim Sci.* (1998) 76:299–308. doi: 10.2527/1998.761299x

6. Rigueiro ALN, Pereira MCS, Squizatti MM, Ferreira MM, Dondé SC, Luiz FP, et al. Different combinations of sodium monensin and virginiamycin during feedlot finishing of Nellore cattle. *Anim Prod Sci.* (2020) 60:18657. doi: 10.1071/AN18657

7. Duffield TF, Merrill JK, Bagg RN. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *J Anim Sci.* (2012) 90:4583–92. doi: 10.2527/jas.2011-5018

8. Rivaroli DC, Guerrero A, Velandia Valero M, Zawadzki F, Eiras CE, Campo MDM, et al. Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Sci.* (2016) 121:278–84. doi: 10.1016/j.meatsci.2016.06.017

9. Giannenas I, Skoufos J, Giannakopoulos C, Wiemann M, Gortzi O, Lalas S, et al. Effects of essential oils on milk production, milk composition, and rumen microbiota in chios dairy ewes. *J Dairy Sci.* (2011) 94:5569–77. doi: 10.3168/jds.2010-4096

10. Calsamiglia S, Castillejos L, Busquet M. Alternatives to antimicrobial growth promoters in cattle. *Recent Adv Anim Nutr.* (2006) 2005:129–67. doi: 10.5661/recadv-05-129

11. Benchaar C, Petit HV, Berthiaume R, Ouellet DR, Chiquette J, Chouinard PY. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *J Dairy Sci.* (2007) 90:886–97. doi: 10.3168/jds.S0022-0302(07)71572-2

12. Parvar R, Ghoorchi T, Kashfi H, Parvar K. Effect of ferulago angulata (Chavil) essential oil supplementation on lamb growth performance and meat quality characteristics. *Small Rumin Res.* (2018) 167:48–54. doi: 10.1016/j.smallrumres.2018.07.026

13. Smeti S, Hajji H, Mekki I, Mahouachi M, Atti N. Effects of dose and administration form of rosemary essential oils on meat quality and fatty acid profile of lamb. *Small Rumin Res.* (2018) 158:62–8. doi: 10.1016/j.smallrumres.2017.10.007

14. McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA, Newbold CJ. Effects of essential oils on ruminal microorganisms and their protein metabolism. *Appl Environ Microbiol.* (2003) 69:5011–4. doi: 10.1128/AEM.69.8.5011-5014.2003

15. Castillejos L, Calsamiglia S, Ferret A. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. *J Dairy Sci.* (2006) 89:2649–58. doi: 10.3168/jds.S0022-0302(06)72341-4

16. Silva APS, Zotti CA, Carvalho RF, Corte RR, Cônsolo NRB, Silva S, et al. Effect of replacing antibiotics with functional oils following an abrupt transition to high-concentrate diets on performance and carcass traits of nellore cattle. *Anim Feed Sci Technol.* (2019) 247:53–62. doi: 10.1016/j.anifeedsci.2018.10.015

17. Melo LQ, Costa SF, Lopes F, Guerreiro MC, Armentano LE, Pereira MN. Rumen morphometrics and the effect of digesta ph and volume on volatile fatty acid absorption. *J Anim Sci.* (2013) 91:1775–83. doi: 10.2527/jas.2011-4999

18. Meschiatti MAP, Gouvea VN, Pellarin LA, Batalha CDA, Biehl MV, Acedo TS, et al. Feeding the combination of essential oils and exogenous alpha-amylase increases performance and carcass production of finishing beef cattle. *J Anim Sci.* (2019) 97:456–71. doi: 10.1093/jas/sky415

19. Nehme R, Andres S, Pereira RB, Ben Jemaa M, Bouhallab S, Ceciliani F, et al. Essential oils in livestock: from health to food quality. *Antioxidants*. (2021) 10:48. doi: 10.3390/antiox10020330

20. Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A. Invited review: essential oils as modifiers of rumen microbial fermentation. *J Dairy Sci.* (2007) 90:2580–95. doi: 10.3168/jds.2006-644

21. Gencoglu H, Shaver RD, Steinberg W, Ensink J, Ferraretto LF, Bertics SJ, et al. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. *J Dairy Sci.* (2010) 93:723–32. doi: 10.3168/jds.2009-2673

22. Noziere P, Steinberg W, Silberberg M, Morgavi DP. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. *J Dairy Sci.* (2014) 97:2319–28. doi: 10.3168/jds.2013-7095

23. Andreazzi ASR, Pereira MN, Reis RB, Pereira RAN, Morais Junior NN, Acedo TS, et al. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. *J Dairy Sci.* (2018) 101:7199–207. doi: 10.3168/jds.2017-14331

24. DiLorenzo N, Galyean ML. Applying technology with newer feed ingredients in feedlot diets: do the old paradigms apply? *J Anim Sci.* (2010) 88:E123–32. doi: 10.2527/jas.2009-2362

25. Jolly-Breithaupt ML, Harris ME, Nuttelman BL, Burken DB, MacDonald JC, Luebbe MK, et al. Effects of syngenta enogen feed corn containing an alpha-amylase trait on finishing cattle performance and carcass characteristics. *Transl Anim Sci.* (2019) 3:504–12. doi: 10.1093/tas/txy121

26. Toseti LB, Goulart RS, Gouvêa VN, Acedo TS, Vasconcellos GSFM, Pires AV, et al. Effects of a blend of essential oils and exogenous α-amylase in diets containing different roughage sources for finishing beef cattle. *Anim Feed Sci Technol.* (2020) 269:643. doi: 10.1016/j.anifeedsci.2020.114643

27. Chemists AOA. *Official Methods of Analysis of Aoac*. Maryland: Association of Official Analytical Chemists (2005).

28. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2

29. Lofgreen GP, Garrett WN, A. system for expressing net energy requirements and feed values for growing and finishing beef cattle. *J Anim Sci.* (1968) 27:793. doi: 10.2527/jas1968.273793x

30. NRC. *Nutrient Requirements of Beef Cattle*. 6 Edn. Washington, DC: National Academy Press (1984).

31. Zinn RA, Shen Y. An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. *J Anim Sci.* (1998) 76:1280–9. doi: 10.2527/1998.7651280x

32. Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, et al. The cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. *Anim Feed Sci Technol.* (2004) 112:29–78. doi: 10.1016/j.anifeedsci.2003.10.006

33. Rigueiro ALN, Squizatti MM, Silvestre AM, Pinto ACJ, Estevam DD, Felizari LD, et al. The potential of shortening the adaptation of nellore cattle to high-concentrate diets using only virginiamycin as sole feed additive. *Front Vet Sci.* (2021) 8:692705. doi: 10.3389/fvets.2021.692705

34. Johnson TR, Combs DK. Effects of prepartum diet, inert rumen bulk, and dietary polyethylene glycol on dry matter intake of lactating dairy cows. *J Dairy Sci.* (1991) 74:933–44. doi: 10.3168/jds.S0022-0302(91)78243-X

35. Pereira MC, Cruz GD, Arrigoni MD, Rigueiro AL, Silva J, Carrara TV, et al. Relationships of feedlot performance, feeding behavior, rumen morphometrics, and carcass characteristics of nellore cattle differing in phenotypic residual feed intake. *J Anim Sci.* (2016) 94:4287–96. doi: 10.2527/jas.2016-0579

36. Carvalho S, Rodrigues MT, Branco RH, Rodrigues CAF. Comportamento ingestivo de cabras alpinas em lactação alimentadas com dietas contendo diferentes níveis de fibra em detergente neutro proveniente da forragem. *Revista Brasileira de Zootecnia.* (2006) 35:562–8. doi: 10.1590/S1516-35982006000200032

37. Heinrichs J KP. Evaluating particle size of forages and tmrs using the penn state particle size separator. *Depart Dairy Anim Sci Pennsyl State Univ.* (1996) 42:1–14.

38. Leonardi C, Armentano LE. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *J Dairy Sci.* (2003) 86:557–64. doi: 10.3168/jds.S0022-0302(03)73634-0

39. Bigham ML, McManus WR. Whole wheat grain feeding of lambs. V* Effects of roughage and wheat grain mixtures. *Aust J Agricult Res.* (1975) 26:53. doi: 10.1071/AR9751053

40. Resende-Junior JC, Pereira MN, Magallanes MGR, Duboc MV, Oliveira EC, Silva S, et al. Efeito do padrão alimentar sobre a morfologia da parede ruminal de vacas e carneiros. *Brazil J Vet Res Anim Sci.* (2006) 43:469. doi: 10.11606/issn.1678-4456.bjvras.2006.26469

41. Daniel JLP, Resende Júnior JC, Cruz FJ. Participação do ruminoretículo e omaso na superfície absortiva total do proventrículo de bovinos. *Brazil J Vet Res Anim Sci.* (2006) 43:579. doi: 10.11606/issn.1678-4456.bjvras.2006.26579

42. Odongo NE, Alzahal O, Lindinger MI, Duffield TF, Valdes EV, Terrell SP, et al. Effects of mild heat stress and grain challenge on acid-base balance and rumen tissue histology in lambs. *J Anim Sci.* (2006) 84:447–55. doi: 10.2527/2006.842447x

43. Pereira FT, Braga FC, Burioli KC, Kfoury JR, Oliveira LJ, Papa PC, et al. Transplacental transfer of iron in the water buffalo (bubalus bubalis): uteroferrin and erythrophagocytosis. *Reprod Domest Anim.* (2010) 45:907–14. doi: 10.1111/j.1439-0531.2009.01462.x

44. Pereira MCS, Dellaqua JVT, Sousa OA, Santi PF, Felizari LD, Reis BQ, et al. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of nellore cattle submitted to strategic diets prior the adaptation period. *Livest Sci.* (2020) 234:985. doi: 10.1016/j.livsci.2020.103985

45. AMSA. *Research Guidelines for Cookery, Sensory Evaluation and Instrumental Tenderness Measurements of Fresh Meat*. Chicago, IL: American Meat Science Association (2015).

46. Wheeler TL, Shackelford SD, Johnson LP, Miller MF, Miller RK, Koohmaraie M, et al. Comparison of warner-bratzler shear force assessment within and among institutions. *J Anim Sci.* (1997) 75:2423–32. doi: 10.2527/1997.7592423x

47. Honikel KO. Reference methods for the assessment of physical characteristics of meat. *Meat Sci.* (1998) 49:447–57. doi: 10.1016/S0309-1740(98)00034-5

48. (CIE) CIdlE. *Colorimetry*. Vienna: Commission Internationale de l'Eclairage (1986).

49. Chemists AoOA. *Official Methods of Analysis of Aoac*. 18 Edn. Washington, DC: Association of Official Analytical Chemists (2007).

50. Meyer NF, Erickson GE, Klopfenstein TJ, Greenquist MA, Luebbe MK, Williams P, et al. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. *J Anim Sci.* (2009) 87:2346–54. doi: 10.2527/jas.2008-1493

51. Burt S. Essential oils: their antibacterial properties and potential applications in foods: a review. *Int J Food Microbiol.* (2004) 94:223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022

52. Gouvêa VN, Meschiatti MAP, Moraes JMM, Batalha CDA, Dórea JRR, Acedo TS, et al. Effects of alternative feed additives and flint maize grain particle size on growth performance, carcass traits and nutrient digestibility of finishing beef cattle. *J Agricult Sci.* (2019) 157:456–68. doi: 10.1017/S0021859619000728

53. Hristov AN, McAllister TA, Van Herk FH, Cheng KJ, Newbold CJ, Cheeke PR. Effect of yucca schidigera on ruminal fermentation and nutrient digestion in heifers. *J Anim Sci.* (1999) 77:2554–63. doi: 10.2527/1999.7792554x

54. Salazar LFL, Nero LA, Campos-Galvao MEM, Cortinhas CS, Acedo TS, Tamassia LFM, et al. Effect of selected feed additives to improve growth and health of dairy calves. *PLoS ONE.* (2019) 14:e0216066. doi: 10.1371/journal.pone.0216066

55. Tricarico JM, Abney MD, Galyean ML, Rivera JD, Hanson KC, McLeod KR, et al. Effects of a dietary aspergillus oryzae extract containing alpha-amylase activity on performance and carcass characteristics of finishing beef cattle. *J Anim Sci.* (2007) 85:802–11. doi: 10.2527/jas.2006-427

56. Oba M, Allen MS. Dose-response effects of intrauminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. *J Dairy Sci.* (2003) 86:2922–31. doi: 10.3168/jds.S0022-0302(03)73889-2

57. Souza KA, Mottin C, Ramos TR, Pinto LAM, Eiras CE, Silva S, et al. Effects of diet supplementation with clove and rosemary essential oils and protected oils (eugenol, thymol and vanillin) on animal performance, carcass characteristics, digestibility, and ingestive behavior activities for Nellore heifers finished in feedlot. *Livestock Sci.* (2019) 220:190–5. doi: 10.1016/j.livsci.2018.12.026

58. Ornaghi MG, Passetti RAC, Torrecilhas JA, Mottin C, Vital ACP, Guerrero A, et al. Essential oils in the diet of young bulls: effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Anim Feed Sci Technol.* (2017) 234:274–83. doi: 10.1016/j.anifeedsci.2017.10.008

59. Marques JDA, Pinto AP, Abrahão JJS, Nascimento WG. Intervalo de tempo entre observações para avaliação do comportamento ingestivo de tourinhos em confinamento. *Semina Ciências Agrárias.* (2008) 29:55. doi: 10.5433/1679-0359.2008v29n4p955

60. Yang WZ, Benchaar C, Ametaj BN, Beauchemin KA. Dose response to eugenol supplementation in growing beef cattle: ruminal fermentation and intestinal digestion. *Anim Feed Sci Technol.* (2010) 158:57–64. doi: 10.1016/j.anifeedsci.2010.03.019

61. Estevam DD, Pereira IC, Rigueiro ALN, Perdigao A, da Costa CF, Rizzieri RA, et al. Feedlot performance and rumen morphometrics of nellore cattle adapted to high-concentrate diets over periods of 6, 9, 14, and 21 days. *Animal.* (2020) 14:2298–307. doi: 10.1017/S1751731120001147

62. Nagaraja TG, Lechtenberg KF. Liver abscesses in feedlot cattle. *Vet Clin North Am Food Anim Pract.* (2007) 23:351–69. doi: 10.1016/j.cvfa.2007.05.002

63. Li YL Li C, Beauchemin KA, Yang WZ. Effects of a commercial blend of essential oils and monensin in a high-grain diet containing wheat distillers' grains on *in vitro* fermentation. *Can J Anim Sci.* (2013) 93:387–98. doi: 10.4141/cjas2013-028

64. Sanz-Fernandez MV, Daniel JB, Seymour DJ, Kvidera SK, Bester Z, Doelman J, et al. Targeting the hindgut to improve health and performance in cattle. *Animals.* (2020) 10:1817. doi: 10.3390/ani10101817

65. Rocha LC, Assuncao ASA, Martins RA, de Carvalho VV, Perdigao A, Buzalaf MAR, et al. Feedlot diets containing different starch levels and additives change the cecal proteome involved in cattle's energy metabolism and inflammatory response. *Sci Rep.* (2022) 12:5691. doi: 10.1038/s41598-022-09715-7

66. Dijkstra J, Boer H, Van Bruchem J, Bruining M, Tamminga S. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, Ph and rumen liquid volume. *Br J Nutr.* (1993) 69:385–96. doi: 10.1079/BJN19930041

67. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell.* (2016) 165:1332–45. doi: 10.1016/j.cell.2016.05.041

68. Vital M, Penton CR, Wang Q, Young VB, Antonopoulos DA, Sogin ML, et al. A gene-targeted approach to investigate the intestinal butyrate-producing bacterial community. *Microbiome.* (2013) 1:8. doi: 10.1186/2049-2618-1-8

69. McRorie JW, McKeown NM. Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. *J Acad Nutr Diet.* (2017) 117:251–64. doi: 10.1016/j.jand.2016.09.021

70. Silvestre AM, Pinto ACJ, Schleifer WF, Miranda LS, Silva LAF, Casali DM, et al. Relationships of the microbial communities with rumen epithelium development of nellore cattle finished in feedlot differing in phenotypic residual feed intake. *Animals.* (2022) 12:820. doi: 10.3390/ani12070820

71. Sakata T, von Engelhardt W. Stimulatory effect of short chain fatty acids on the epithelial cell proliferation in rat large intestine. *Comp Biochem Physiol A Comp Physiol.* (1983) 74:459–62. doi: 10.1016/0300-9629(83)90631-X

72. Sakata T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Br J Nutr.* (1987) 58:95–103. doi: 10.1079/BJN19870073

73. Ichikawa H, Sakata T. Effect of L-lactic acid, short-chain fatty acids, and Ph in cecal infusate on morphometric and cell kinetic parameters of rat cecum. *Dig Dis Sci.* (1997) 42:1598–610. doi: 10.1023/a:1018884625737

74. Edwards CA, Wilson RG, Hanlon L, Eastwood MA. Effect of the dietary fibre content of lifelong diet on colonic cellular proliferation in the rat. *Gut.* (1992) 33:1076–9. doi: 10.1136/gut.33.8.1076

75. Duval SM, McEwan NR, Graham RC, Wallace RJ, Newbold CJ. Effect of a blend of essential oil compounds on the colonization of starch-rich substrates by bacteria in the rumen. *J Appl Microbiol.* (2007) 103:2132–41. doi: 10.1111/j.1365-2672.2007.03455.x

76. Park SJ, Beak SH, Jung DJS, Kim SY, Jeong IH, Piao MY, et al. Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle: a review. *Asian-Aust J Anim Sci.* (2018) 31:1043–61. doi: 10.5713/ajas.18.0310

77. Benchaar C, Greathead H. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Anim Feed Sci Technol.* (2011) 166–167:338–55. doi: 10.1016/j.anifeedsci.2011.04.024

78. Cardozo PW, Calsamiglia S, Ferret A, Kamel C. Screening for the effects of natural plant extracts at different ph on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. *J Anim Sci.* (2005) 83:2572–9. doi: 10.2527/2005.83112572x

79. Smith SB, Crouse JD. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. *J Nutr.* (1984) 114:792–800. doi: 10.1093/jn/114.4.792

80. Rhoades RD, Sawyer JE, Chung KY, Schell ML, Lunt DK, Smith SB. Effect of dietary energy source on *in vitro* substrate utilization and insulin sensitivity of muscle and adipose tissues of angus and wagyu steers. *J Anim Sci.* (2007) 85:1719–26. doi: 10.2527/jas.2006-498

81. Lee B, Choi YM. Correlation of marbling characteristics with meat quality and histochemical characteristics in longissimus thoracis muscle from hanwoo steers. *Food Sci Anim Resour.* (2019) 39:151–61. doi: 10.5851/kosfa.2019.e12

82. Puolanne E, Halonen M. Theoretical aspects of water-holding in meat. *Meat Sci.* (2010) 86:151–65. doi: 10.1016/j.meatsci.2010.04.038

83. Prado IN, Campo MM, Muela E, Valero MV, Catalan O, Olleta JL, et al. Effects of castration age, dietary protein level and lysine/methionine ratio on animal performance, carcass and meat quality of friesian steers intensively reared. *Animal.* (2014) 8:1561–8. doi: 10.1017/S1751731114001591

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 20 March 2023
DOI 10.3389/fvets.2023.1090107

OPEN ACCESS

EDITED BY
Abdelfattah Zeidan Mohamed Salem,
Universidad Autónoma del Estado de México, Mexico

REVIEWED BY
Ian John Lean,
Scibus, Australia
Neeta Agarwal,
Indian Veterinary Research Institute (IVRI), India
Anusorn Cherdthong,
Khon Kaen University, Thailand

*CORRESPONDENCE
Danilo D. Millen
✉ danilo.millen@unesp.br

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 04 November 2022
ACCEPTED 28 February 2023
PUBLISHED 20 March 2023

CITATION
Pacheco RDL, Souza JM, Marino CT, Bastos JPST, Martins CL, Rodrigues PHM, Arrigoni MDB and Millen DD (2023) Ruminal fermentation pattern of acidosis-induced cows fed either monensin or polyclonal antibodies preparation against several ruminal bacteria. *Front. Vet. Sci.* 10:1090107. doi: 10.3389/fvets.2023.1090107

Ruminal fermentation pattern of acidosis-induced cows fed either monensin or polyclonal antibodies preparation against several ruminal bacteria

Rodrigo D. L. Pacheco[1], Johnny M. Souza[2], Carolina T. Marino[3], João Paulo S. T. Bastos[1], Cyntia L. Martins[1], Paulo H. M. Rodrigues[4], Mario D. B. Arrigoni[1] and Danilo D. Millen[2]*

[1]Department of Breeding and Animal Nutrition, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, Brazil, [2]Department of Animal Production, College of Agricultural and Technological Sciences, São Paulo State University (UNESP), Dracena, Brazil, [3]Embrapa Beef Cattle, Campo Grande, Brazil, [4]School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil

This study was designed to evaluate a spray-dried multivalent polyclonal antibody preparation (PAP) against lactate-producing bacteria as an alternative to monensin (MON) to control ruminal acidification. Holstein cows (677 ± 98 kg) fitted with ruminal cannulas were allocated in an incomplete Latin square design with two 20 days period. Cows were randomly assigned to control (CTL), PAP, or MON treatments. For each period, cows were fed a forage diet in the first 5 days (d−5 to d−1), composed of sugarcane, urea and a mineral supplement, followed by a 74% concentrate diet for 15 days (d 0 to d 14). There were no treatment main effects ($P > 0.05$) on dry matter intake (DMI) and microbial protein synthesis. However, there was a large peak ($P < 0.01$) of intake on d 0 (18.29 kg), followed by a large decline on d 1 (3.67 kg). From d2, DMI showed an increasing pattern (8.34 kg) and stabilized around d 8 (12.96 kg). Higher mean pH was measured ($P < 0.01$) in cattle-fed MON (6.06 vs. PA$P = 5.89$ and CTL = 5.91). The ruminal NH_3-N concentration of CTL-fed cows was lower ($P < 0.01$) compared to those fed MON or PAP. The molar concentration of acetate and lactate was not affected ($P > 0.23$) by treatments, but feeding MON increased ($P = 0.01$) propionate during the first 4 days after the challenge. Feeding MON and PAP reduced ($P = 0.01$) the molar proportion of butyrate. MON was effective in controlling pH and improved ruminal fermentation of acidosis-induced cows. However, PAP was not effective in controlling acidosis. The acidosis induced by the challenge was caused by the accumulation of SCFAs. Therefore, the real conditions for evaluation of this feed additive were not reached in this experiment, since this PAP was proposed to work against lactate-producing bacteria.

KEYWORDS

additives, rumen, *Streptococcus bovis*, *Fusobacterium necrophorum*, *Lactobacillus ssp*

1. Introduction

Intensive cattle management systems often include increased amounts of cereal grains in the diet to increase energy input and improve performance (1). Thus, energy-dense diets typically fed to highly productive ruminants can lead to digestive disorders, such as ruminal acidosis, in response to the rapid ruminal fermentation of starch and sugars (2). Ruminal acidosis is the most economically important metabolic disorder in intensive cattle production systems, and that exists in both acute and subacute forms (3). Ruminal acidosis has been reported as the second most common health problem affecting feedlot cattle in Brazil (1).

Sub-acute ruminal acidosis (SARA) is an ongoing problem in the dairy and feedlot sector, responsible for the onset of different pathologies, such as rumenitis, parakeratosis, laminitis, and metabolic acidosis. *Streptococcus bovis* was the major initiators of ruminal acidosis by producing lactate as the major fermentation product under low ruminal pH (4). This results in consistent economic losses in both beef and dairy industries primarily due to decreased efficiency of milk production, premature culling, and reduction in milk yield and milk fat (5, 6) as well as a reduction on efficiency and performance of beef cattle (6, 7).

In this context, ionophore antibiotics, such as monensin (MON), are widely used in feedlot diets for acidosis control by modulating rate the of ruminal fermentation reducing dry matter intake (8, 9). Moreover, this ionophore modify rumen fermentation dynamics by inhibiting growth of Gram-positive bacteria, including lactate-producing rumen bacteria such as *Streptococcus bovis* (10), reducing ruminal lactate production and the risk of acidosis. Moreover, studies reported that monensin reduces ruminal protein degradation and decreased microbial protein synthesis (11, 12), due to inhibitory effects on hyper-ammonia-producing bacteria (13). Despite the beneficial effects of monensin, the use of antibiotics and growth promoters has raised the concern about the risk of these products in increasing bacterial resistance to antibiotics and, consequently, possible risks to human health (14). However, these mechanisms of resistance are not fully understood, since the genes responsible for ionophore resistance in ruminal bacteria have not been identified (15).

As a result, there is a search for antibiotic replacements to modulate ruminal fermentation to control acidosis and increase animal performance (16). An alternative to antibiotics is passive immunization with polyclonal antibodies preparations (PAP) against specific groups of ruminal bacteria, such as lactate-producing ruminal bacteria (*Streptococcus bovis*) and bacteria related to liver abscesses (*Fusobacterium necrophorum*). In this context, the use of PAP against lactate-producing ruminal bacteria have led to a reduction in the concentration of rumen lactate (17–19), and also in the control of acidosis in animals during the rapid transition to a high-concentrate diet (16, 20).

Recent ruminal metabolism studies with PAP were conducted under the most extreme condition of abrupt transition to high-concentrate diets (18, 20). However, most experiments carried out with acidosis induction for the evaluation of additives were designed to monitor rumen fermentation in short periods (between 1 and 2 d; 3.5–7 g PAP/d (18, 20). In this context, there is a lack of information on the persistence of action of feed additives, as well as the administration time necessary to reduce the negative effects of this nutritional disorder on ruminal fermentation. Furthermore, this allows the comparison of PAP with other additives, such as MON, under these conditions of the longer evaluation period.

Therefore, it was hypothesized that, in a situation of induction of ruminal acidosis, MON reduces the flow of microbial protein to the intestine. Furthermore, PAP would be effective in preventing lactic acidosis after an abrupt change to high-concentrate diet. Thus, the present study aimed to evaluate the spray-dried multivalent polyclonal antibody preparation against lactate-producing bacteria (*Streptococcus bovis*, *Fusobacterium necrophorum*, and *Lactobacillus ssp*.) as an alternative to monensin to control ruminal acidification in cow fed high concentrate diets.

2. Materials and methods

All protocols and procedures followed in this study were approved by the São Paulo State University Ethical Committee for Animal Research (CONCEA, 2013), São Paulo State University, Botucatu campus, Brazil.

2.1. Polyclonal antibody preparation

Polyclonal antibodies were produced by CAMAS Inc. (Le Center, MN, USA). The commercial product contains 39.5% immunoglobulins against *Streptococcus bovis* (ATCC 9809), 17.6% against *Lactobacillus ssp.* (ATCC 4356; 14917; 9649 and 7469), 13.20% against *Fusobacterium necrophorum* (ATCC 27852), 17.6% against *E. coli* O157:H7, and endotoxins. For PAP preparation, the procedures were similar to those described by DiLorenzo et al. (17), with the exception that a multivalent PAP was tested, rather than antibodies to specific organisms. *Streptococcus bovis* and *Lactobacillus ssp.* are the main lactic acid-producing bacteria during the process of acidosis; *Fusobacterium necrophorum* is strongly associated with liver abscess in cattle; while *E. coli* is a commensal in the rumen and can be opportunistically pathogenic in humans. Endotoxins are the lipopolysaccharides that are present in the walls of gram-negative ruminal bacteria, being released in situations of bacterial death. The PAP in solid form was obtained by spray drying and was maintained in hermetically sealed packages Protected from heat light during the experimental period.

2.2. Animals and experimental facilities

The experiment was conducted at the School of Veterinary Medicine and Animal Science at the University of São Paulo (USP), Campus of Pirassununga, São Paulo, Brazil. Nine non-pregnant and non-lactating Holstein cows with an average live weight of 677 ± 98 kg previously fitted with ruminal cannulas were used. The animals were housed in individual stalls (Stall size: 1.5 m bunk space per 9 m. 13.5 m2 per animal) with sand bedding, feed bunk, and access to drinking water. The facility had fans suspended from the ceiling that was turned on automatically during the hottest hours of the day, to mitigate the effects of ambient temperature.

10.3389/fvets.2023.1090107

2.3. Experimental design and treatments

An incomplete Latin square design was used, divided into two experimental periods of 20 d each (d−5 to d 14). The choice for only two experimental periods is attributed to the attempt to avoid diluting the challenge effect of high-concentrate diets by potential metabolic memory in an attempt to avoid large intakes of concentrate. Cattle were submitted to the following treatments: (1) Control (CTL); (2) Monensin (MON); (3) Polyclonal Antibody Preparations (PAP) against *Streptococcus bovis*, *Fusobacterium necrophorum*, and *Lactobacillus ssp*. The PAP and Monensin were inserted through the ruminal cannula twice a day, before each meal and inside envelopes made of absorbent paper, between days 0 and 14 of each experimental period. The MON was administered at a dose of 300 mg/d, which corresponds to 3 g/d of the commercial product Rumensin (Elanco Animal Health, Indianapolis, IN). This commercial product contains 10% sodium monensin per kilogram of product. The PAP (CAMAS Inc, Le Centre, MN, USA) was administered at a dose of 3 g/d (corresponding to 10 mL of liquid product).

The experiment had a total duration of 55 d, divided into two periods of 20 d each (d−5 to d 14), with an interval of readaptation to the roughage diet of 15 d between periods. The washout period was used to reestablish normal ruminal pH conditions. During d−5 to−1, the cows received only a roughage diet, composed of sugarcane, urea (1.4% DM), and a mineral supplement to supply the following levels of crude protein (CP), NDF, and ADF (DM basis): 14.05, 43.98, and 22.18%, respectively. From d 0 to d 14, a 74% concentrate diet was offered (Table 1), composed of sugarcane, high-moisture corn silage, soybean meal, and vitamin and mineral premix.

Between d−5 to d 14, the following variables were measured: individual dry matter intake; ruminal pH; ruminal concentration of total lactate and short-chain fatty acids (SCFA); NH_3-N concentrations, and microbial protein synthesis.

Before the two experimental periods, ∼20 kg of rumen content was extracted from each animal and these portions were mixed. After this procedure, the same amount of rumen content, removed and already mixed, was returned to each animal. This procedure aimed to homogenize the ruminal microbial population before the application of the experimental treatment.

2.4. Nutritional management

Diets were offered twice a day, at 800 h and 1,600 h. The experimental diet was administered as a total mixed ration (TMR), with a roughage: concentrate ratio of 26:74, in which the roughage source used was fresh sugarcane (2.9% CP, 47.48% NDF, and 25.68% ADF, DM basis) chopped with a theoretical mean particle size of 1.14 cm (21). The concentrate was composed of soybean meal (44.12% CP, 20.57% NDF, and 7.30% ADF, DM basis), and high-moisture corn silage (7.91% CP, 6.22% NDF, and 3.31% ADF, DM basis; Table 1). The DM, mineral matter (MM), CP, ether extract (EE), calcium, and phosphorus analyzes were performed according to AOAC (22), while the NDF corrected for ash and ADF were performed according to Van Soest et al. (23). For NDF analysis, α-amylase and urea were added. The starch concentration was carried out according to Pereira and Rossi Jr. (24), in which extraction of carbohydrates was performed according to Hendrix (25). The diet was formulated according to the NRC (26) and evaluated in the Cornell Net Carbohydrate and Protein System (CNCPS program, version 5.0.40) (27).

TABLE 1 Feed ingredients and chemical composition of the experimental diet.

Item	Experimental diet
Ingredients, % of DM	
Sugarcane, fresh and chopped	26.5
High-moisture corn silage	53.6
Soybean meal	17.9
Vitamin and mineral premix[a]	1.0
Calcitic limestone	1.0
Nutrient content	
Dry matter (%)	55.0
Crude protein (%DM)	14.8
Rumen degradable protein (% CP)	72.0
Rumen undegradable protein (%CP)	28.0
Neutral detergent fiber (% DM)	23.3
Physically effective neutral detergent fiber (% DM)[b]	13.0
Non-fiber carbohydrates (% DM)	55.0
Starch (% DM)	32.6
TDN (% DM)	80.0
Ca (%DM)	0.6
P (%DM)	0.4

[a]Composition of vitamin and mineral premix per kilogram of product: 230 g of Ca, 90 g of P, 15 g of S, 20 g of Mg, 48 g of Na, 100 mg of Co, 700 mg of Cu, 2.000 mg of Fe, 80 mg of I, 1.250 mg of Mn, 20 mg of Se, 2.700 mg of Zn, 900 mg of F (maximum), 200.000 UI of vitamin A, 60.000 UI of vitamin D3, 60 UI of vitamin E.
[b]Estimated by equations according to CNCPS, Cornell version 5.0.40.

2.5. Dry matter intake

To evaluate dry matter intake in kg (DMI), DMI expressed as % of BW (DMI % BW), DMI expressed as g/kg of metabolic weight (DMI g/kg BW0,75), the amount of diet offered and refused were collected and weighed daily, from d−5 to d 15. All feed bunks were examined every morning. If there was no feed remaining, the amount offered was raised by 10%. If up to 10% remained, the amount of feed offered was not changed and if the surplus was >10%, the feed offered was reduced by 10%. Additionally, the fluctuation of dry matter intake (DMIF) was calculated for each animal, as the difference in dry matter intake between consecutive days,

according to the methodology proposed by Bevans et al. (28), as follows:

$$DMIF = [\frac{(DMI\ current\ day - DMI\ previous\ day)}{DMI\ previous\ day}] * 100$$

The DMIF was performed between days−1 and 3 of each experimental period.

2.6. Ruminal fermentation parameters

At each daily collection, at least 500 mL of rumen content was removed at three different points of the rumen (through an electric vacuum pump), which were returned to the rumen-reticulum after collecting the appropriate aliquots for determination of lactate, NH_3-N, and SCFA molar concentrations. The collections were carried out daily, from d−5 to d 14 at 1,100 h (3 h after the morning feeding, carried out at 800 h) (29). Immediately after collection, 100 mL of rumen fluid was used for pH determination in a portable digital potentiometer (HANNA instruments HI8424), calibrated with pH 4.0 and pH 7.0 buffer solutions. Regarding the determination of the days on which the animals presented acidosis (DEA), according to definitions created by several authors (6, 9, 30), sub-acute acidosis was considered to occur when the pH was $\leq$ 5.6. So, the number of days in which the ruminal pH of each animal was pH $<$ 5.66 was counted. The adoption of the second decimal place is due to the sensitivity of the pH measuring device. Thus, pH values starting at 5.66 were considered pH 5.7 and discarded as acidotic pH. Only the post-challenge experimental phase was accounted for (d 0 to d 14), and the results were expressed as a percentage of this phase.

To determine the ruminal total lactate, 2 mL of rumen fluid was placed in test tubes and subsequently measured by the colorimetric technique according to Pryce (31). For short-chain fatty acid (SCFA) analyses that included acetate, propionate, and butyrate, a fraction of ∼100 mL of rumen content was centrifuged at 2000 x g for 20 min; 2 mL of the supernatant was added to 0.4 mL of formic acid and frozen at−20 °C for further analyses, according to Erwin et al. (32). The SCFA were measured by gas chromatography (Thermo Scientific®, model Focus GC) with an automatic sample injector (Thermo Electron Corporation®, model AS-3000) equipped with a 2 m long, and 1/5" diameter glass column was used, packed with Carbopack B-DA/4% Carbowax® 20M 80-120 (Supelco®) and flame ionization detector (FID) maintained at 270°C. The gas chromatograph oven was maintained at 190°C during the analysis and the injector temperature was 220°C. The carrier gas was high-purity H2, maintained in a flow of 30 mL/min. The number of repetitions per sample was the one necessary for the difference between readings to be <5%.

To determine the concentration of NH_3-N, fractions of 2 mL of rumen fluid were placed in test tubes containing 1 mL of 1N sulfuric acid solution and stored under refrigeration until the analysis by colorimetry (Kjeltec 2300 Analyzer Unit, Tecator, Hoganas, Sweden), according to the method described by Kulasek (33) and adapted by Foldager (34).

2.7. Estimation of microbial protein synthesis in the rumen

Analyses to determine microbial protein synthesis were performed at the Laboratory of Animal Biochemistry and Physiology of the VNP-FMVZ/USP, based on the quantification of urinary purine derivatives (PD), according to the methodology described by Valadares et al. (35) and Rennó (36), considering the absorption of purines from the formula suggested by Verbic et al. (37).

Urine samples (50 mL, spot sample) were collected from all animals on d−3; 3, and 14 of each experimental period, ∼3 h after feeding. The urine was filtered and 10 ml aliquots were immediately diluted in 40 mL of 0.018M sulfuric acid to avoid bacterial destruction of purine derivatives and uric acid precipitation, then stored at −15 °C for further analysis of allantoin and acid. uric. A pure urine sample was stored for the determination of total nitrogen compounds, urea and creatinine.

Creatinine concentrations were determined by commercial kits (Laborlab®), using an enzymatic reaction in a spectrophotometer (SBA-200 Celm®). The total daily urinary volume was estimated by dividing the daily urinary excretions of creatinine by the observed values of creatinine concentration in the urine of the spot samples according to Oliveira et al. (38).

The daily urinary excretion of creatinine was estimated from the established mean daily excretion of 24.05 mg/kg body weight for dairy cows (39). Thus, with the average daily excretion of creatinine and the concentration of creatinine (mg/dL) in the spot urine sample, the total daily volume of urine, in liters per cow, was estimated. The levels of allantoin and uric acid in the urine were determined by the colorimetric method, according to the methodology of Fujihara et al. (40), described by Chen and Gomes (41).

The total excretion of PD was calculated as the sum of allantoin and uric acid excreted in the urine, expressed in mmol/day. Absorbed microbial purines (AP, mM/day) were calculated from the urinary excretion of purine derivatives (PD, mM/day), using the equation:

$$AP = (PD\ 0.236 * BW^{0.75})/0.84$$

where 0.84 is the recovery of purines absorbed as purine derivatives and 0.236 is the endogenous excretion of PD (42).

Absorbed microbial purines were also assessed, considering the endogenous excretion of $0.512*BW^{0.75}$ and the recovery of 0.70 found by Gonzalez-Ronquillo et al. (43). Microbial protein synthesis (Pmic, g of N/day) was calculated based on the AP (absorbed microbial purines, mM/day), using the Equation (41):

$$Pmic = (70*AP)/(0.83*0.134*1000)$$

where 70 is the N content in the purines (mgN/mol); 0.134, the purine N: total N ratio in bacteria (35); and 0.83, the intestinal digestibility of microbial purines. To obtain the microbial crude protein synthesis, the Pmic data were multiplied by the Kjeldahl factor of 6.25.

2.8. Statistical analysis

The experimental design was an incomplete Latin square. Data were analyzed by Statistical Analysis System software (SAS version 9.2; SAS Inst., Inc., Cary, NC, USA), in which the model included the effects of treatments as fixed, and period and animal as random. Before the analysis of variance, the normality of the residuals was verified by the SHAPIRO-WILK Test (PROC UNIVARIATE), and the variances were compared by the "F" Test. Data (dependent variable) that did not meet these premises were submitted to logarithmic [Log (X+1)] or square root [SR (X+1/2)] transformation. The original or transformed data, when the latter procedure was necessary, were subjected to analysis of variance that separated the effects of treatments and period as sources of variation, plus the factor repeated measures over time, referring to the different sampling days. Such analysis was performed using the MIXED procedure of SAS. The effect of time analysis was only reported when the interaction between time and treatment effects was significant. The differences between means were performed using the Tukey test. Effects were considered significant at $P < 0.05$.

3. Results

3.1. Dry matter intake

No interactions were found between day and treatment for DMI ($P = 1.00$), DMI % BW ($P = 0.99$), and DMI g/kg BW0,75 ($P = 0.99$). The administration of feed additives *via* ruminal cannula did not result in changes ($P > 0.11$) in DMI when compared to the control (Tables 2, 3). However, a day effect ($P < 0.01$) was found for all dry matter intake variables mentioned above. There was a large peak of intake on d 0 (18.29 kg or 2.60% of BW), the day when the experimental diet was started, followed by a large decline on d 1 (3.67 kg or 0.54 % of BW; Figure 1). From d 2, DMI showed an increasing pattern (8.34 kg or 1.23 % of BW) and stabilized around d 8 (12.96 kg or 1.86 % of BW) for all treatments. The mean difference in intake between the forage diet on d−1 and the experimental diet on d 0 was 10.61 kg. On the other hand, cows ingested 14.63 kg less feed on d 1, compared to d 0. Additionally, a decrease in the variation of DMI was observed from d 2 (4.67 kg) and d 3 (1.29 kg), which corroborates the increasing pattern found in DMI and DMI % BW.

3.2. Rumen fermentation

No interaction was observed between day and treatment ($P = 0.19$) for rumen pH 3 h after feeding. Intraruminal addition of MON resulted in higher pH ($P < 0.01$) at the third postprandial hour compared to the other treatments (MON = 6.06 vs. CTL = 5.91 and PA$P = 5.89$; Table 4). Additionally, a day effect was found ($P < 0.01$) for rumen pH, where the decline in pH commenced on day 0 and the maximum decline was on day 1 (Figure 2A) regardless of treatments.

There was no interaction between day and treatment ($P = 0.78$) for rumen lactate. The molar concentration of lactate remained low throughout the experimental period (0.23 mM). The molar concentration of SCFA was higher in cows treated with PAP ($P = 0.02$) but there was no interaction between day and treatment ($P = 0.20$). Furthermore, a treatment effect was observed ($P = 0.02$), in which cows receiving PAP presented the greatest concentration. Similarly, a day effect was detected ($P < 0.01$), in which the total SCFA increased from ~90 mM in the pre-challenge period to a maximum value of 135 mM on d 1, stabilizing around 129 mM from d 8 (data not shown).

The interaction between day and treatment was not significant ($P = 0.38$) for the molar concentration of acetate. Additionally, neither feed additive influenced acetate concentrations ($P = 0.33$; Table 4). There was a time effect ($P < 0.01$) for the molar proportion of this SCFA (Figure 2B), where the molar proportions of acetate went from ~60% to around 55% on d 1, after the abrupt change to the experimental diet.

There was an interaction between day and treatment ($P < 0.01$; Figure 2C), in which an increase in the molar proportions of propionate was observed in cows receiving MON during the first 4 days following the challenge, an increase of 78% on d 4 when compared to control (MON = 30.42 vs. PA$P = 21.63$ and CTL = 17.07 mM on d 4). An interaction between day and treatment ($P = 0.01$) was also observed for the acetate/propionate ratio, in which MON reduced this proportion in the three first days following the challenge (Figure 2D).

There was no interaction between day and treatment ($P = 0.25$) for the molar concentration of butyrate, but both feed additives were effective ($P < 0.01$; Table 4) in decreasing ruminal butyrate (MON = 15.42 and PA$P = 16.35$ vs. CTL = 18.43).

The interaction between day and treatment was not significant ($P = 0.90$) for NH_3-N concentration. However, a treatment effect was found (<0.01), in which animals treated with additives had higher concentrations than CTL animals (MON = 14.74 and PA$P = 13.64$ vs. CTL = 11.20). Moreover, the day effect was significant ($P < 0.0001$), where the animals went from a concentration of around 19 mg/dL when fed a forage diet, to 27 mg/dL on d 0, 3.56 on d 1, and remaining around 12 mg/dL (with large variations) until the end of the experiment (Figure 2E).

3.3. Microbial protein synthesis

No interactions were observed between day and treatment for any of the evaluated experimental variables ($P > 0.05$). Moreover, the treatments with feed additives did not result in significant differences ($P > 0.05$, Table 5) for any experimental variable related to microbial protein synthesis (Figure 3).

4. Discussion

The results of the current study indicate that monensin was effective in controlling subacute ruminal acidosis in cattle abruptly shifted to a high-concentrate diet, and also in improving rumen fermentation by altering SCFA molar proportions. In addition, the acidosis challenge induced by the abrupt increase in diet energy levels was caused by the accumulation of SCFAs. As a result, PAP was not effective in controlling acidosis, which can be explained by the fact that this product targets the control of

TABLE 2 Dry matter intake in kg (DMI, kg/d), DMI as a percentage of body weight (DMI, %BW), and DMI based on metabolic BW (DMI, g/kg BW0.75) of cows induced to ruminal acidosis receiving polyclonal antibody preparations (PAP) or monensin (MON).

Item	Treatments			Mean[a]	SEM[b]	*P*-value
	CTL	MON	PAP			
DMI, kg	10.71	10.87	11.04	10.88	0.20	0.50
DMI, % BW[c]	1.59	1.58	1.51	1.56	0.03	0.11
DMI, g/kg $BW^{0,75}$	80.90	80.67	78.48	80.02	1.43	0.44

[a]Mean across treatments: CTL, Control; MON, Monensin; PAP, Polyclonal Antibody Preparation.
[b]SEM, standard error of mean.
[c]BW, body weight = 677 ± 98 kg.

TABLE 3 Fluctuation in dry matter intake (DMIF, kg) of cows induced to ruminal acidosis receiving polyclonal antibody preparations (PAP) or monensin (MON).

Day[a]	Treatments			Mean[b]	SEM[c]	*P*-value
	CTL	MON	PAP			
0	10.16	10.22	11.45	10.61	0.81	0.79
1	−13.88	−15.25	−14.76	−14.63	1.16	0.90
2	5.88	3.95	4.19	4.67	0.56	0.33
3	0.72	1.47	1.68	1.29	0.62	0.81
Overall	0.72	0.10	0.64	0.49	1.18	0.82

[a]Represents the difference in DMI (kg) between consecutive days.
[b]Mean across treatments: CTL, Control; MON, Monensin; PAP, Polyclonal Antibody Preparation.
[c]SEM, standard error of mean.

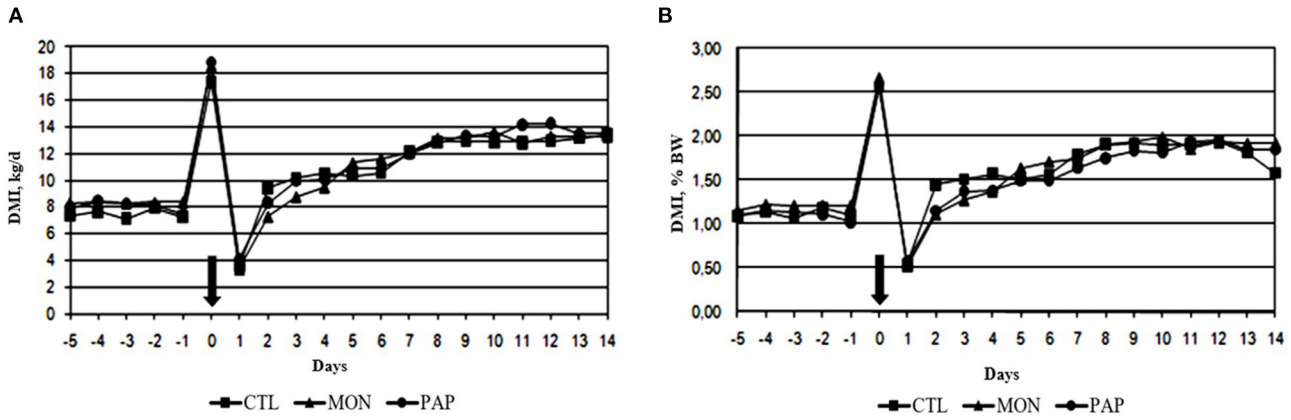


FIGURE 1
Dry matter intake expressed in kg [DMI, **(A)**] and as a percentage of body weight [DMI %BW, **(B)**] of cows induced to ruminal acidosis. The bold arrow represents the change from the 100% forage diet to the 74% concentrate diet containing the treatments.

lactate-producing bacteria. However, it is worth mentioning that the experimental design, as well as the protocol used in the present study, which included a simultaneous increase in diet density and different treatments inserted through the rumen cannulae, may have influenced the results. Since cows get used to consecutive acidosis challenges, an incomplete Latin square was adopted. Despite of these limitations, and considering the complexity of acidosis induction studies, the experimental protocol used in the study was efficient for inducing acidosis.

The reduction in DMI and increases in feed efficiency in animals fed high-concentrate diets in response to MON supplementation are well documented in the literature (8, 9). Similarly, reductions in intake in animals receiving MON have been found in comparative performance studies with PAP (44, 45). However, in the present study, MON did not reduce the DMI after an abrupt transition to high-concentrate diets, and this corroborates the absence of difference in DMI reported by Rodrigues et al. (46) where they found no reduction in dry matter intake in animals that received MON, also compared to PAP. From a metabolic point of view, the evaluation of the intake behavior of animals fed high-concentrate diets with MON may provide better information than the simple quantification of the total daily intake, because this negative effect of MON on DMI is related to a lower DMI per meal (47, 48), which consequently leads to an increase

TABLE 4 Ruminal fermentation variables of cows induced to acidosis and receiving polyclonal antibodies preparations (PAP) or monensin (MON).

Item	Treatments			Mean[1]	SEM[2]	*P*-value
	CTL	MON	PAP			
pH	5.91[b]	6.06[a]	5.89[b]	5.95	0.03	<0.01
DEA[3], %	60.00	32.22	58.89	50.37	6.93	0.14
Lactate, m*M*	0.23	0.21	0.25	0.23	0.009	0.23
Acetate, mol/100 mol	57.08	56.44	57.37	56.97	0.29	0.33
Propionate, mol/100 mol*	24.48[b]	28.08[a]	26.28[b]	26.28	0.45	0.04
Butyrate, mol/100 mol	18.43[a]	15.42[b]	16.35[b]	16.74	0.29	<0.01
Total SCFA, m*M*	115.81[b]	115.04[b]	120.17[a]	117.00	1.23	0.02
NH_3-N, mg/dL	11.20[b]	14.74[a]	13.64[a]	13.19	0.53	<0.01

[1]Mean across treatments: CTL, Control; MON, Monensin; PAP, Polyclonal Antibody Preparation.
[2]SEM, standard error of mean.
[3]DEA = percentage of days from the challenge with the high-concentrate diet whose pH measured at the third postprandial hour was <5.66.
*Significant interaction between day and treatment ($P < 0.01$).
[a,b]Values within a row with different superscripts differ ($P < 0.05$).

in the number of meals without changing the total amount of dry matter ingested daily (49, 50). In this context, this is the best mode of action for a feed additive to prevent subacute acidosis, associated with increased concentrations of SCFA (9), and, consequently, the control of dry matter intake prevents excessive fermentation of high starch content. These facts may explain the faster recovery of pH to normal rumen conditions in animals treated with MON in the present study.

However, the low DMI observed on day 1 may be explained by the increase in rumen osmolarity, as a result of reduced absorption and an increase in substances that contribute to an increase in osmolarity, such as glucose, SCFAs, and lactate, may lead to an influx of fluid from the blood into the rumen and, consequently, decrease DMI (51). In the present study, the greatest fluctuations in DMI occurred between day−1 and day 1, where the lowest average daily pH (5.43) was observed on day 1. Increases in DMI variation have been identified as an indicator of subacute acidosis (52, 53). However, the fluctuations decreased over the days, and instead of a cyclic pattern, as would be expected in animals that experienced ruminal acidosis, an increase in DMI was observed from day 2 onwards. The large amount of feed consumed by cows on the day of the challenge (d 0) can be explained by the fact that ruminants show a preference for feeds or diets that compensate for nutrient deficiencies (54). During the pre-challenge phase, cows were fed a high-forage diet. This may have increased the avidity for the concentrate after the challenge, increasing the DMI from day 2.

Furthermore, by evaluating pH and lactate concentration, it is possible to classify the acidosis caused by the challenge in this study as subacute (9). However, it is worth noting that in the present study, a single sample was collected 3 h after feeding on each day of the experimental period (29), but a recent study reported that rumenocentesis should be performed in the late afternoon or evening to maximize the probability of detecting animals with pH values below the threshold level (55, 56). Subacute ruminal acidosis (SARA) is characterized by a decline in ruminal pH below 5.8 or 5.6 (57). However, the diagnosis of SARA should not be made based on rumen pH alone, but in combination with symptoms to make SARA identification more accurate and feasible, including fecal consistency, rumen motility, and inflammatory markers (57). Nevertheless, there is not complete agreement on the etiology and symptoms of SARA (57, 58). In recent years, the development of sequencing technologies has enriched the study of SARA by expanding the understanding of the rumen microbiota (59). Changes in the structure and function of the rumen microbiota have been reported during SARA, including decreased bacterial richness and diversity, decreased relative abundance of fibrolytic bacteria and increased levels of amylolytic bacteria, and increased levels of propionate and total SCFA (59, 60). In the present study, feeding MON was more effective in minimizing reductions in rumen pH when compared to PAP and CTL. It is well documented in the literature that MON improve feed efficiency by reducing dry matter intake (47), reducing ruminal lactate production because this ionophore shifts the rumen microbial population by inhibiting growth of Gram-positive bacteria, including lactate-producing rumen bacteria such as *Streptococcus bovis* (10). However, considering only the average pH from day 0 to day 14, it was observed that cattle fed MON had a minimized risk of ruminal acidosis (pH 5.84), whereas cows from the other two treatments presented, on average, acidotic pH (pH 5.60).

In addition, the high-grain diet challenge resulted in some clinical manifestations, which are indicative of SARA, such as diarrhea in all animals on day 1. The change in feces could be due to the large flow of readily fermentable carbohydrates from the rumen to the intestine, causing excessive fermentation in these organs (61). Also, the high osmolarity promoted by the experimental diet described in animals with subacute ruminal acidosis, may retain fluid in the lumen and alter fecal consistency (62). The increase in total SCFA concentration in the rumen of cows fed PAP was not sufficient to reduce ruminal pH to a level below that of cows fed no feed additive. Therefore, feeding PAP may play a role in controlling rumen

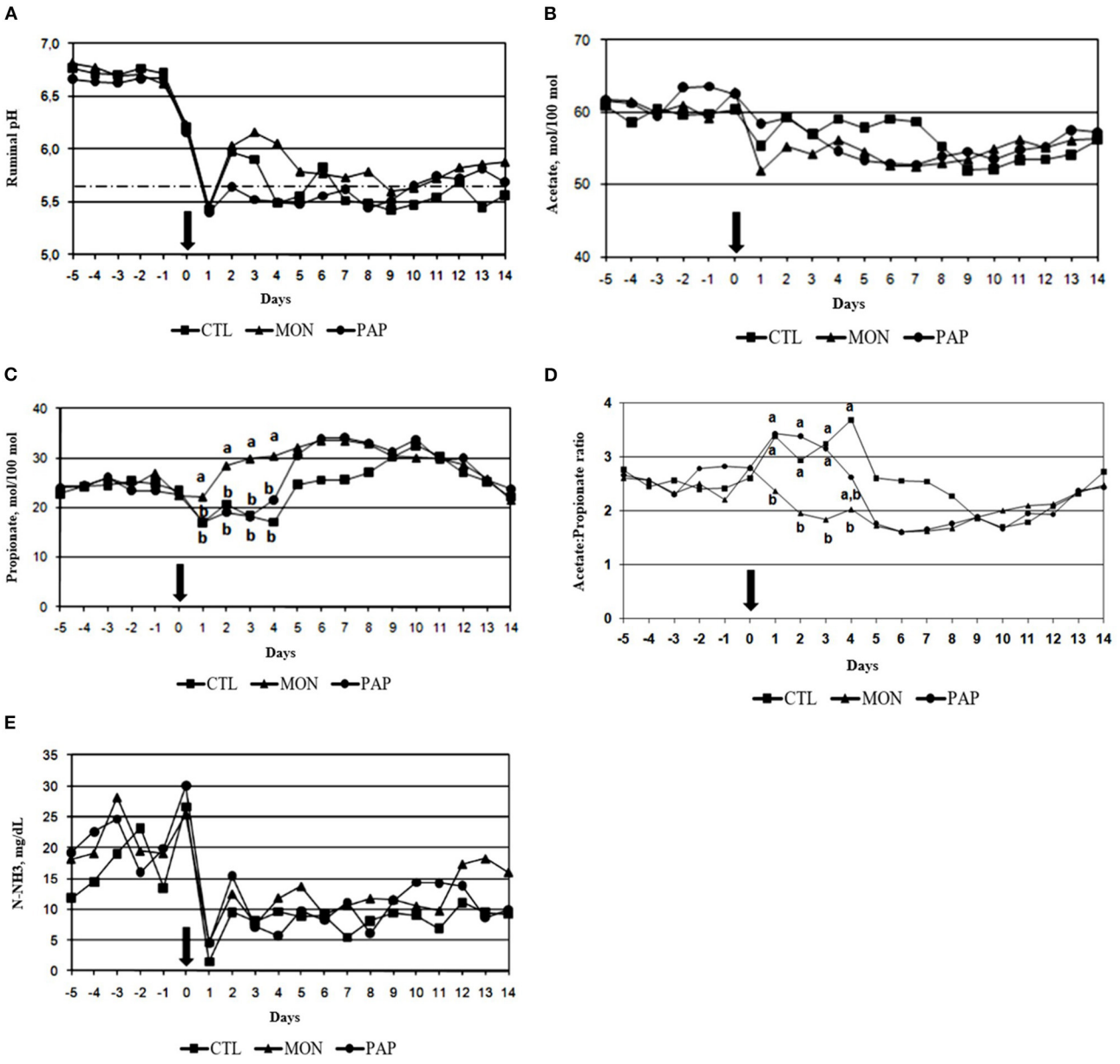


FIGURE 2
Ruminal pH **(A)**, acetate [% molar proportion, **(B)**], propionate [% molar proportion, **(C)**], acetate: propionate ratio **(D)**, and N-NH3 concentration **(E)** of cows induced to ruminal acidosis receiving polyclonal antibodies (PAP) or monensin (MON). The bold arrow represents the change from the 100% forage diet to the 74% concentrate diet containing the treatments.

acidification during an abrupt change from a high-forage to a high-concentrate diets.

In this context, the effect of MON in modulating the lactate-producing bacteria population *in vivo* and *in vitro* in acute acidosis situations is well described in the literature (51). The microbiological changes in acute and lactic acidosis are also well documented in the literature (9), but very little is known about the changes that occur in ruminants with subacute acidosis (63). This may be because MON is a feed additive with a broad-spectrum of activity as it reduces the rumen fermentation rate, which may be explained by the effect of treatment on the total SCFA concentration. It is worth mentioning that the lactate concentration was low in the present study. Based on this fact, the lactate levels in the ruminal fluid of cattle with subacute ruminal acidosis are usually not increased, which shows that the total concentration of SCFA is more important in subacute acidosis and the lactate concentration is more important in acute acidosis (6, 57, 64). Also, SARA may promote a gene expression change on rumen epithelium, due to an accumulation of intracellular cholesterol and its metabolites in response to a higher substrate supply (total SCFA). This could stimulate cell proliferation, increase membrane permeability, and induce epithelial inflammation, that

10.3389/fvets.2023.1090107

TABLE 5 Microbial protein synthesis in cattle induced to ruminal acidosis receiving polyclonal antibodies (PAP) or monensin (MON).

Item	Treatments			Mean[a]	SEM[b]	*P*-value
	CTL	MON	PAP			
Total urine excreted, L/day	6.57	7.76	5.72	6.70	0.47	0.12
Urinary allantoin, mM/day	104.72	106.51	89.92	100.38	6.20	0.41
Urinary uric acid, mM/day	7.04	6.40	5.81	6.42	0.59	0.61
Total purines, mM/day	111.74	112.90	95.75	106.80	6.41	0.38
Allantoin in relation to total purine, %	92.80	94.74	93.58	93.58	0.54	0.31
Absorbed purines, mM/day	95.58	90.0	74.66	86.68	7.35	0.42
Microbial protein synthesis (Pmic), g/day	60.15	60.90	46.99	56.01	4.76	0.43
Microbial crude protein, g/day	375.93	354.03	293.66	340.95	28.92	0.43

[a]Mean across treatments: CTL, Control; MON, Monensin; PAP, Polyclonal Antibody Preparation. [b]SEM, standard error of mean.

eventually disrupts rumen homeostasis and negatively affects cow health (59).

In contrast, the lack of effect of PAP in controlling rumen pH may have been due to the high specificity of these antibodies. Since SARA was induced by high concentrations of SCFA, the favorable conditions for a true evaluation of this additive were not presented because the target microorganisms were mostly acid-tolerant bacteria. In clinical and sub-clinical acidosis, the rumen pH decreases to a point where cellulolytic bacteria are inhibited and lactate-producing bacteria predominate, particularly *Streptococcus bovis* and *Lactobacillus* sp. (65). However, the observation of the lack of results in studies performed with PAP in solid form (66) raises doubts about whether the loss of antibody activity occurs during the conversion of the liquid to solid phase. Cassiano et al. (18) reported that neither liquid nor powdered forms of PAP altered rumen acidosis variables in adapted or unadapted animals. In this context, further comparative studies between the two forms of product presentation and new drying techniques are recommended.

The effects of MON in manipulating rumen fermentation are due to changes in rumen microbial ecology (67, 68). The increase in the molar proportion of propionate, the decrease in the acetate/propionate ratio, and the decrease in the molar proportions of butyrate in animals treated with MON can be explained by the inhibition of the growth of the population of Gram-positive bacteria, which are sensitive to ionophores and produce mainly acetate, butyrate, H2, and formate (67). However, when the above changes are verified, a decrease in the molar proportion of acetate would be expected, an effect confirmed by others studies (67–69). Probably, the decrease in the molar proportions of acetate occurs in experimental situations where rumen fermentation is already stabilized, which was not the case in this study. Thus, given the abrupt change in diet and the start of administration of the treatments on the day of challenge, it may have taken some time for rumen bacterial community to change. In addition, low acetate/propionate ratios are desirable to some extent in cattle, to maintain the necessary daily weight gain (48, 70). However, low acetate/propionate ratios can lead to a decrease in DMI and weight gain in unstable situations (53). In dairy cattle, low acetate/propionate ratios may decrease milk fat and an increase in body condition scores (71).

Furthermore, SARA has been characterized as a condition of elevated SCFA concentration that can lead to a critical rumen pH because of the imbalance between the production and absorption of these acids (57, 72), causing reduced microbial protein synthesis (73). In the present study, no changes in microbial protein synthesis were observed in response to the treatments. Studies have reported a reduction in microbial protein flux into the gut and decreased efficiency of microbial protein synthesis in response to MON (74). However, these studies show results in situations in which fermentation would theoretically be stabilized, in contrast to the present study in which animals were induced to acidosis. A possible explanation for the lack of effect of the treatments would be that abrupt changes, promoted by both diet and additives, contributed to a greater growth of the ruminal microbial population, and shortened the time for MON to contain bacterial growth. As a result, a linear increase in purine derivatives excretion and an increase in microbial protein were observed during the experimental period, explained by the increase in energy density of the diet and the greater supply of rapidly fermentable carbohydrates (75).

Contrary to expectations, animals treated with both MON and PAP had higher concentrations of ruminal NH_3-N. Both additives affect the population of *Streptococcus bovis*, a highly proteolytic gram-positive rumen bacterium (76) and therefore may indirectly affect rumen ammonia concentrations. In addition, MON may act on the partitioning of protein metabolism by decreasing the production of NH_3-N in the rumen and increasing escape of dietary protein from ruminal degradation [increasing the passage rate of protein from 22 to 55%; (77) and (78), respectively]. However, despite decades of widespread use of this ionophore, the protein partitioning effect has never been fully explained (79), largely because of the observation that most isolated ammonia-producing rumen bacteria are Gram-negative (80). Chen and Russell (79, 81) were able to obtain three isolates of gram-positive bacteria (*Peptostreptococcus anaerobius*, *Clostridium sticklandii* and *Clostridium aminophilum*) with highly specific activities for the production of ammonia, which are

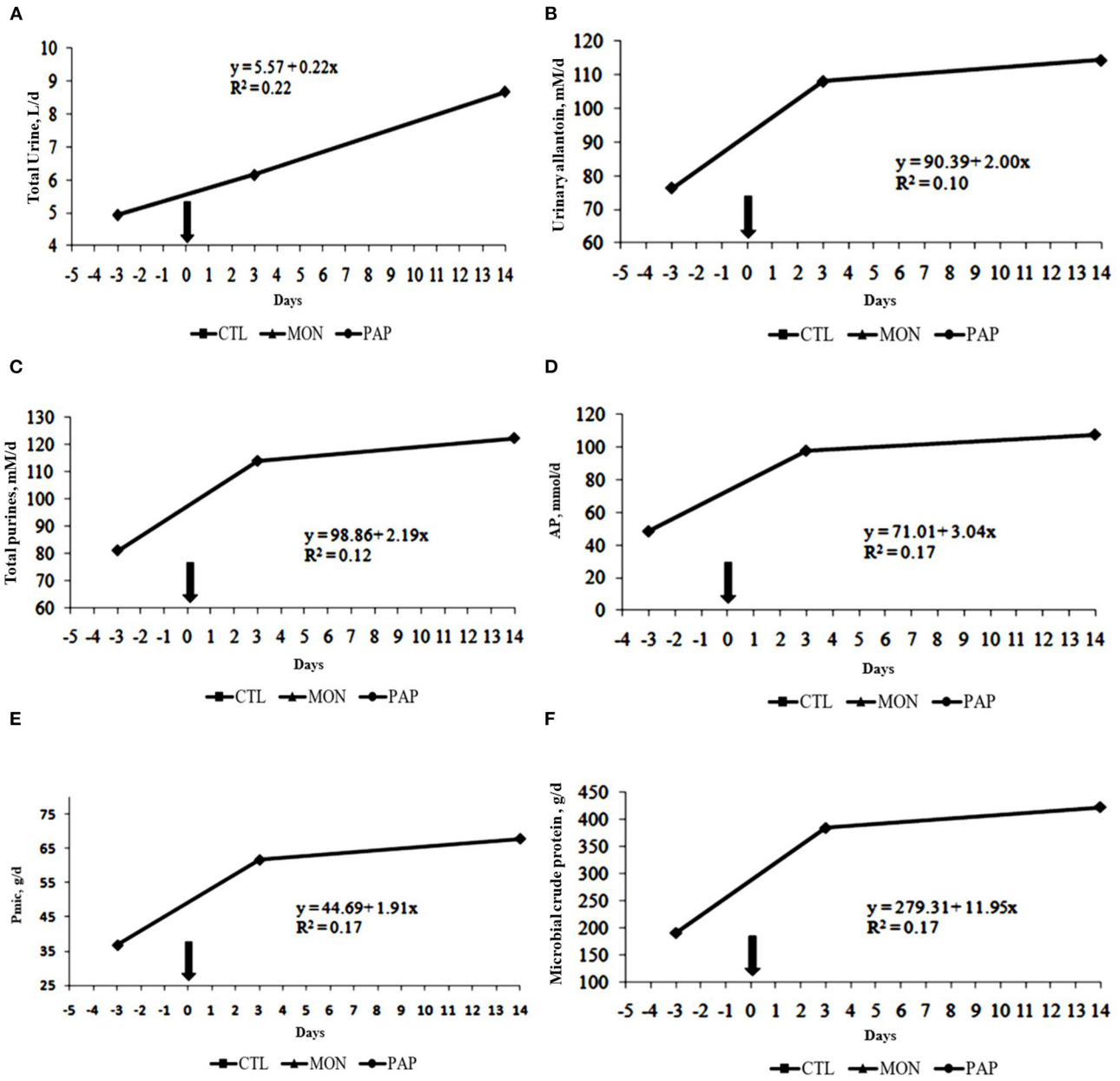


FIGURE 3
Total urine excreted **(A)**, urinary allantoin **(B)**, total purines **(C)**, and absorbed microbial purines **(D)**, microbial-N **(E)** and microbial crude protein **(F)** of cows induced to ruminal acidosis. The bold arrow represents the change from the 100% forage diet to the 74% concentrate diet containing the treatments.

also sensitive to MON in *in vitro* studies. However, further studies are needed to explain the increase in rumen NH_3-N concentration in animals. However, it is noteworthy to mention that the protocol used in the present study, which involved a simultaneous increase in diet density and different treatments, may have influenced the results. Ammonia is the predominant base in the rumen (51); therefore the sharp decrease in the concentration of NH_3-N observed on day 1 could be due to the low rumen pH, along with the decrease in DMI on that day. In addition, much of the NH_3-N may have been incorporated by the bacteria, which may have had a high growth rate during this experimental period.

5. Conclusion

Monensin was effective in controlling subacute acidosis in cattle challenged with the abrupt transition to a high-concentrate diet and improved rumen fermentation by altering SCFA molar proportions. The PAP was not effective in controlling acidosis, which can be explained by the fact that this product targets the control of lactate-producing bacteria. However, the acidosis induced by the challenge was caused by the accumulation of SCFAs. Therefore, the real conditions for evaluation of this feed additive were not reached in this experiment, which opens the possibility of new studies under the conditions of

lactic acidosis. It is worth mentioning that the protocol used in the present study, which included a simultaneous increase in diet density and different treatments, may have influenced the results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by São Paulo State University Ethical Committee for Animal Research (CONCEA, 2013), São Paulo State University, Botucatu campus, Brazil.

Author contributions

RP and DM: conceived and designed study, collected and complied, and analyzed data. CTM, CLM, JB, MA, and PR: provided intellectual input. RP, JS, and DM: provided intellectual input and drafted and edited manuscript. All authors contributed to the article and approved the submitted version.

Funding

This project was financially supported by Fapesp, The São Paulo Research Foundation (Grant Number: 09/01968-3).

Acknowledgments

We wish to acknowledge the FAPESP (São Paulo Research Foundation) for the scholarship provided to conduct this study.

Conflict of interest

CTM is a Post Doctoral Researcher at Embrapa Beef Cattle under a scholarship.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Silvestre AM, Millen DD. The 2019 brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia*. (2021) 50:e20200189. doi: 10.37496/rbz5020200189

2. McCarthy MM, Yasui T, Ryan CM, Mechor GD, Overton TR. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. *J Dairy Sci.* (2015) 98:3335–50. doi: 10.3168/jds.2014-8820

3. Aikman PC, Henning PH, Humphries DJ, Horn CH. Rumen Ph and fermentation characteristics in dairy cows supplemented with Megasphaera Elsdenii Ncimb 41125 in early lactation. *J Dairy Sci.* (2011) 94:2840–9. doi: 10.3168/jds.2010-3783

4. Kim H, Park T, Kwon I, Seo J. Specific inhibition of streptococcus Bovis by Endolysin Lyjh307 supplementation shifts the rumen microbiota and metabolic pathways related to carbohydrate metabolism. *J Anim Sci Biotechnol.* (2021) 12:93. doi: 10.1186/s40104-021-00614-x

5. Abdela N. Sub-acute ruminal acidosis (Sara) and its consequence in dairy cattle: a review of past and recent research at global prospective. *Achiev Life Sci.* (2016) 10:187–96. doi: 10.1016/j.als.2016.11.006

6. Krause KM, Oetzel GR. Understanding and preventing subacute ruminal acidosis in dairy herds: a review. *Anim Feed Sci Technol.* (2006) 126:215–36. doi: 10.1016/j.anifeedsci.2005.08.004

7. Schwartzkopf-Genswein KS, Beauchemin KA, Gibb DJ, Crews DH Jr, Hickman DD, Streeter M, et al. Effect of bunk management on feeding behavior, ruminal acidosis and performance of feedlot cattle: a review. *J Anim Sci.* (2003) 81:E149–E58. doi: 10.2527/2003.8114_suppl_2E149x

8. Goodrich RD, Garrett JE, Gast DR, Kirick MA, Larson DA, Meiske JC. Influence of monensin on the performance of cattle. *J Anim Sci.* (1984) 58:1484–98. doi: 10.2527/jas1984.5861484x

9. Nagaraja TG, Titgemeyer EC. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci.* (2007) 90 (Suppl. 1):E17–38. doi: 10.3168/jds.2006-478

10. Weimer PJ, Stevenson DM, Mertens DR, Thomas EE. Effect of monensin feeding and withdrawal on populations of individual bacterial species in the rumen of lactating dairy cows fed high-starch rations. *Appl Microbiol Biotechnol.* (2008) 80:135–45. doi: 10.1007/s00253-008-1528-9

11. Andrade-Montemayor H, García Gasca T, Kawas J. Ruminal fermentation modification of protein and carbohydrate by means of roasted and estimation of microbial protein synthesis. *Revista Brasileira de Zootecnia.* (2009) 38:277–91. doi: 10.1590/S1516-35982009001300028

12. Yang CM, Russell JB. The effect of monensin supplementation on ruminal ammonia accumulation in vivo and the numbers of amino acid-fermenting bacteria. *J Anim Sci.* (1993) 71:3470–6. doi: 10.2527/1993.71123470x

13. Eschenlauer SC, McKain N, Walker ND, McEwan NR, Newbold CJ, Wallace RJ. Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. *Appl Environ Microbiol.* (2002) 68:4925–31. doi: 10.1128/AEM.68.10.4925-4931.2002

14. rd CoS. [Decision No. 1786/Ec of the European Parliament and of the Council of September 23rd, concerning the adoption of a program of community action in the field of public health (2003-2008)]. *An Sist Sanit Navar.* (2003) 26:109-19. doi: 10.23938/ASSN.0466

15. Russell JB, Houlihan AJ. Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiol Rev.* (2003) 27:65–74. doi: 10.1016/S0168-6445(03)00019-6

16. Blanch M, Calsamiglia S, DiLorenzo N, DiCostanzo A, Muetzel S, Wallace RJ. Physiological changes in rumen fermentation during acidosis induction and its control using a multivalent polyclonal antibody preparation in heifers. *J Anim Sci.* (2009) 87:1722–30. doi: 10.2527/jas.2008-1184

17. DiLorenzo N, Diez-Gonzalez F, DiCostanzo A. Effects of feeding polyclonal antibody preparations on ruminal bacterial populations and ruminal ph of steers fed high-grain diets. *J Anim Sci.* (2006) 84:2178–85. doi: 10.2527/jas.2005-489

18. Cassiano ECO, Junior FP, Barros TA, Marino CT, Pacheco RDL, Ferreira FA, et al. Evaluation of liquid and powdered forms of polyclonal antibody preparation against streptococcus bovis and fusobacterium necrophorum in cattle adapted or not adapted to highly fermentable carbohydrate diets. *Anim Biosci.* (2021) 34:74–84. doi: 10.5713/ajas.19.0761

19. Millen DD, Pacheco RD, DiLorenzo N, Martins CL, Marino CT, Bastos JP, et al. Effects of feeding a spray-dried multivalent polyclonal antibody preparation on feedlot performance, feeding behavior, carcass characteristics, rumenitis, and blood gas profile of brangus and nellore yearling bulls. *J Anim Sci.* (2015) 93:4387–400. doi: 10.2527/jas.2015-9227

20. Barros TA, Cassiano ECO, Marino CT, Pacheco RDL, Ferreira FA, Junior FP, et al. Polyclonal antibodies as a feed additive for cattle adapted or not adapted to highly fermentable carbohydrate diets. *J Appl Anim Res.* (2019) 47:565–72. doi: 10.1080/09712119.2019.1691561

21. Lammers BP, Buckmaster DR, Heinrichs AJ. A simple method for the analysis of particle sizes of forage and total mixed rations. *J Dairy Sci.* (1996) 79:922–8. doi: 10.3168/jds.S0022-0302(96)76442-1

22. Chemists AoOA. *Official Methods of Analysis of Aoac*. 18 ed. Maryland, MD: Association of Official Analytical Chemists (2005).

23. Van Soest PJ. *Nutritional Ecology of the Ruminant*. 2nd ed. Ithaca, NY: Cornell Univ Press (1994).

24. Pereira JRA, Rossi Jr P. *Practical Manual of Feedstuffs Nutritional Evaluation*. 1st ed. Piracicaba, SP: FEALQ (1995).

25. Hendrix DL. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Sci.* (1993) 33:1306–11. doi: 10.2135/cropsci1993.0011183X003300060037x

26. NRC. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Washington, DC: Natl. Acad. Press (2001).

27. Fox DG, Sniffen CJ, O'Connor JD, Russell JB, Van Soest PJ, A. Net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *J Anim Sci.* (1992) 70:3578–96. doi: 10.2527/1992.70113578x

28. Bevans DW, Beauchemin KA, Schwartzkopf-Genswein KS, McKinnon JJ, McAllister TA. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. *J Anim Sci.* (2005) 83:1116–32. doi: 10.2527/2005.8351116x

29. Nordlund KG EF. Rumenocentesis: a technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. *Bovine Pract.* (1994) 28:109–12.

30. Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: a review. *J Anim Sci.* (1998) 76:275–86. doi: 10.2527/1998.761275x

31. Pryce JD. A Modification of the barker-summerson method for the determination of lactic acid. *Analyst.* (1969) 94:1151–2. doi: 10.1039/an9699401151

32. Erwin ES, Marco GJ, Emery EM. Volatile fatty acids analyses of blood and rumen fluid by gas chromatography. *J Dairy Sci.* (1961) 44:1768–71. doi: 10.3168/jds.S0022-0302(61)89956-6

33. Kulasek GA. A micromethod for determination of urea in plasma, whole blood cells using urease and phenol reagent. *Pol Arch Weter.* (1972) 15:801–10.

34. Foldager J. *Protein Requirement and Non Protein Nitrogen for High Producing Cow in Early Lactation*. East Lasing, MI: PhD Diss. Michigan State University (1977).

35. Valadares RFD, Broderick GA, Filho SCV, Clayton MK. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J Dairy Sci.* (1999) 82:2686–96. doi: 10.3168/jds.S0022-0302(99)75525-6

36. Rennó LN. *Consumo, Digestibilidade Total E Parcial, Produção Microbiana, Parâmetros Ruminais E Excreções De Uréia E Creatinina Em Novilhos Alimentados Com Dietas Contendo Quatro Níveis De Uréia Ou Dois De Proteína*. Viçosa: Universidade Federal de Viçosa (2003).

37. Verbic J, Chen XB, MacLeod NA, Ørskov ER. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. *J Agri Sci.* (2009) 114:243–8. doi: 10.1017/S0021859600072610

38. Oliveira AS, Valadares RFD. Valadares Filho SdC, Cecon PR, Rennó LN, Queiroz ACd, et al. Produção De Proteína Microbiana E Estimativas Das Excreções De Derivados De Purinas E De Uréia Em Vacas Lactantes Alimentadas Com Rações Isoprotéicas Contendo Diferentes Níveis De Compostos Nitrogenados Não-Protéicos. *Revista Brasileira de Zootecnia*. (2001) 30:1621–9. doi: 10.1590/S1516-35982001000600032

39. Chizzoti ML, Valadares Filho SC, Valadares RFD, Marcondes MI, Fonseca MA, Pina DS, et al. *Excreção De Creatinina Em Novilhos E Novilhas Anais da 41o Reunião Anual da Sociedade Brasileira de Zootecnia*. Campo Grande (2004).

40. Fujihara T, Ørskov ER, Reeds PJ, Kyle DJ. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. *J Agric Sci.* (2009) 109:7–12. doi: 10.1017/S0021859600080916

41. Chen XB, Gomes MJ. *Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives – an Overview of Technical Details: Rowett Research Institute*. Bucksburnd: International Feed Resources Unit (1992).

42. Orellana Boero P, Balcells J, Martin-Orúe SM, Liang JB, Guada JA. Excretion of purine derivatives in cows: endogenous contribution and recovery of exogenous purine bases. *Livestock Prod Sci.* (2001) 68:243–50. doi: 10.1016/S0301-6226(00)00231-1

43. Gonzalez-Ronquillo M, Balcells J, Guada JA, Vicente F. Purine derivative excretion in dairy cows: endogenous excretion and the effect of exogenous nucleic acid supply. *J Dairy Sci.* (2003) 86:1282–91. doi: 10.3168/jds.S0022-0302(03)73712-6

44. Millen DD, Pacheco RD, Arrigoni MD, Galyean ML, Vasconcelos JT, A. Snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. *J Anim Sci.* (2009) 87:3427–39. doi: 10.2527/jas.2009-1880

45. Millen DD, Pacheco RDL, Arrigoni MB, Parrili M, Matsuhara SA, Fossa MV, et al. Feedlot performance and rumen parakeratosis incidence in bos indicus type bullocks fed high grain diets and monensin or polyclonal antibodies preparations against rumen bacteria. In: 2007 Joint Annual Meeting, editor. *Asas – American Society of Animal Science, Vol. 85*. San Antonio, TX: Journal of Animal Science (2007). p. 552.

46. Rodrigues M, Deschk M, Santos GGF, Perri SHV, Merenda VR, Hussni CA, et al. Avaliação Das Características Do Líquido Ruminal, Hemogasometria, Atividade Pedométrica E Diagnóstico De Laminite Subclínica Em Vacas Leiteiras. *Pesquisa Veterinária Brasileira*. (2013) 33:99–106. doi: 10.1590/S0100-736X2013001300016

47. Duffield TF, Merrill JK, Bagg RN. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *J Anim Sci.* (2012) 90:4583–92. doi: 10.2527/jas.2011-5018

48. Rigueiro ALN, Pereira MCS, Squizatti MM, Ferreira MM, Dondé SC, Luiz FP, et al. Different combinations of sodium monensin and virginiamycin during feedlot finishing of nellore cattle. *Anim Prod Sci*. (2020) 60:1061–72. doi: 10.1071/AN18657

49. Erickson GE, Milton CT, Fanning KC, Cooper RJ, Swingle RS, Parrott JC, et al. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle. *J Anim Sci.* (2003) 81:2869–79. doi: 10.2527/2003.8111 2869x

50. Mariani TM, Millen DD, Pacheco RDL, Lauritano RD, Arrigoni MDB, Martins CL, et al. Feeding behavior of nellore and brangus cattle fed monensin or polyclonal antibody preparation against lactate-producing rumen bacteria. In: ADSA-CSAS-ASAS, editor. *2009 Joint Adsa-Csas-Asas Annual Meeting, Vol. 87*. Montreal, QC (2009). p. 283.

51. Owens FDG. *AL Ruminal Fermentation. The Ruminant Animal: Digestive Physiology and Nutritio*. Wavelend: Long Grove (1988). p. 145–71.

52. Bauer ML, Herold DW, Britton RA, Stock RA, Klopfenstein TJ, Yates DA. Efficacy of laidlomycin propionate to reduce ruminal acidosis in cattle. *J Anim Sci.* (1995) 73:3445–54. doi: 10.2527/1995.73113445x

53. Stock R. Acidosis in cattle: an overview. In: *33rd Annual Convention of the American Association of Bovine Practitioners Proceedings*. Rapid City, SD (2000). p. 30–7.

54. Forbes JM, Provenza F. D. Integration of learning and metabolic signals into a theory of dietary choice and food intake. In: Cp B, editor. *Ruminant Physiology Digestion, Metabolism, Growth and Reproduction*. New York, NY: CABI Publishing (2000). p. 3–20.

55. Jonsson NN, Kleen JL, Wallace RJ, Andonovic I, Michie C, Farish M, et al. Evaluation of reticuloruminal Ph measurements from individual cattle: sampling strategies for the assessment of herd status. *Vet J.* (2019) 243:26–32. doi: 10.1016/j.tvjl.2018.11.006

56. Denwood MJ, Kleen JL, Jensen DB, Jonsson NN. Describing temporal variation in reticuloruminal Ph using continuous monitoring data. *J Dairy Sci.* (2018) 101:233–45. doi: 10.3168/jds.2017-12828

57. Plaizier JC, Mulligan FJ, Neville EW, Guan LL, Steele MA, Penner GB. Invited review: effect of subacute ruminal acidosis on gut health of dairy cows. *J Dairy Sci.* (2022) 105:7141–60. doi: 10.3168/jds.2022-21960

58. Plaizier JC, Danesh Mesgaran M, Derakhshani H, Golder H, Khafipour E, Kleen JL, et al. Review: enhancing gastrointestinal health in dairy cows. *Animal*. (2018) 12:s399–418. doi: 10.1017/S1751731118001921

59. Mu Y, Qi W, Zhang T, Zhang J, Mao S. Multi-omics analysis revealed coordinated responses of rumen microbiome and epithelium to high-grain-induced subacute rumen acidosis in lactating dairy cows. *mSystems*. (2022) 7:e0149021. doi: 10.1128/msystems.01490-21

60. Mu YY Qi WP, Zhang T, Zhang JY, Mao SY. Gene function adjustment for carbohydrate metabolism and enrichment of rumen microbiota with antibiotic resistance genes during subacute rumen acidosis induced by a high-grain diet in lactating dairy cows. *J Dairy Sci.* (2021) 104:2087–105. doi: 10.3168/jds.2020-19118

61. Oetzel GR, editor. Clinical Aspects of Ruminal Acidosis in Dairy Cattle. In: *Proceedings of the 33rd Annual Convention of the American Association of Bovine Practitioner*. Rapid City, SD (2000).

62. Garry FB. Indigestion in ruminants. In: Smith BP, editor. *Large Animal Internal Medicine* St Louis, MO: Mosby (2002). p. 722–47.

63. Goad DW, Goad CL, Nagaraja TG. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. *J Anim Sci.* (1998) 76:234–41. doi: 10.2527/1998.761234x

64. Meyer NF, Bryant TC. Diagnosis and management of rumen acidosis and bloat in feedlots. *Vet Clin North Am Food Anim Pract.* (2017) 33:481–98. doi: 10.1016/j.cvfa.2017.06.005

65. Khafipour E, Li S, Plaizier JC, Krause DO. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. *Appl Environ Microbiol.* (2009) 75:7115–24. doi: 10.1128/AEM.00739-09

66. Barducci RS, Arrigoni MDB, Pacheco RDL, Millen DD, Martins CL, Baldin PFS, et al. Feedlot performance of brangus cattle fed monensin or polyclonal antibody preparation against lactate- producing rumen bacteria. In: ADSA-CSAS-ASAS, editor. *2009 Joint Adsa-Csas-Asas Annual Meeting, Vol. 87*. Montreal, QC (2009). p. 283–4.

67. Bergen WG, Bates DB. Ionophores: their effect on production efficiency and mode of action. *J Anim Sci.* (1984) 58:1465–83. doi: 10.2527/jas1984.5861465x

68. Bell NL, Anderson RC, Callaway TR, Franco MO, Sawyer JE, Wickersham TA. Effect of monensin inclusion on intake, digestion, and ruminal fermentation parameters by bos taurus indicus and bos taurus taurus steers consuming bermudagrass hay. *J Anim Sci.* (2017) 95:2736–46. doi: 10.2527/jas.2016.1011

69. Ruiz R, Albrecht GL, Tedeschi LO, Jarvis G, Russell JB, Fox DG. Effect of monensin on the performance and nitrogen utilization of lactating dairy cows consuming fresh forage. *J Dairy Sci.* (2001) 84:1717–27. doi: 10.3168/jds.S0022-0302(01)74607-3

70. Kleen JL, Hooijer GA, Rehage J, Noordhuizen JP. Subacute ruminal acidosis (Sara): a review. *J Vet Med A Physiol Pathol Clin Med.* (2003) 50:406–14. doi: 10.1046/j.1439-0442.2003.00569.x

71. Urrutia NL, Harvatine KJ. Acetate dose-dependently stimulates milk fat synthesis in lactating dairy cows. *J Nutr.* (2017) 147:763–9. doi: 10.3945/jn.116.245001

72. Allen MS, Voelker JA, Oba M. Physically effective fiber and regulation of ruminal Ph: more than just chewing. In: NP Joshi TH, editor. *Production Diseases in Farm Animals*. Wageningen: Wageningen Academic Publishers (2006). p. 270–8.

73. Kolver ES, de Veth MJ. Prediction of ruminal ph from pasture-based diets. *J Dairy Sci.* (2002) 85:1255–66. doi: 10.3168/jds.S0022-0302(02)74190-8

74. Fereli F, Branco AF, Jobim CC, Coneglian SM, Granzotto F, Barreto JC. Monensina Sódica E Saccharomyces Cerevisiae Em Dietas Para Bovinos: Fermentação Ruminal, Digestibilidade Dos Nutrientes E Eficiência De Síntese Microbiana. *Revista Brasileira de Zootecnia.* (2010) 39:183–90. doi: 10.1590/S1516-35982010000100024

75. Pina DS, Valadares Filho SC, Tedeschi LO, Barbosa AM, Valadares RF. Influence of different levels of concentrate and ruminally undegraded protein on digestive variables in beef heifers. *J Anim Sci.* (2009) 87:1058–67. doi: 10.2527/jas.2008-1069

76. Russell JB, Bottje WG, Cotta MA. Degradation of protein by mixed cultures of rumen bacteria: identification of streptococcus bovis as an actively proteolytic rumen bacterium. *J Anim Sci.* (1981) 53:242–52. doi: 10.2527/jas1981.531242x

77. Owens FN, Lusby KS, Mizwicki K, Forero O. Slow ammonia release from urea: rumen and metabolism studies. *J Anim Sci.* (1980) 50:527–31. doi: 10.2527/jas1980.503527x

78. Poos MI, Hanson TL, Klopfenstein TJ. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. *J Anim Sci.* (1979) 48:1516–24. doi: 10.2527/jas1979.4861516x

79. Chen G, Russell JB. More monensin-sensitive, ammonia-producing bacteria from the rumen. *Appl Environ Microbiol.* (1989) 55:1052–7. doi: 10.1128/aem.55.5.1052-1057.1989

80. Bladen HA, Bryant MP, Doetsch RN. A study of bacterial species from the rumen which produce ammonia from protein hydrolyzate. *Appl Microbiol.* (1961) 9:175–80. doi: 10.1128/am.9.2.175-180.1961

81. Chen GJ, Russell JB. Fermentation of peptides and amino acids by a monensin-sensitive ruminal peptostreptococcus. *Appl Environ Microbiol.* (1988) 54:2742–9. doi: 10.1128/aem.54.11.2742-2749.1988

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 29 March 2023
DOI 10.3389/fvets.2023.1089903

OPEN ACCESS

EDITED BY
Muhammad Saeed,
Cholistan University of Veterinary and Animal Sciences, Pakistan

REVIEWED BY
Yosra Ahmed Soltan,
Alexandria University, Egypt
Suban Foiklang,
Maejo University, Thailand

*CORRESPONDENCE
Danilo D. Millen
✉ danilo.millen@unesp.br

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 04 November 2022
ACCEPTED 10 March 2023
PUBLISHED 29 March 2023

CITATION
Squizatti MM, Rigueiro ALN, Silvestre AM, Soares CHG, Assumpção AHPM, Dias EFF, Felizari LD, Silva LAF, Souza KLR, Carvalho VM, Demartini BL, Souza JM and Millen DD (2023) Shortening the adaptation of Nellore cattle to high-concentrate diets using only virginiamycin as sole feed additive negatively impacts ruminal fermentation and nutrient utilization. *Front. Vet. Sci.* 10:1089903. doi: 10.3389/fvets.2023.1089903

Shortening the adaptation of Nellore cattle to high-concentrate diets using only virginiamycin as sole feed additive negatively impacts ruminal fermentation and nutrient utilization

Mariana M. Squizatti[1], André L. N. Rigueiro[1], Antonio M. Silvestre[1], Carlos H. G. Soares[2], Alice H. P. M. Assumpção[2], Evandro F. F. Dias[2], Luana D. Felizari[1], Leandro A. F. Silva[1], Katia L. R. Souza[2], Victor M. Carvalho[2], Breno L. Demartini[1], Johnny M. Souza[2] and Danilo D. Millen[2]*

[1]School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, [2]College of Agricultural and Technological Sciences, São Paulo State University (UNESP), Dracena, São Paulo, Brazil

Feedlot cattle are usually adapted to high-concentrate diets containing sodium monensin (MON) in more than 14 days. However, considering that the dry matter intake DMI is usually lower during adaptation when compared to the finishing period, the use of MON during adaptation may decrease even further the DMI, and virginiamycin (VM) may be an alternative. This study was designed to investigate the effects of shortening the adaptation length from 14 to 9 or 6 days on ruminal metabolism, feeding behavior, and nutrient digestibility of Nellore cattle fed high-concentrate diets containing only VM as the sole feed additive. The experimental design was a 5 × 5 Latin square, where each period lasted 21 days. Five 17 mo-old Nellore yearling bulls were used (415 ± 22 kg of body weight), which were assigned to five treatments: (1) MON (30 mg/kg) and adaptation for 14 days; (2) MON (30 mg/kg) + VM (25 mg/kg) and adaptation for 14 days; (3) VM (25 mg/kg) and adaptation for 14 days; (4) VM (25 mg/kg) and adaptation for 9 days, and (5) VM (25 mg/kg) and adaptation for 6 days. A quadratic effect for adaptation length when only VM was fed was observed for mean pH ($P = 0.03$), duration of pH below 5.2 ($P = 0.01$) and 6.2 ($P = 0.01$), where cattle consuming VM adapted for 9 days had higher mean pH and shorter period of pH below 5.2 and 6.2. Cattle that consumed only MON had a lower concentration of butyrate ($P = 0.02$) and a higher concentration of propionate ($P = 0.04$) when compared to those consuming VM and adapted for 14 days. As the adaptation length decreased for animals consuming only VM, the rumen degradability of dry matter ($P < 0.01$), neutral detergent fiber ($P < 0.01$), and starch ($P < 0.01$) decreased; however, protozoa numbers of *Entodinium* and total protozoa increased. It is not recommended to shorten the adaptation length of these animals to either 6 or 9 days without negatively impacting nutrient disappearance and ruminal fermentation patterns.

KEYWORDS

rumen, metabolism, feedlot, protozoa, degradability

1. Introduction

Ruminant animals, including beef cattle, evolved as herbivores, consuming diets where forages were predominant. However, to supply the increasing demand worldwide for beef, the adoption of feedlot systems became a reality, and grains and concentrate feedstuffs became most of the diets consumed by beef cattle in these systems (1, 2). Furthermore, high-concentrate or starch-rich diets modify the ruminal microflora (3), and this transition from a forage-based to a high-grain diet should take place gradually to allow microorganisms, as well as the ruminal epithelium, to adapt properly to the increasing amount of readily fermentable carbohydrates in the rumen (4).

Several studies have been conducted in North America (5, 6) and Brazil (7–11) to investigate the most appropriate adaptation length to high-concentrate diets for feedlot cattle. Brown et al. (5) compiled several studies from North America and reported that feedlot cattle should not be adapted in <14 d to high-concentrate diets without impairing overall performance and health. Concerning Brazilian feedlots, based on the results of the studies cited above, it was found that, regardless of the type of protocol used, corn processing method, and nutritional background, the adaptation period for Nellore cattle in Brazil should not be in <14 d as well.

For feedlot systems, typically, sodium monensin (MON) is included in the diets in North America and Brazil (1, 2) to improve feed efficiency (12) by reducing dry matter intake (DMI), and to minimize ruminal acidification. Furthermore, all adaptation studies from Brazil mentioned earlier fed high-concentrate diets containing MON as the sole feed additive.

Therefore, it is well documented in the literature that the DMI is usually lower during adaptation when compared to the finishing period (13) and the use of a feed additive, such as the MON, may decrease even further the DMI in this period. Since finishing diets in Brazil contain less energy (1.22 vs. 1.52 Mcal/kg of diet dry matter) than a typical feedlot diet in North America (1, 2), the use of feed additives that decrease DMI, especially during adaptation, may not be recommended. In this context, Virginiamycin (VM) is a growth promoter, and a potential MON replacement in this scenario, that may improve feedlot performance without negatively impacting DMI (14), and it was the second feed additive mostly used by Brazilian nutritionists in 2019 (2). In a Brazilian study, authors reported that Nellore bulls fed VM, as the sole feed additive during adaptation, reached DMI of 2% of body weight (BW) in 4.3 d, on average; whereas that fed MON needed 20.7 d to reach a similar intake (13). In this context, the faster cattle reach a DMI of 2% of BW, the more adapted they are to the diets, since DMI is an important indicator to evaluate how well cattle are either accepting or adapted to the diets (5). As a result, the use of VM as the sole feed additive in feedlot diets containing a moderate amount of energy may allow the adaptation period to be shortened to nine or even 6 days, which may represent a greater economic return, since the animals will be adapted to the finishing diet earlier. Thus, this study was designed to investigate the effects of shortening the adaptation length from 14 to 9 or 6 days on ruminal metabolism, feeding behavior, and nutrient digestibility of Nellore cattle fed high-concentrate diets containing only VM as the sole feed additive.

2. Materials and methods

All the procedures involving the use of animals in this study were in accordance with the guidelines established by the São Paulo State University Ethical Committee for Animal Research (protocol number 02/2017.R1- CEUA).

2.1. Animals and treatments

The trial was conducted at the São Paulo State University feedlot, Dracena campus, Brazil. Five 22-mo-old yearling Nellore steers (414.86 ± 21.71 kg) fitted with ruminal cannulas were randomly assigned to a 5 × 5 Latin square design. Cattle were randomly assigned to a different treatment in each period, which lasted 21 days. Therefore, the experimental treatments were as follows: (1) MON [27 mg/kg of dry matter (DM)] and 14-d adaptation (MON14); (2) MON (27 mg/kg of DM) + VM (25 mg/kg of DM) and 14-d adaptation (MONVM14); (3) VM (25 mg/kg of DM) and 14-d adaptation (VM14); (4) VM (25 mg/kg of DM) and 9-d adaptation (VM9); e (5) VM (25 mg/kg of DM) and 6-d adaptation (VM6).

2.2. Feeding and management description

At the beginning of the study, all steers were dewormed and vaccinated (tetanus, bovine viral diarrhea virus, 7-way *Clostridium sp.*; Cattlemaster and Bovishield, Pfizer Animal Health, New York, NY). Nellore steers were housed in individual pens (72 m^2) equipped with 6 m of linear bunk space and free water access to a drinking fountain (3.00 × 0.80 × 0.20 m) shared by two animals. Steers were fed *ad libitum* with a total mixed ration (TMR) once a day at 0800 h, and DMI was calculated daily by weighing ration offered and orts, before the next morning delivery, and expressed both in kilograms and as a percentage of BW. The dietary DM was determined daily following the procedures from (method 934.01; AOAC, 1990). The amount of feed offered was adjusted daily based on the targeted amount of orts (3 to 5%) left before morning feed delivery (0700 h). The BW was measured at the beginning (day 1) and at the end (day 21) of each period at 0700 h.

The basal diets were formulated according to the Large Ruminant Nutrition System (15) and are shown in Table 1. Basal diets were composed of sugarcane bagasse, *Cynodon dactylon hay*, corn grain (finely ground), soybean meal, mineral supplement, and urea. The step-up adaptation program consisted of *ad libitum* intake with increasing levels of concentrate ingredients until reaching the concentrate level of the finishing diet (84%). Adaptation diets 1, 2, and 3 contained 66, 72, and 78% concentrate. Moreover, the management of the adaptation diets was performed according to treatments as follows: cattle adapted for 6 d were fed adaptation diets for two d each; animals adapted for 9 d received adaptation diets for three d each; whereas cattle adapted for 14

TABLE 1 Feed ingredients and chemical composition of high-concentrate diets fed to Nellore yearling bulls during adaptation and finishing periods.

Item	Percent of concentrate			
	66	72	78	84
Ingredients, % of DM[a]				
Sugarcane bagasse	20.00	18.00	16.00	12.00
Cynodon dactylon hay	15.00	10.00	5.00	2.00
Finely ground corn grain	41.80	50.00	59.60	70.00
Soybean meal	20.00	18.70	16.00	12.55
Supplement[b]	2.50	2.50	2.50	2.50
Urea	0.70	0.80	0.90	0.95
Nutrient content, % of DM[c]				
DM, as % of organic matter	46.00	48.00	51.00	57.00
Total digestible nutrients	64.00	67.00	70.00	74.00
Crude protein	15.60	15.60	15.20	14.60
Neutral detergent fiber	41.40	36.60	31.40	14.60
Non-fiber carbohydrates	38.00	43.00	49.00	55.00
peNDF[d]	28.00	23.00	18.00	13.00
NEg[e], Mcal/kg[e]	1.00	1.08	1.15	1.26
Ca	0.60	0.58	0.56	0.54
P	0.40	0.41	0.42	0.42

[a]Dry matter; [b]Supplement contained: Ca: 182g/kg of DM; P: 40.5g/kg of DM; Mg: 7.7g/kg of DM; K: 0.5g/kg of DM; Na: 82.2g/kg of DM; Cl: 126.5g/kg of DM; S: 16g/kg of DM; Co: 27.50 mg/kg of DM; Cu: 754.17 mg/kg of DM; Fe: 2498 mg/kg of DM; I: 37.29 mg/kg of DM; Mn: 740 mg/kg of DM; Se: 6.20 mg/kg of DM; Zn: 1790 mg/kg of DM. Monensin (Bovensin 200; Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil) was added at 1000 mg/kg of supplement and Virginiamycin (V-Max 2; Phibro Animal Health Corporation, Guarulhos, São Paulo, Brazil) was added at 833 mg/kg of supplement and offered to yearling bulls according to the treatments; [c]Estimated by equations according to Large Ruminant Nutrition System Fox et al., (15); [d]Phisically effective NDF determined according to method described by Heinrichs and Kononoff (18); [e]Net energy for gain, Mcal.

d were fed 66, 72, and 78% concentrate for 5 d, 4 d, and 5 d, respectively.

2.3. Experimental period

All data and samples in this study were collected, from each experimental period, according to the timeline shown in Figure 1. The description of each method employed to process and analyze samples and data collected are described in the following sections.

2.4. Feeding behavior and particle sorting

Cattle were submitted to visual observations to evaluate feeding behavior, every 5 min, over 24 h on day 07 (only animals fed VM6; 6-d adaptation), day 10 (only animals fed VM9; 9-d adaptation), and day 15 (all animals) of each experimental period 15 of each experimental period. The visual observations were performed according to Robles et al. (16). Feeding behavior data were recorded for each animal as follows: time spent eating, ruminating, and resting (expressed in minutes), and the number of meals per day. A meal was considered the non-interrupted time cattle stayed in the feed bunk eating the ration.

The meal length in minutes was calculated by dividing the time spent eating by the number of meals per day. The DMI per meal in kilograms was calculated by dividing DMI by the number of meals per day. In addition, time spent eating and time spent ruminating data were used to calculate the eating rate of DM (ERDM; time spent eating/DMI) and rumination rate of DM (RRDM; time spent ruminating/DMI), both expressed in minutes per kilogram of DM. Moreover, samples of diets and orts were collected on the days of feeding behavior data collection for chemical analysis of neutral detergent fiber (NDF) (17) to determine the intake of NDF. The eating rate of NDF (ERNDF) was then calculated by dividing the time spent eating by NDF intake. Likewise, the rumination rate of NDF (RRNDF) was determined by dividing the time spent ruminating by NDF intake. Both ERNDF and RRNDF were expressed in minutes per kilogram of NDF.

Samples of diets and orts were also collected for determination of particle-size distribution, which was performed by sieving using the Penn State Particle Size Separator and reported on an as-fed basis as described by Heinrichs and Kononoff (18). Particle sorting was determined as follows: n intake/n predicted intake, in which n = particle fraction screens of 19 mm (long), 8 mm (medium), 1.18 mm (short), and a pan (fine). Particle sorting values equal to 1 indicate no sorting, those <1 indicate selective refusals (sorting against), and those >1 indicate preferential consumption [sorting for; (19)].

2.5. *In situ* degradability

The *in situ* degradability determination was performed on days 8, 9 and 10 (only animals fed VM6; 6-d adaptation); days 11, 12 and 13 (only animals fed VM9; 9-d adaptation); and days 16, 17 and 18 (all animals) of each experimental period, according to methodology adapted from Mehres and Ørskov (20). About 15 g of diet samples, previously dried at 65°C for 72 h, were added to nylon bags with a porosity of 50 microns, measuring 10.0 × 19.0 cm. Bags were inserted into the rumen and incubated for 72 h. After rumen retrieval, nylon bags were washed with cold running water, and then oven-dried at 65°C for 72 h. Samples not incubated in the rumen were also washed as described above. Samples were analyzed for DM [method 934.01; (21)]; crude protein (CP), by total N determination using the micro-Kjeldahl technique[method 920.87; (21)], NDF, with heat-stable α-amylase according to Van Soest et al. (17), and acid detergent fiber (ADF) according to Van Soest et al. (17). Starch analysis was performed according to Pereira and Rossi (22), with the previous extraction of soluble carbohydrates, as proposed by Hendrix (23). Ether extract (EE), was determined gravimetrically after extraction using petroleum ether in a Soxhlet extractor [method 920.85 (21)]. The value of nitrogen-free extract (NFE) and total digestible nutrients (TDN) was estimated according to NRC (24). The apparent coefficient of nutrient degradability was calculated by the following equation: 100 × [01 – (bag weight after incubation – empty bag weight) / (bag weight before incubation – empty bag weight)].

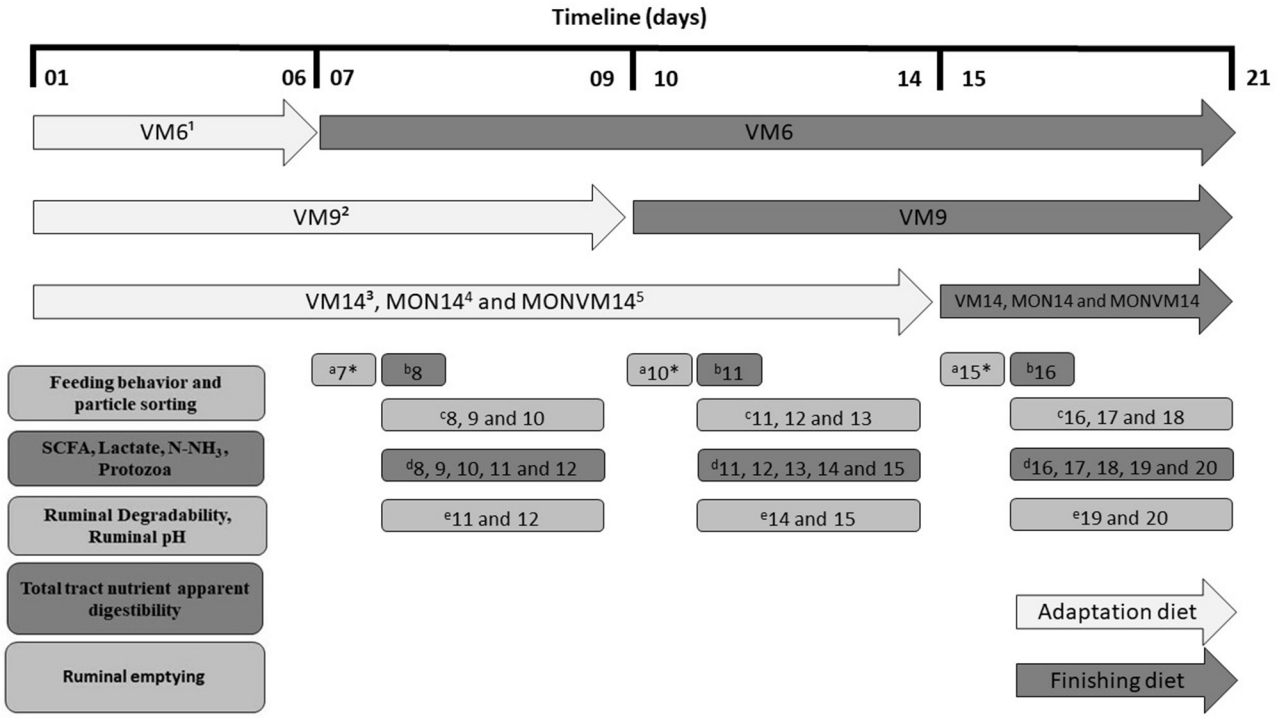


FIGURE 1
Timeline of samples and data collection from Nellore steers cannulated in the rumen and fed Virginiamycin as sole feed additive in each experimental period. [1]VM6: 25 mg of VM/kg of DM and adaption for 6 d; [2]VM9: 25 mg of VM/kg of DM and adaption for 9 d; [3]VM14: 25 mg of VM/kg of DM and adaption for 14 d; [4]MON14: 27 mg of MON/kg of DM and adaption for 14 d; [5]MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; [a]Feeding behavior and particle sorting; [b]Short chain fatty acids, lactate, N-NH_3 and Protozoa; [c]Ruminal degradability and Ruminal pH; [d]Total tract nutrient apparent digestibility; [e]Ruminal emptying. *Sampling was performed at days 7 to 12 for animals fed VM6 only (6-d adaptation); days 10 to 15 for animals fed VM9 only (9-d adaptation); days 15 to 16 for all animals (VM14, MON14, and MONVM14).

2.6. Ruminal fermentation variables

Ruminal pH was continuously measured every 10 min, on days 8, 9 and 10 (only animals fed VM6; 6-d adaptation); days 11, 12 and 13 (only animals fed VM9; 9-d adaptation); and days 16, 17 and 18 (all animals) of each experimental period, using a Lethbridge Research Center Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA) as described by Penner et al. (25). The pH electrode (model T7-1 LRCpH, Dascor, Escondido, CA model S650) was covered by a shroud that allowed particle and liquid passage but kept the pH electrode from contacting the surface of the ruminal epithelium. The capsule was attached to the ruminal cannula plug to aid in system location within the rumen and to help maintain the electrode in a vertical position. Two 900-g weights were fastened to the bottom of the electrode shroud to maintain the electrode in the ventral sac of the rumen. Readings in pH buffers 4 and 7 were recorded before placing the LRCpH system in the rumen. The daily ruminal pH data were averaged and summarized as minimum pH, mean pH, and maximum pH, as well as the area under the curve, and the duration of time in which pH was below 6.2, 6.0, and 5.8. The area under the curve was calculated by multiplying the absolute value of deviations in pH by the time (min) spent below the established threshold for each measure divided by 60 and expressed as pH unit × hour. Likewise, data loggers recorded rumen temperature and ox-redox potential (25).

Ruminal fluid samples were collected *via* cannula at 0, 3, 6, 9, and 12 h after the morning meal on days 8, 9 and 10 (only animals fed VM6; 6-d adaptation); days 11, 12 and 13 (only animals fed VM9; 9-d adaptation); and days 16, 17 and 18 (all animals) of each experimental period. Approximately 500 mL of rumen fluid was collected, at each sampling time, from 3 different parts of the rumen. After the collection of samples, the remaining ruminal fluid was returned to the rumen immediately after the collection. For short-chain fatty acid (SCFA) analyses that included acetate, propionate, and butyrate, a fraction of approximately 100 mL of ruminal fluid was centrifuged at 2,000 × g for 20 min at room temperature, and 2 mL of the supernatant was added to 0.4 mL of formic acid and frozen at −20°C for further analyses, according to Erwin et al. (26). The SCFA were measured by gas chromatography (Finnigan 9001, Thermo Scientific, West Palm Beach, FL) using a glass column Ohio Valley Megabore, model 1 OV-351 of 1 Micron, being 30 mm long and 0.53 mm in diameter. Lactic acid concentration was measured by a colorimetric technique, according to Erwin et al. (26). For NH_3-N concentration determination, 2 mL of the supernatant was added to 1 mL of 1 N of H_2SO_4 solution and the centrifuge tubes were immediately frozen until the colorimetric analyses, according method described by Kulasek (27) and adapted by Foldager (28).

2.7. Ruminal protozoa counting

For the differential counting of rumen ciliated protozoa, the ruminal content was manually collected by sweeping the floor of this organ, and 10 ml of this material was stored in a vial containing 20 ml of 50% (v / v) formaldehyde. The collections were performed on day 10 (only animals fed VM6; 6-d adaptation); day 13 (only animals fed VM9; 9-d adaptation); and day 18 (all animals) of each experimental period, at 0, 4, 8, and 12 hours after morning feeding. In 1-mL sample was added two drops of 2% brilliant green and diluted with 9 ml of 30% glycerol. Protozoa were identified (genus *Isotricha*, *Dasytricha*, *Entodinium* and *Diplodiniinae* subfamily) and counted using a Sedgwick counting chamber Rafter with internal dimensions of 50 mm × 20 mm × 1 mm (capacity 1 mL) by optical microscopy (Olympus CH-2®, Japan) (29).

2.8. Total tract apparent digestibility

The apparent total tract digestibility was determined using titanium dioxide (TiO_2) as an external marker according to Pezzato et al. (30). Cattle received 12 g of titanium dioxide daily, through the ruminal cannula, from day 11 to day 20 of each experimental period. Diet and orts samples were collected from each pen from day 16 to 20 once a day at 0800 h. Samples of feces were collected from day 16 to 20 of each experimental period twice a day at 0800 h and at 1600 h. Feed and fecal samples were dried at 65°C for 72 h and ground to pass a 1-mm screen. After being individually ground, each daily sample was appropriately weighed and an aliquot of each day was taken to compose an individual composite sample per animal per period (∼200 g). Composite samples per animal were used to determine DM [method 934.01; (21)]; CP [method 920.87; (21)]; NDF and ADF (17); starch (22) as proposed by Hendrix (23); EE [method 920.85; (21)]; NFE and total TDN (24), as described above for *in situ* degradability of nutrients. Titanium dioxide concentration was determined according to Pezzato et al. (30). The digestibility coefficients were calculated based on the titanium dioxide (TiO_2) content of feces samples. The excretion of DM and nutrients, as well as nitrogen excretion, were calculated from the digestibility coefficient data of DM and their fractions, multiplying the nutrient intake by the respective digestibility coefficients and dividing by 100.

2.9. Ruminal dynamics

The ruminal digesta was removed manually from each steer through the rumen cannula to determine the disappearance rate in the rumen as described by Dado and Allen (31). On day 11 (VM6); day 14 (VM9); and day 19 (all treatments) of each experimental period, steers had their rumens emptied at 11:00, which was about 3 h after delivering the morning meal, based on assumption that the rumen is at the highest level of volume. The same procedure was done on day 12 (VM6); day 15 (VM9); and day 20 (all treatments) of each experimental period, at 8:00, immediately before the morning meal delivery, assuming that the rumen is at its lowest volume. During the emptying procedure of ruminal contents, liquid and solid phases were separated, weighed, and then a 1 kg sample from each steer was homogenized taking into account the proportion of liquid and solid phases for determination of DM. Consequently, rumen digesta was reconstituted and placed back in the rumen of the steer it originally came from. The rumen pool of DM and its disappearance rate were calculated based on the dry weight of each sample (55°C for 72 h). The DM disappearance rate was considered equal to the intake rate, and they were estimated using the formula (32): DM disappearance rate (%/h) = Daily DM intake (kg) / DM Ruminal contents (kg) /24.

2.10. Statistical analysis

First, data related to days 15 to 20 were analyzed, in which all treatments were in the finishing phase, after 14 days of adaptation (minimum period to adapt cattle to finishing diets). Subsequently, data were analyzed shortly after the start of finishing phase, which would be days 7 to 11 and 10 to 14 for animals treated only with VM and adapted for 6 and 9 days, respectively; and from the 15th to the 20th days for the other treatments. In this way, there are two analyzes in which the data were compared. Data were analyzed by PROC MIXED of SAS (2003), where residual normality (Shapiro–Wilk's and Kolmogorov–Smirnov's) and variance heterogeneity (GROUP option of SAS) tests were performed before the analysis of variance. The effect of the treatments was considered fixed; however, the effects of period and animal were considered random factors in the model. Response variables, such as the molar proportion of SCFA, NH_3-N concentration, and protozoa counting were analyzed with repeated measures over time (33). The model included the same effects just described plus time and its interactions with treatments. Each variable analyzed as repeated measures was subjected to 8 covariance structures: unstructured, compound symmetric, heterogeneous compound symmetric, autoregressive of order one [AR (1)], heterogeneous first-order autoregressive [ARH (1)], Toeplitz, heterogeneous Toeplitz, and ante-dependence of order one [ANTE (1)]. The covariance structure that yielded the smaller Akaike and Schwarz's Bayesian criterion based on their −2 res log-likelihood was considered to provide the best fit.

For all response variables analyzed, the following contrasts were tested: (1) linear relationship between days of adaptation when only VM was fed (6, 9, and 14 d) and the dependent variable; (2) quadratic relationship between days of adaptation when only VM was fed (6, 9, and 14 d) and the dependent variable; (3) MONVM14 vs. VM14, and (4) MON14 vs. VM14. As days of adaptation were unequally spaced, we used a SAS macro (ORPOLY), which finds contrast coefficients for orthogonal polynomials for testing a quantitative factor variable and constructs CONTRAST statements using these values. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Dry matter intake and ruminal pH

The results of DMI and ruminal pH on the 16th day of the experimental period are presented in Table 2. When VM was fed as the sole feed additive, no effect was observed ($P > 0.69$) on DMI;

TABLE 2 Dry matter intake and ruminal pH of rumen cannulated Nellore cattle fed high concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on day 16 of the experimental period (or both during finishing periods).

Item	Treatments[a]						*P-value*			
	MON	MONVM	VM				MONVM14	MON14	VM effect[e]	
	14	14	6	9	14	SEM[d]	vs. VM14	vs. VM14	L	Q
DMI[b]										
Kg	8.99	8.88	9.37	9.76	9.62	0.4	0.01	0.04	0.78	0.69
% of BW[c]	2.18	2.16	2.33	2.35	2.33	0.74	0.02	0.03	0.94	0.73
pH measurement										
Mean pH	5.83	5.91	6.12	6.29	5.96	0.08	0.57	0.20	0.16	0.01
Maximum pH	6.83	6.75	6.90	7.04	7.03	0.10	0.03	0.11	0.33	0.51
Minimum pH	4.96	5.06	5.15	5.18	5.15	0.12	0.48	0.15	0.97	0.76
Duration pH <5.2, h	1.14	2.30	0.77	0.82	1.59	0.77	0.50	0.27	0.15	0.43
Duration pH <5.6, h	8.40	5.79	2.51	2.38	5.53	1.47	0.89	0.16	0.05	0.27
Duration pH <6.2 h	18.62	16.72	10.11	8.14	15.34	1.64	0.64	0.35	0.05	0.01
Area < 5.2 pH × h	0.15	0.40	0.08	0.11	0.19	0.12	0.23	0.15	0.05	0.39
Area < 5.6 pH × h	1.87	1.98	0.71	0.70	1.60	0.58	0.62	0.88	0.05	0.32
Area < 6.2 pH × h	10.01	8.49	4.13	3.52	7.65	1.51	0.64	0.40	0.02	0.05
Temperature	39.07	39.20	39.40	39.12	39.24	0.07	0.71	0.02	0.03	0.04
Ox-redox potential	−372.09	−355.67	−376.33	−385.3	−382.9	12.76	0.05	0.51	0.70	0.69

[a]MON14: 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14: 25 mg of VM/kg of DM and adaption for 14 d; VM9: 25 mg of VM/kg of DM and adaption for 9 d; VM6: 25 mg of VM/kg of DM and adaption for 6 d; [b]Dry matter intake; [c]Body weight; [d]Standard Error of Mean; [e]L: linear and Q: quadratic responses for the effect of adaptation length in cattle fed only VM.

however, steers fed either MON14 or MONVM14 decreased DMI ($P \leq 0.04$) in kg and as % of BW when compared to those fed VM14.

For rumen pH variables, no effects of treatments were observed ($P > 0.15$) for minimum pH and time below pH 5.2. However, time below pH < 5.6, time below pH < 6.2, area under pH 5.2, area under pH 5.6, and area under pH 6.2 decreased linearly ($P \leq 0.05$; Table 2) by shortening the adaptation period from 14 to 6 d for cattle fed VM as a sole feed additive. Furthermore, mean rumen pH and temperature were affected quadratically ($P \leq 0.04$) when the adaptation period was shortened from 14 to 6 d for cattle fed only VM as a feed additive. No differences ($P > 0.15$) in mean and minimum pH, as well as in time below pH 5.2, 5.6, and 6.2, and area under pH 5.2, 5.6, and 6.2 were observed when cattle fed VM14 was compared to steers consuming either MON14 or MONVM14. Besides that, cattle fed VM14 had greater maximum rumen pH ($P = 0.03$), and lower ox-redox potential ($P = 0.02$), than animals fed MONVM14. In addition, steers receiving VM14 slightly increased rumen temperature when compared to those fed MON14.

The results of DMI and ruminal pH on the 2nd day after adaptation period are presented in Supplementary Table A. As adaptation length was increased for animals fed VM, intake in kg and% BW increased linearly ($P < 0.01$; 8.37, 9.31 and 9.77 kg; 2.02, 2.21, 2.32 %BW for VM6, VM9 or VM14, respectively), with no differences for MON14 when compared to VM14 (9.17 kg and 2.29 %BW, respectively; $P > 0.13$). Furthermore, on day 2 after adaptation, as the adaptation time increased for animals fed only VM, the mean pH variable was quadratically affected ($P = 0.03$), and the highest value was observed for animals adapted at 9 days (5.94, 6.16 and 5.96 for VM6, VM9 or VM14, respectively). Moreover, a quadratic effect was observed for time below pH <5.2 h ($P = 0.01$) and pH <6.2 h ($P = 0.01$), and the lowest value was observed for animals adapted at 9 days for both pH < 5.2h (1.11, 0.72, and 1.59 for VM6, VM9 or VM14, respectively) and pH <6.2 h (5.44, 3.11 and 5.53 for VM6, VM9, or VM14, respectively).

3.2. Feeding behavior and particle sorting

The results of feeding behavior and particle sorting on the 16th day of the experimental period are presented in Table 3. When cattle receiving VM14 were compared to animals fed MONVM14 and MON14, a decrease in RRDM and RRNDF was observed ($P \leq 0.05$). Moreover, cattle consuming diets containing MON14 or VMMON14 had lesser DMI ($P \leq 0.02$) than that fed VM14. Cattle fed MONVM14 also decreased ($P < 0.01$) NDF intake when compared to animals receiving VM14. It was observed that cattle receiving only VM as a feed additive increased RRDM and RRNDF quadratically ($P \leq 0.05$) when the adaptation period was shortened from 14 to 6 d. Furthermore, NDF intake was linearly decreased ($P = 0.05$) by shortening the adaptation period from 14 to 6 d. No other differences ($P > 0.08$) were detected in terms of feeding behavior by shortening the adaptation period for cattle-fed VM as the sole feed additive. Regarding particle sorting, no differences were detected ($P > 0.15$) among cattle adapted for 14 days regardless of the feed additive fed. On the other hand, the sorting of

TABLE 3 Feeding behavior and feed selectivity of rumen cannulated Nellore cattle fed high concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period.

Item	Treatments[a]					SEM[h]	*P-value*			
	MON	MONVM	VM				MONVM14	MON14vs.	VM effect[i]	
	14	14	6	9	14		vs. VM14	VM14	L	Q
Feeding behavior										
Time spent resting, min	847	875	832.43	785	851	35.02	0.56	0.92	0.68	0.14
Time spent ruminating, min	417	387	433.5	455	392	23.93	0.87	0.42	0.21	0.13
Time spent eating, min	169	166	163.66	186	184	18.27	0.22	0.3	0.2	0.34
Meal length, min	15.78	18.45	17.63	18.98	17.28	2.59	0.5	0.39	0.85	0.33
DMI[b], Kg	9.88	9.72	10.92	10.36	11.08	0.46	0.01	0.02	0.74	0.13
DMI per meal, Kg	1.02	1.18	1.23	1.19	1.06	0.24	0.37	0.79	0.26	0.68
ERDM[c], min/kg de DM	17.99	17.25	14.81	18.22	16.61	2.26	0.64	0.32	0.24	0.05
RRDM[d], min/kg de DM	43.22	40.41	39.11	43.95	35.37	3.11	0.05	0.02	0.25	0.02
NDF[e] intake	4.09	3.69	4.15	4.25	4.51	0.2	<0.01	0.07	0.05	0.69
ERNDF[f], min/kg de DM	43.91	45.84	38.82	45.02	40.96	6.23	0.29	0.52	0.67	0.22
RRNDF[g], min/kg de DM	104.39	106.41	103.25	107.73	87.17	7.69	0.03	0.05	0.08	0.05
Particle sorting										
Long	0.91	0.96	0.87	1.02	0.87	0.1	0.46	0.75	0.98	0.05
Medium	1.07	1.05	1.08	1.16	1.02	0.05	0.66	0.45	0.47	0.04
Short	1.03	1.06	1.03	1.07	1.02	0.02	0.15	0.79	0.68	0.11
Fine	0.96	0.95	0.96	0.9	0.96	0.02	0.66	0.92	0.99	0.02

[a]MON14: 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14: 25 mg of VM/kg of DM and adaption for 14 d; VM9: 25 mg of VM/kg of DM and adaption for 9 d; VM6: 25 mg of VM/kg of DM and adaption for 6 d; [b]Dry matter intake; [c]Eating rate of dry matter; [d]Rumination rate of dry matter; [e]Neutral detergent fiber; [f]Eating rate of NDF; [g]Rumination rate of NDF; [h]Standard Error of Mean; [i]L: linear and Q: quadratic responses for the effect of adaptation length in cattle fed only VM.

TABLE 4 Evaluation of ruminal fermentation products and differential protozoan counting of rumen cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM), or both on the 16th day of the experimental period.

Item	Treatments[a]					SEM[b]	*P*-value			
	MON	MONVM	VM				MONVM14	MON14	VM effect[c]	
	14	14	6	9	14		vs. VM14	vs. VM14	L	Q
Acetate, mol/ 100 mol	60.03	58.75	65.04	61.68	59.98	2.13	0.74	0.99	0.17	0.78
Propionate, mol/ 100 mol	30.77	27.32	25.98	22.96	24.28	2.31	0.33	0.04	0.61	0.43
Butyrate, mol/ 100 mol	11.82	14.79	14.79	14.02	15.09	0.9	0.92	0.01	0.74	0.38
Total SCFA, mM*	102.62	100.86	106.16	98.66	99.34	4.21	0.81	0.6	0.31	0.46
Acet.: Prop.	2.14	2.24	2.6	2.8	2.61	0.17	0.05	0.04	0.99	0.45
Lactate mM	0.05	0.06	0.05	0.05	0.07	0.06	0.85	0.53	0.56	0.54
N-NH_3 mg/ dl	7.03	8	7.99	6.07	7.11	0.62	0.18	0.99	0.26	0.04
Dasytricha $\times$ 10^c/ ml	0.67	0.82	0.63	0.53	0.38	0.2	0.15	0.34	0.25	0.9
Isotricha $\times$ 10^c/ ml*	1.63	1.2	2.74	2.88	1.3	0.29	0.78	0.48	<0.01	0.02
Entodinium $\times$ 10^c/ ml*	216.19	214.75	297.66	296.11	214.46	4.87	0.96	0.82	<0.01	0.01
Diplodinium $\times$ 10^c/ ml	29.28	27.07	23.29	26.83	16.99	0.92	<0.01	<0.01	<0.01	<0.01
Total $\times$ 10^c / ml*	247.78	243.84	325.09	326.35	233.14	5.64	0.23	0.11	<0.01	<0.01

[a]MON14: 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14: 25 mg of VM/kg of DM and adaption for 14 d; VM9: 25 mg of VM/kg of DM and adaption for 9 d; VM6: 25 mg of VM/kg of DM and adaption for 6 d; *Treatment vs. collection time; [b]Standard Error of Mean; [c]L: linear and Q: quadratic responses for the effect of adaptation length in cattle fed only VM.

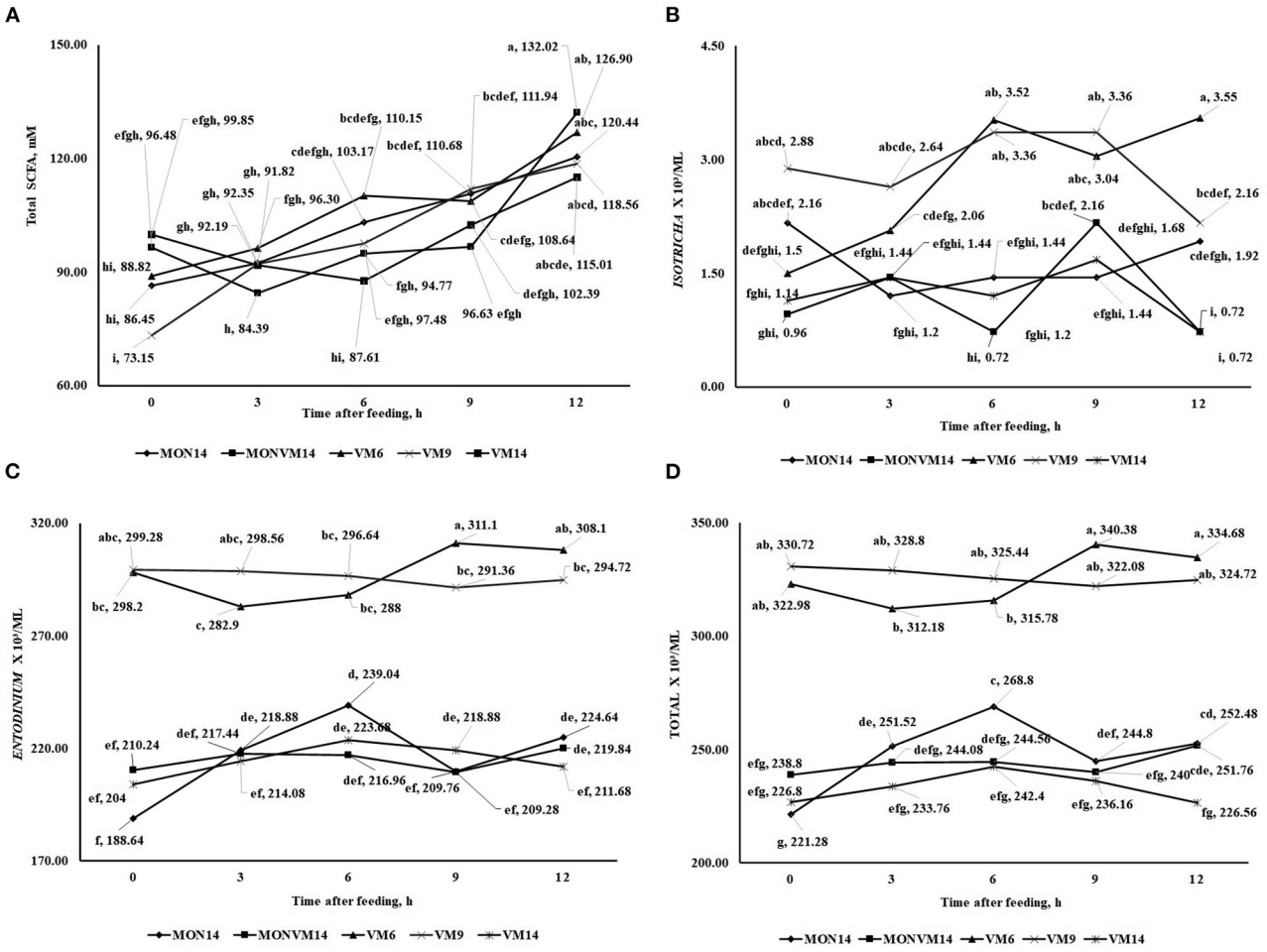


FIGURE 2
This is a figure with sub figures. **(A)** Interaction between treatments and hours after the treatment[1] on the concentration of total (SCFA) fatty acid chain (mM) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period. **(B)** Interaction between treatments and hours after treatment on total ruminal Isotricha count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period. **(C)** Interaction between treatments and hours after treatment on total ruminal Entodinium count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period. **(D)** Interaction between treatments and hours after treatment on total ruminal protozoan count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period.

long, medium, and fine particles was impacted quadratically by shortening the adaptation period from 14 to 6 days in cattle fed only VM.

The results of feeding behavior and particle sorting on the 2nd day after adaptation period are presented in Supplementary Table B. The RRDM was linearly decreased ($P = 0.02$) by shortening the adaptation period from 14 to 6 d (42.23, 41.29 and 35.37 for VM6, VM9 or VM14, respectively). Furthermore, time spent ruminating was affected quadratically ($P = 0.05$) when the adaptation period was shortened from 14 to 6 d for cattle fed only VM as a feed additive (428.00, 452.00 and 392.00 min for VM6, VM9 or VM14, respectively). Similarly, time spent eating was linearly decreased ($P = 0.03$) by shortening the adaptation period from 14 to 6 d (228.00, 214.00 and 184.00 min for VM6, VM9 or VM14, respectively).

3.3. Ruminal fermentation and protozoa counting

The results of ruminal fermentation end-products and total and differential counting of protozoa on the 16th day of the experimental period are presented in Table 4. Cattle receiving MON14 showed ($P = 0.04$) a greater concentration of propionate, but a lower ($P = 0.01$) concentration of butyrate when compared to animals fed VM14. Furthermore, the acetate:propionate ratio decreased ($P \leq 0.05$) for cattle consuming MON14 and MONVM14 when compared to that fed VM14. It's noteworthy to mention that no differences in concentration of Acetate ($P > 0.74$), Lactate ($P > 0.53$), and N-NH_3 ($P > 0.18$) were observed among animals adapted for 14 days. Regarding ruminal protozoa, Nellore cattle receiving MON14 and MONVM14 had greater populations of protozoa from the genus *Diplodinium* ($P < 0.01$) when compared to those from the

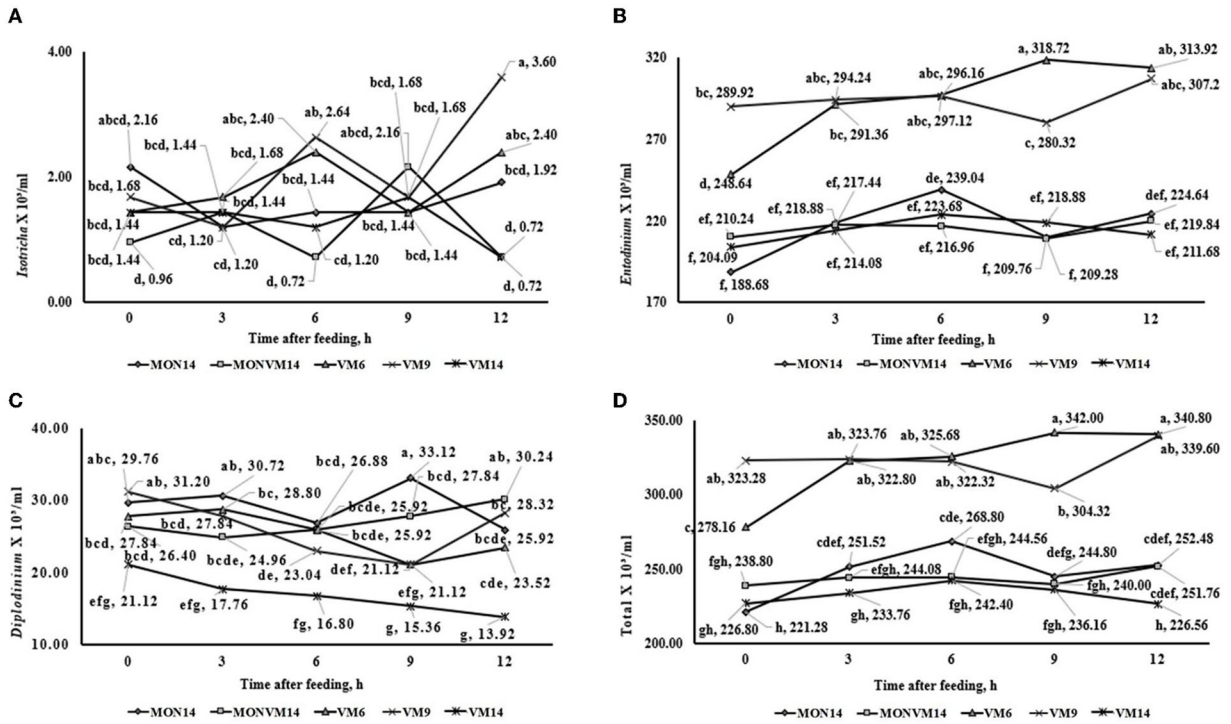


FIGURE 3
This is a figure with sub figures. **(A)** Interaction between treatments[2] and hours after treatment on total ruminal Isotricha count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 2th day after adaptation period. **(B)** Interaction between treatments and hours after treatment on total ruminal Entodinium count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 2th day after adaptation period. **(C)** Interaction between treatments and hours after treatment on total ruminal Diplodinium count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 2th day after adaptation period. **(D)** Interaction between treatments and hours after treatment on total ruminal protozoan count (ml3/ml) of rumen-cannulated Nellore cattle fed high-concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 2th day after adaptation period.

control group fed VM14; however, no differences were noted in populations of protozoa from the genus *Dasytricha* ($P > 0.15$) in this study.

There was no effect of shortening the adaptation period from 14 to 6 days for Nellore cattle consuming only VM as feed additive ($P > 0.10$) for most variables evaluated, except for N-NH_3 ($P = 0.04$) and populations of protozoa from the genus *Diplodinium* ($P < 0.01$) that presented a quadratic response. Moreover, it was observed an interaction between treatments and time for Total SCFA ($P < 0.01$), and populations of protozoa from the genus, *Isotricha* ($P < 0.01$), *Entodinium* ($P < 0.01$), and total protozoa ($P < 0.01$), which are shown in Figure 1. For total SCFA concentration, at hours 0 and 6 cattle receiving VM9 decreased the total concentration of SCFA (Figure 2A). For the variables of ciliated protozoa, cattle in VM6 and VM9 groups presented greater populations of *Isotricha* (Figure 2B), *Entodinium* (Figure 2C), and total protozoa (Figure 2D).

The results of ruminal fermentation end-products and total and differential counting of protozoa on the 2nd day after adaptation period are presented in Supplementary Table C. No effects of treatments were observed ($P > 0.10$) in concentration of Acetate, Lactate and total SCFA. Furthermore, N-NH3 concentration was affected quadratically ($P = 0.05$) when the adaptation period was shortened from 14 to 6 d for cattle fed only VM as a feed additive (6.72, 5.67 and 7.11 mg/ dl for VM6, VM9 or VM14, respectively). Regarding ruminal protozoa, it was observed an interaction between treatments and time for populations of protozoa from the genus *Isotricha* ($P < 0.01$), *Entodinium* ($P < 0.01$), *Diplodinium* ($P < 0.01$), and total protozoa ($P < 0.01$), which are shown in Figure 3. For the variables of ciliated protozoa, cattle in VM6 and VM9 groups presented greater populations of *Isotricha* (Figure 3A), *Entodinium* (Figure 3B), and total protozoa (Figure 3D). However, cattle fed VM14 presented smaller populations of *Diplodinium* (Figure 3C).

1 MON14, 27 mg of MON/kg of DM and adaption for 14 d; MON+VM148 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14, 25 mg of VM/kg of DM and adaption for 14 d; VM9, 25 mg of VM/kg of DM and adaption for 9 d; VM6, 25 mg of VM/kg of DM and adaption for 6 d. [a–i]Means without a common superscript letter differ ($P < 0.01$); pooled SEM (2.41, 0.29, 4.87, and 5.64).

2 MON14, 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14, 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14, 25 mg of VM/kg of DM and adaption for 14 d; VM9, 25 mg of VM/kg of DM and adaption for 9 d; VM6, 25 mg of VM/kg of DM and adaption for 6 d. [a–h]Means without a common superscript letter differ (P < 0.01); pooled SEM (0.28, 4.88, 0.97, and 5.06).

3.4. *In situ* degradability and total tract apparent digestibility

The results of *in situ* ruminal degradability of nutrients on the 16th day of the experimental period are presented in Table 5. Cattle fed MON14 decreased ($P \leq 0.05$) ruminal degradability of DM, NDF, ADF, starch, NFE, and TDN, but increased CP degradability, when compared to animals consuming VM14. Likewise, cattle receiving MONVM14 had lower ($P \leq 0.04$) ruminal degradability of DM, NDF, ADF, EE, starch, NFE, and TDN than those fed VM14. Furthermore, as the adaptation length was shortened for cattle consuming only VM as a feed additive, the degradability of DM, NDF, ADF, EE, and TDN decreased linearly ($P \leq 0.05$) for animals that consumed only VM. In addition, it was observed ($P = 0.05$) a quadratic response for CP degradability when the adaptation length was shortened, in which cattle fed VM9 presented greater CP degradability.

Concerning the total tract apparent digestibility, the results are in Table 5. Animals receiving MON14 decreased ($P \leq 0.05$) digestibility of NDF, ADF, EE, and CP, but increased ($P < 0.01$) NFE digestibility when compared to cattle consuming VM14. Moreover, cattle receiving MONVM14 had lower ($P \leq 0.01$) digestibility of DM, EE, NFE, and TDN, but greater ($P \leq 0.04$) digestibility of NDF, ADF, and CP, when compared to animals fed VM14. When the adaptation period was shortened, the digestibility of DM, ADF, and EE responded quadratically ($P < 0.01$), in which cattle fed VM9 presented lower digestibility of DM and EE, and greater digestibility of ADF. Moreover, the digestibility of starch, NFE, and TDN decreased linearly ($P < 0.01$), but NDF digestibility increased linearly ($P = 0.05$), as the adaptation length was shortened.

The results of *in situ* ruminal degradability of nutrients on the 2nd day after adaptation period are presented in Supplementary Table D. Ruminal degradability of DM, NDF, ADF, and EE was affected linearly ($P < 0.01$) when the adaptation period was increased from 6 to 14 d for cattle fed only VM as a feed additive. Furthermore, when the adaptation period was shortened, the total apparent digestibility of DM, NDF, ADF, EE, CP, Starch, NFE and TDN responded quadratically ($P < 0.01$), in which cattle fed VM9 presented lower digestibility for all variables.

3.5. Ruminal dynamics

The results of ruminal dynamics on the 16th day of the experimental period are presented in Table 6. Cattle fed MON14 increased ($P = 0.05$) total mass in the rumen, expressed as % of BW, as well as lower ($P = 0.03$) Kt, expressed as %/h, when compared to animals receiving VM14. Likewise, animals fed MONVM14 showed a lower ($P = 0.03$) Kt, expressed as %/h than that fed VM14. In addition, cattle consuming MONVM14 presented greater ($P = 0.05$) ruminal DM content than cattle fed VM14. Regarding adaptation length, no effect was observed ($P > 0.10$) on ruminal dynamics when the adaptation period was shortened to either 9 or 6 days.

The results of ruminal dynamics on the 2nd day after adaptation period are presented in Supplementary Table E. There was no linear or quadratic effect of shortening the adaptation period from 14 to 6 days for Nellore cattle fed only VM as feed additive ($P > 0.09$) for the variables evaluated on ruminal dynamics.

4. Discussion

The first part of this discussion section was to verify the effectiveness of VM in promoting a safe adaptation for cattle adapted to high-concentrate diets in 14 days when compared to MON and MON + VM. Based on the fact that the feeding of VM was as effective as MON and MON + VM to assure a good adaptation for Nellore cattle, the second part of the discussion was to evaluate the potential of shortening the adaptation period from 14 to 6 days when VM was added into high-concentrate diets as the sole feed additive.

4.1. Adaptation in 14 days

Considering only 14 days of adaptation, the DMI was higher for cattle that received only VM compared to animals that consumed diets containing MONVM or MON, and this result is clearly explained by the fact that VM does not decrease DMI (13). In contrast, the addition of MON in feedlot diets, associated or not with VM, leads to a reduction in DMI. This reduction in intake may be due to the longer ruminal retention time of dry matter, and to the increased production of propionic acid, which in ruminants is responsible for the regulation of animal satiety (14). These data differ from the reported by Salinas-Chavira (34), where Dutch steers consuming 88% concentrate containing different levels of VM (0, 16, 22.5 ppm) presented similar DMI when compared to cattle fed MON (28 ppm). Furthermore, the positive effect on DMI promoted by feeding only VM had no impact on ruminal pH, which emphasizes the efficiency of VM in controlling rumen fermentation. The replacement of MON and MONVM by only VM as the sole feed additive in the diet increased NDF intake, and also the RRDM and RRNDF, which may have helped cattle receiving VM to cope with ruminal acidification at greater DMI.

On the other hand, cattle consuming only MON had higher concentrations of propionic acid, which is in agreement with Duffield et al. (12). In a companion study, considering 14 days of adaptation, Rigueiro et al. (35) reported that cattle fed only MON improved feed efficiency when compared to those fed only VM, showing that ionophores, such as MON, increase energy availability when added to high-energy diets. According to Lanna and Medeiros (36), the increase in propionate concentration in the rumen is associated with the reduction of DMI, a phenomenon known by the chemostatic mechanism. In addition, cattle receiving either MON or VM did not differ in terms of total SCFA concentrations overall; however, these feed additives tested in this study slightly shift concentrations of specific SCFA, such as the reduced acetate:propionate ratio presented by cattle fed MON. The rumen pH and N-NH3 concentration have a great impact on the main end products of rumen fermentation, which are the SCFA and the microbial protein, main sources of energy and amino

TABLE 5 *In situ* degradability and total apparent digestibility for rumen cannulated cattle fed high concentrate diets containing sodium monensin (MON), virginiamycin (VM) or both on the 16th day of the experimental period.

Item	Treatments[a]						*P*-value			
	MON	MONVM	VM				MONVM14	MON14	VM effects[e]	
	14	14	6	9	14	SEM[d]	vs. VM14	vs. VM14	L	Q
***In situ* degradability**										
Dry Matter, %	69.90	63.39	71.46	73.81	74.55	0.60	<0.01	<0.01	0.01	0.53
Neutral Detergent Fiber, %	36.59	33.70	35.40	44.03	45.11	2.89	0.01	0.04	0.03	0.29
Acid Detergent Fiber[d], %	25.93	23.60	20.06	28.36	32.70	3.05	0.04	0.05	0.01	0.58
Ethereal extract	84.88	71.90	78.02	81.05	84.32	3.16	0.04	0.89	0.04	0.95
Crude protein, %	75.35	61.78	62.26	71.55	66.20	2.90	0.28	0.03	0.36	0.05
Starch, %	86.50	80.51	93.89	93.23	95.51	1.26	<0.01	<0.01	0.35	0.31
NFE[b], %	82.76	78.64	90.24	90.24	93.11	1.57	<0.01	<0.01	0.77	0.17
TDN[c], %	69.74	63.10	71.01	72.65	73.02	0.68	<0.01	<0.01	0.05	0.43
Total apparent digestibility										
Dry Matter, %	75.10	74.16	74.15	71.97	75.45	0.29	<0.01	0.30	<0.01	<0.01
Neutral Detergent Fiber, %	58.93	63.44	62.22	61.57	60.56	0.58	<0.01	0.05	0.05	0.79
Acid Detergent Fiber, %	49.04	53.60	48.28	54.79	51.33	0.81	0.04	0.04	0.01	<0.01
Ethereal extract	74.63	74.79	79.51	70.22	79.47	0.48	<0.01	<0.01	0.93	<0.01
Crude protein, %	77.63	81.81	78.25	77.87	78.44	0.28	<0.01	0.04	0.63	0.15
Starch, %	92.45	92.23	86.13	92.74	92.70	0.20	0.09	0.35	<0.01	<0.01
NFE, %	86.77	80.20	81.88	80.24	84.72	0.47	<0.01	<0.01	<0.01	<0.01
TDN, %	77.01	75.91	75.87	73.80	76.95	0.22	<0.01	0.79	<0.01	<0.01

[a]MON14: 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14: 25 mg of VM/kg of DM and adaption for 14 d; VM9: 25 mg of VM/kg of DM and adaption for 9 d; VM6: 25 mg of VM/kg of DM and adaption for 6 d; [b]Nitrogen free extract; [c]Total digestible nutrients; [d]Standard Error of Mean; 5L: linear and Q: quadratic responses for the effect of adaptation length in cattle fed only VM.

TABLE 6 Ruminal dynamics of rumen cannulated cattle fed high concentrate diets containing sodium monensin (MON), virginiamycin (VM), or both on the 16th day of the experimental period.

Item	Treatments[a]						*P-value*			
	MON	MONVM	VM				MONVM14	MON	VM effect[e]	
	14	14	6	9	14	SEM[d]	vs. VM14	vs. VM 14	L	Q
Body weight, kg	413.40	412.59	414.55	419.89	412.62	21.21	0.99	0.85	0.67	0.11
Total liquid mass, Kg	33.23	32.42	33.24	32.64	31.94	1.60	0.67	0.27	0.30	0.96
Total solid mass, Kg	5.94	5.99	5.91	5.58	5.42	0.36	0.11	0.15	0.20	0.79
Total mass, Kg	39.17	38.42	39.14	38.23	37.36	1.91	0.45	0.20	0.24	0.98
Total liquid mass, % BW[b]	8.13	7.87	8.07	7.93	7.73	0.50	0.58	0.11	0.21	0.88
Total solid mass, % BW	1.44	1.46	1.43	1.36	1.32	0.11	0.11	0.14	0.23	0.90
Total mass, % BW	9.57	9.32	9.50	9.30	9.05	0.60	0.37	0.05	0.18	0.93
DM[c] disappearance rate, Kg/h	0.39	0.39	0.44	0.45	0.43	0.67	0.22	0.18	0.88	0.41
Solid disappearance rate, %/ h	6.62	6.61	7.81	8.30	8.04	0.03	0.03	0.03	0.72	0.48
DM of rumen content, %	15.13	15.68	15.13	14.57	14.49	0.44	0.05	0.21	0.49	0.69

[a]MON14: 27 mg of MON/kg of DM and adaption for 14 d; MON+VM14: 27 mg of MON/kg of DM and 25 mg of VM/kg of DM and adaption for 14 d; VM14: 25 mg of VM/kg of DM and adaption for 14 d; VM9: 25 mg of VM/kg of DM and adaption for 9 d; VM6: 25 mg of VM/kg of DM and adaption for 6 d; [b]Body weight, [c]Dry matter; [d]Standard Error of Mean; [e]L: linear and Q: quadratic responses for the effect of adaptation length in cattle fed only VM.

acids for the animal. In this context, when SCFA accumulates and ruminal pH declines, urea cycling is compromised, resulting on a reduced urea infux and N-NH3 concentrations in the rumen (37, 38). In the present study, no effect of adaptation period was observed for SCFA concentrations in animals fed only VM. In addition, animals adapted for 14 days had higher N-NH3 concentrations, because the higher DMI observed for this treatment, resulting in more N-NH3 availability in the rumen; whereas for animals adapted for 6 days this higher N-NH3 concentration can be explained by not having enough microorganisms, due to the higher count of ciliated protozoa for this treatment, which use bacteria as a source of amino acids and nucleic acids (39).

Moreover, animals that consumed only VM had smaller numbers of *Diplodinium* genus in the rumen, which may be associated with an increased passage rate resulting from greater DMI presented by these animals, since there was no difference involving rumen pH variables of these animals. According to Frazolin and Dehority (40), diets containing high levels of energy may lead to a reduction in the protozoan population due to either reduced rumen pH or increased passage rate. It is noteworthy to mention that VM-fed cattle presented greater Kt, as well as increased ruminal degradability and total tract digestibility of most nutrients than those fed either MON or MONVM, which may lead to the inference that *Diplodinium* protozoa have been washed out of the rumen. Also, the feeding of VM decreased ruminal DM content, which is in agreement with the fact that the rumen passage rate was faster for these animals, but not enough to negatively impact the ruminal degradability of nutrients. These results corroborate the data from Poos et al. (41), who reported that in sheep diets with increasing levels of MON (0, 22, and 38 ppm), there was a reduction in ruminal degradability of DM and fiber. Ruminal degradation of CP may be also reduced with the inclusion of ionophores resulting in an increase in the amount of protein that bypasses the rumen (42). However, this study cannot confirm this.

When the adaptation period of 14 days was adopted, changes in ruminal fermentation patterns and in nutrient disappearance across the digestive tract were detected when MON and MONVM were replaced by VM as the sole feed additive in the diet; however, none of these changes negatively impacted the variables related to the adaptation period in this study. Thus, cattle fed VM can be safely adapted for 14 days to high-concentrate diets.

4.2. Adaptation in <14 days

This section will discuss the effects of shortening the adaptation period from 14 to either 6 or 9 days using VM as the sole feed additive.

The shortening of the adaptation period did not affect the DMI, however, it changed the feeding behavior of Nellore that were adapted in <14 days since they sorted for long and medium particles and against fine particles. Dado and Allen (31) reported that cattle may sort for longer diet particles and avoid diet fines to cope with ruminal acidification. Likewise, cattle adapted for either 9 or 6 days took longer to consume and ruminate a kg of DM, which is a sign of unproper ruminal function (43). This mechanism of animal defense against ruminal acidosis may have collaborated to decrease the pH duration below 5.6 and 6.2, resulting in a smaller area under pH 5.2, 5.6, and 6.2 for cattle adapted in <14 days. As a result of increasing ruminal pH, ciliated protozoa numbers, such as *Isotricha, Entodinium*, and *Diplodinium* also increased, since they are sensitive to low pH (3), in cattle adapted in <14 days.

The change observed in feeding behavior and particle sorting when cattle were adapted for either 6 or 9 days also negatively impacted ruminal degradability and total tract digestibility of most nutrients evaluated. Furthermore, this decrease in ruminal degradability of nutrients may have contributed to reducing ruminal fermentation, resulting in an increase in ruminal pH, and in the numbers of ciliated protozoa as well. Nagaraja and Tigtemeyer (3) reported that ruminal protozoa consume bacteria in order to control ruminal acidification to survive, and this may be one of the factors to explain the decrease in nutrient disappearance across the cattle's digestive tract. It's noteworthy to mention that the shortening of the adaptation did not negatively impact Kt, and the lower ruminal degradability of nutrients may have been offset by a faster passage rate since cattle adapted for either 6 sorted for long and medium particles. In a companion study, Rigueiro et al. (35) reported that shortening the adaptation period from 14 to either 9- or 6-days compromises carcass fat deposition, disrupts feeding behavior patterns, and does not promote any positive effect on animal performance and on development of both the rumen and cecum epithelium.

Therefore, despite maintaining adequate ruminal pH, the pH-related variables evaluated in this study only demonstrate that cattle were able to self-regulate to avoid acidosis. Moreover, it is noteworthy to mention that during the experimental period, an animal while on VM6 treatment presented intensive bloat when promoted to the finishing diet. The steer returned to the study in the next experimental period without presenting any signs of clinical bloat or acidosis for the rest of the study. The short time of 6 days to adapt to the finishing diet may have not allowed the animal self-regulate its behavior to avoid ruminal acidosis.

5. Conclusion

Nellore cattle adapted well to finishing diets, containing only VM as a feed additive, for a period of 14 days. However, it is not recommended to shorten the adaptation length of these animals to either 6 or 9 days without negatively impacting nutrient disappearance and ruminal fermentation patterns.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the São Paulo State University Ethical Committee for Animal Research (protocol number 02/2017.R1- CEUA).

Author contributions

MS and DM designed the experiment and wrote the manuscript. MS, AR, AS, CS, AA, ED, LF, LS, KS, VC, and BD conducted the experiment. MS performed the laboratory and data analyses. MS, JS, and DM provided intellectual input. All authors edited and approved the manuscript submission.

Funding

This project was financially supported by the FAPESP, The São Paulo State Foundation (Grant Number: 2017/17896-8), and Phibro Animal Health.

Acknowledgments

We would like to thank lab and feedlot staff and personnel for providing such outstanding support during the conduction of this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2023.1089903/full#supplementary-material

References

1. Samuelson KL, Hubbert ME, Galyean ML, Loest CA. Nutritional recommendations of feedlot consulting nutritionists: the 2015 new mexico state and texas tech university survey. *J Anim Sci.* (2016) 94:2648–63. doi: 10.2527/jas.2016-0282

2. Silvestre AM, Millen DD. The 2019 brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Rev Brasileira de Zootecnia.* (2021) 50:189. doi: 10.37496/rbz5020200189

3. Nagaraja TG, Titgemeyer EC. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. *J Dairy Sci.* (2007) 90:E17–38. doi: 10.3168/jds.2006-478

4. Bevans DW, Beauchemin KA, Schwartzkopf-Genswein KS, McKinnon JJ, McAllister TA. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. *J Anim Sci.* (2005) 83:1116–32. doi: 10.2527/2005.8351116x

5. Brown MS, Ponce CH, Pulikanti R. Adaptation of beef cattle to high-concentrate diets: performance and ruminal metabolism. *J Anim Sci.* (2006) 84:E25–33. doi: 10.2527/2006.8413_supplE25x

6. Choat WT, Krehbiel CR, Brown MS, Duff GC, Walker DA, Gill DR. Effects of restricted versus conventional dietary adaptation on feedlot performance, carcass characteristics, site and extent of digestion, digesta kinetics, and ruminal metabolism 1,2. *J Anim Sci.* (2002) 80:2726–39. doi: 10.1093/ansci/80.10.2726

7. Barducci RS, Sarti LMN, Millen DD, Putarov TC, Franzói MCS, Ribeiro FA, et al. Restricted versus step-up dietary adaptation in nellore bulls: effects over periods of 9 and 14 days on feedlot performance, feeding behavior and rumen morphometrics. *Anim Feed Sci Technol.* (2019) 247:222–33. doi: 10.1016/j.anifeedsci.2018.11.012

8. Estevam DD, Pereira IC, Rigueiro ALN, Perdigao A, da Costa CF, Rizzieri RA, et al. Feedlot performance and rumen morphometrics of nellore cattle adapted to high-concentrate diets over periods of 6, 9, 14 and 21 days. *Animal.* (2020) 14:2298–307. doi: 10.1017/S1751731120001147

9. Parra FS, Ronchesel JR, Martins CL, Perdigão A, Pereira MCS, Millen DD, et al. Nellore bulls in brazilian feedlots can be safely adapted to high-concentrate diets using 14-day restriction and step-up protocols. *Anim Prod Sci.* (2019) 59:207. doi: 10.1071/AN18207

10. Perdigao A, Millen DD, Brichi ALC, Vicari DVF, Franzoi MCS, Barducci RS, et al. Effects of restricted vs. step up dietary adaptation for 6 or 9 days on feedlot performance, feeding behaviour, ruminal and blood variables of nellore cattle. *J Anim Physiol Anim Nutr.* (2018) 102:224–34. doi: 10.1111/jpn.12681

11. Pereira MCS, Dellaqua JVT, Sousa OA, Santi PF, Felizari LD, Reis BQ, et al. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of nellore cattle submitted to strategic diets prior the adaptation period. *Livestock Sci.* (2020) 234:985. doi: 10.1016/j.livsci.2020.103985

12. Duffield TF, Merrill JK, Bagg RN. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *J Anim Sci.* (2012) 90:4583–92. doi: 10.2527/jas.2011-5018

13. Rigueiro ALN, Pereira MCS, Squizatti MM, Ferreira MM, Dondé SC, Luiz FP, et al. Different combinations of sodium monensin and virginiamycin during feedlot finishing of nellore cattle. *Animal Prod Sci.* (2020) 60:8. doi: 10.1071/AN18657

14. Rogers JA, Branine ME, Miller CR, Wray MI, Bartle SJ, Preston RL, et al. Effects of dietary virginiamycin on performance and liver abscess incidence in feedlot cattle. *J Anim Sci.* (1995) 73:9–20. doi: 10.2527/1995.7319

15. Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, et al. The cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. *Anim Feed Sci Technol.* (2004) 112:29–78. doi: 10.1016/j.anifeedsci.2003.10.006

16. Robles V, Gonzalez LA, Ferret A, Manteca X, Calsamiglia S. Effects of feeding frequency on intake, ruminal fermentation, and feeding behavior in heifers fed high-concentrate diets. *J Anim Sci.* (2007) 85:2538–47. doi: 10.2527/jas.2006-739

17. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2

18. Heinrichs J KP. Evaluating particle size of forages and tmrs using the penn state particle size separator. *State Univ.* (1996) 42:1–14.

19. Leonardi C, Armentano LE. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *J Dairy Sci.* (2003) 86:557–64. doi: 10.3168/jds.S0022-0302(03)73634-0

20. Mehrez AZ, Ørskov ER. A study of artificial fibre bag technique for determining the dig estibility of feeds in the rumen. *J Agric Sci.* (2009) 88:645–50. doi: 10.1017/S0021859600037321

21. Chemists AoOA. *Official Methods of Analysis of Aoac. 15th Edn.* Arlington, VA: Association of Official Analytical Chemists (1990).

22. Pereira JRA, Rossi, P. *Practical Manual of Feedstuffs Nutritional Evaluation.* Piracicaba-SP: FEALQ (1995).

23. Hendrix DL. Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. *Crop Sci.* (1993) 33:1306–11. doi: 10.2135/cropsci1993.0011183X003300060037x

24. NRC NRC-. *Nutrient Requirements of Dairy Cattle. 7th Edn.* Washington, DC: Natl. Acad. Press (2001).

25. Penner GB, Beauchemin KA, Mutsvangwa T. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal ph measurement system. *J Dairy Sci.* (2006) 89:2132–40. doi: 10.3168/jds.S0022-0302(06)72284-6

26. Erwin ES, Marco GJ, Emery EM. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J Dairy Sci.* (1961) 44:1768–71. doi: 10.3168/jds.S0022-0302(61)89956-6

27. Kulasek GA. A micromethod for determination of urea in plasma, whole blood cells using urease and phenol reagent. *Pol Arch Weter*. (1972) 31:15.

28. Foldager J. *Protein Requirement and Non Protein Nitrogen for High Producing Cow in Early Lactation: PhD Diss*. Michigan State University, East Lasing, MI. (1977).

29. Dehority BA. *Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa*. Florida: CRC Press. (1993).

30. Pezzato LE, Miranda EC, Pinto LGQ, Furuya WM, Barros MM, Rosa GJM, et al. Avaliação de dois métodos de determinação do coeficiente de digestibilidade aparente com a tilápia do nilo (*Oreochromis niloticus*). *Acta Sci Anim Sci*. (2008) 24:2452. doi: 10.4025/actascianimsci.v24i0.2452

31. Dado RG, Allen MS. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. *J Dairy Sci.* (1995) 78:118–33. doi: 10.3168/jds.S0022-0302(95)76622-X

32. Robinson PH, Tamminga, S., van Vuuren, A.M. Influence of declining level of feed intake and varying proportion of starch in the concentrate on milk production and whole tract digestibility in dairy cows. *Livestock Production Sci.* (1987) 17:9. doi: 10.1016/0301-6226(87)90050-9

33. Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using sas procedures. *J Anim Sci.* (1998) 76:1216–31. doi: 10.2527/1998.7641216x

34. Salinas-Chavira J, Lenin J, Ponce E, Sanchez U, Torrentera N, Zinn RA. Comparative effects of virginiamycin supplementation on characteristics of growth-performance, dietary energetics, and digestion of calf-fed holstein steers. *J Anim Sci.* (2009) 87:4101–8. doi: 10.2527/jas.2009-1959

35. Rigueiro ALN, Squizatti MM, Silvestre AM, Pinto ACJ, Estevam DD, Felizari LD, et al. The potential of shortening the adaptation of nellore cattle to high-concentrate diets using only virginiamycin as sole feed additive. *Front Vet Sci.* (2021) 8:692705. doi: 10.3389/fvets.2021.692705

36. Lanna DDP, Medeiros, S.R. *Uso De Aditivos Na Bovinocultura De Corte*. Piracicaba-SP: FEALQ (2007). p. 297–324.

37. Aschenbach JR, Penner GB, Stumpff F, Gabel G. Ruminant nutrition symposium: role of fermentation acid absorption in the regulation of ruminal Ph. *J Anim Sci.* (2011) 89:1092–107. doi: 10.2527/jas.2010-3301

38. Silva GM, Podversich F, Schulmeister TM, Santos ERS, Sanford C, Siqueira MCB, et al. Polyclonal antibody preparations from avian origin as a feed additive to beef cattle: ruminal fermentation during the step-up transition diets. *Transl Anim Sci.* (2022) 6:txac070. doi: 10.1093/tas/txac070

39. Williams CL, Thomas BJ, McEwan NR, Rees Stevens P, Creevey CJ, Huws SA. Rumen protozoa play a significant role in fungal predation and plant carbohydrate breakdown. *Front Microbiol.* (2020) 11:720. doi: 10.3389/fmicb.2020.00720

40. Franzolin R, Dehority BA. Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. *J Anim Sci.* (1996) 74:2803–9. doi: 10.2527/1996.74112803x

41. Poos MI, Hanson TL, Klopfenstein TJ. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. *J Anim Sci.* (1979) 48:1516–24. doi: 10.2527/jas1979.4861516x

42. Bergen WG, Bates DB. Ionophores: their effect on production efficiency and mode of action. *J Anim Sci.* (1984) 58:1465–83. doi: 10.2527/jas1984.5861465x

43. Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: a review. *J Anim Sci.* (1998) 76:275–86. doi: 10.2527/1998.761275x

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 30 March 2023
DOI 10.3389/fvets.2023.1041479

OPEN ACCESS

EDITED BY
Rodrigo Marques,
Montana State University, United States

REVIEWED BY
Nuh Ocak,
Ondokuz Mayis University, Türkiye
Daniel Polizel,
University of São Paulo, Brazil
Georgios Papadopoulos,
Aristotle University of Thessaloniki, Greece

*CORRESPONDENCE
Rodrigo Dias Lauritano Pacheco
✉ rodrigo@campanelli.agr.br

SPECIALTY SECTION
This article was submitted to Animal Nutrition and Metabolism, a section of the journal Frontiers in Veterinary Science

RECEIVED 11 September 2022
ACCEPTED 13 February 2023
PUBLISHED 30 March 2023

CITATION
Pacheco RDL, Oliveira Gusmão J, Moura GA, Capelari M, Greco L, Carvalho JCFd, Cervieri RdC, Castro PA, de Alencar Beserra V, Campanelli VPC, Cabral LdS, Carvalho LB, Lanna DPD, Neto MC, Galyean M and Maia ASC (2023) Lysolecithin-derived feed additive improves feedlot performance, carcass characteristics, and muscle fatty acid profile of *Bos indicus*-influenced cattle fed in a tropical environment. *Front. Vet. Sci.* 10:1041479. doi: 10.3389/fvets.2023.1041479

Lysolecithin-derived feed additive improves feedlot performance, carcass characteristics, and muscle fatty acid profile of *Bos indicus*-influenced cattle fed in a tropical environment

Rodrigo Dias Lauritano Pacheco[1]*, Jessica Oliveira Gusmão[1], Gustavo André Moura[2], Matheus Capelari[3], Leandro Greco[3], João Carlos Fontanari de Carvalho[4], Rafael da Costa Cervieri[4], Patrick André Castro[1], Valquíria de Alencar Beserra[1], Victor Paschoal Consentino Campanelli[1], Luciano da Silva Cabral[5], Laura Barbosa Carvalho[5], Dante Pazzanese Duarte Lanna[6], Marcos Chiquitelli Neto[7], Michael Galyean[8] and Alex Sandro Campos Maia[2]

[1]Agro-Pastoril Paschoal Campanelli, Altair, SP, Brazil, [2]Laboratory of Bioclimatology, INOBIO-MANERA, Department of Animal Science, São Paulo State University, Jaboticabal, São Paulo, Brazil, [3]Kemin Industries, Valinhos, São Paulo, Brazil, [4]Nutribeef Consulting, Botucatu, São Paulo, Brazil, [5]Department of Animal Science, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil, [6]Laboratory of Animal Nutrition and Growth, Department of Animal Science, University of São Paulo, Piracicaba, São Paulo, Brazil, [7]Department of Biology and Animal Science, São Paulo State University, Ilha Solteira, São Paulo, Brazil, [8]Department of Veterinary Sciences, Texas Tech University, Lubbock, TX, United States

Lysolecithin might increase ruminal and intestinal emulsification, leading to increased digestibility, but there is minimum information about which is the most appropriate phase to start supplementation and its impacts on feedlot performance and muscle fatty acid profile. Two experiments were conducted to evaluate the effects of phase-feeding of Lysoforte™ eXtend (LYSO). In the first experiment, 1,760 predominantly *Bos indicus* bullocks (initial body weight of 400 ± 0.561 kg) were allocated in a complete randomized block design. LYSO was supplemented at 1 g/1% of ether extract from the diet. Treatments were no LYSO supplementation (NON); LYSO starting during the growing period and continuing during the finishing period; LYSO starting during the finishing period (FIN); and LYSO during adaptation, growing, and finishing periods (ALL). In the second experiment, the same treatments were evaluated with 96 bullocks (64 Nellore and 32½ Nellore × ½ Angus) in a 4 × 2 factorial arrangement (treatments × genotype). For both studies, intake and average daily gain were accessed; carcass characteristics were evaluated in the first experiment, while digestibility of nutrients and profile of muscle fatty acids were measured in the second experiment. In the first experiment, LYSO increased final body weight ($P < 0.022$) and average daily gain (GRO and FIN; $P < 0.05$). In the second study, a treatment × breed × feeding phase interaction was observed with Nellore having a greater average daily gain ($P < 0.05$) than crossbreds in every feeding phase that LYSO was introduced to the diet. A treatment × feeding phase interaction was observed for digestibility, such that LYSO increased total dry matter ($P = 0.004$), crude protein ($P = 0.043$), and NDF ($P = 0.001$) digestibility during the finishing

period. A treatment × breed × day classification was observed ($P < 0.05$). During the finishing phase, crossbreds treated with LYSO had greater DMI ($P < 0.05$) on very hot days than NON. Also, animals treated with LYSO presented a greater C18:3 n3 concentration ($P = 0.047$) in the *longissimus*. Overall, feeding LYSO during GRO and FIN enhanced feedlot performance and should lead to higher intakes during very hot days of the finishing feeding period.

KEYWORDS

feed additives, lysolecithin, zebu, phase-feeding, Nellore, heat stress

1. Introduction

Fat inclusion is a worldwide nutritional strategy recommended by nutritionists (1–3), especially as a means of increasing dietary energy density. Moreover, a fraction of rapidly fermented carbohydrates can be replaced when fat is added to finishing diets, thereby decreasing the risk of metabolic disorders (4, 5).

Generally, fat sources can be classified as non-protected (tallow, yellow grease, vegetable oils, and seeds such as whole cottonseed) or protected (rumen bypass sources, which are mainly calcium salts of soybean and palm oil). Limitations to fat digestion and absorption exist and are inherent in the ruminant animal (6). In the rumen, microorganisms perform biohydrogenation of unsaturated fatty acids into more saturated forms (7), whereas bypass sources are emulsified and digested in the intestines. Independent of the site, microbial and host animal metabolism and digestion of fatty acids have limits that decrease the potential for fat inclusion in diets (8). Therefore, if lipid digestive capacity can be improved in ruminants, feed conversion, and costs could be decreased in feedlot operations.

Lysoforte^{TM} eXtend (LYSO) is an emulsifier additive composed of lysolecithin, synthetic emulsifier, and monoglycerides that increase lipid emulsification and emulsion stability (9), which can lead to higher fat digestibility, alter ruminal biohydrogenation, and increase ruminal lipid passage rate associated with the liquid phase (10). Hence, it may also lead to muscle fatty acid metabolism alterations in feedlot animals, as a greater flux of dietary unsaturated fatty acids is expected to be available for absorption in the intestines. Rumen-protected fatty acids sources, such as calcium salts, have been previously reported to increase feedlot performance (11), downregulate mRNA expression of enzymes related to lipid metabolism (12), and improve marbling (13). However, it has not yet been described how emulsifiers alter carcass characteristics, especially the *longissimus* fatty acids profile.

Calves receiving LYSO had improved fat digestion and absorption of nutrients (14). Also, in lactating cows, greater milk fat yield was observed in high-fiber and lower-fat diets but not in low-fiber and higher-fat diets (10). On the contrary, Drago (15) observed improvements in feedlot performance when a higher-fat diet was offered to Nellore bullocks. Thus, taking into consideration that in feedlot nutrition programs higher fiber and lower fat diets are usually fed during the initial and intermediary days of feeding, conversely to the finishing periods, it is necessary to determine which feeding protocol is the most appropriate to start LYSO administration (i.e., adaptation, growing, or finishing diets) and its consequences in dry matter intake (DMI), digestibility, and performance.

Feedlot cattle raised in open dry lots often are exposed to heat events that directly affect animal bioenergetics (16), with the potential to decrease feed and energy intakes, ADG, and efficiency (17, 18). As a result, it is necessary to monitor meteorological variables during the feeding period. To better describe and understand the effects of supplemental feed additives on DMI, especially during heat events, we evaluated and proposed an innovative methodology to monitor the thermal comfort of feedlot cattle.

We hypothesized that fat and fiber digestion, as well as animal performance, would be increased when LYSO was added to the diet of finishing *Bos indicus*-influenced cattle. The objectives of this study were (1) to evaluate three different strategies of LYSO supplementation on feedlot performance, carcass characteristics, nutrient digestion, and the *longissimus* fatty acid profile of *Bos indicus* and *Bos indicus* crosses and (2) to validate an innovative environmental index named InComfort (InCl) as a tool to evaluate DMI during natural heat stress events in the course of conducting open dry-lot feedlot experiments.

2. Materials and methods

All procedures and protocols involving the use of animals were approved by the Ethics Committee on animal use of the São Paulo State University "Julio de Mesquita Filho" (UNESP; Protocol number: 016339/19).

Two experiments were simultaneously conducted at the Agro-Pastoril Paschoal Campanelli Research Center in Altair, São Paulo, Brazil (20° 31′26″S 49° 03′32″O; average annual temperature is 22.9°C and annual rainfall is 1,287 mm). The first experiment (Exp. 1) was conducted in a large pen setting with the goal of evaluating performance variables, and the second experiment (Exp. 2) was conducted in electronically monitored pens to access digestive, physiological, and metabolic variables. The experiments were carried out from 1 June to 23 September 2020.

2.1. Pre-experimental procedure

From 21 March to 8 May 2020, a total of 1,970 predominantly *Bos indicus* and *Bos indicus* × *Bos taurus* crossbred bullocks

10.3389/fvets.2023.1041479

TABLE 1 Diet composition (Experiments 1 and 2).

	Diets			
Item	Pre exp[1]	Adaptation	Growing	Finishing
Ingredient (%, DM basis)				
Sugar cane silage	30.1	–	–	–
Sugar cane bagasse	35.8	–	–	–
Corn silage	–	31.5	17.3	9.42
Snaplage	–	19.5	25.9	27.1
Citrus pulp	–	18.7	25.7	31.2
Soybean molasses	14.3	6.80	6.10	6.54
Peanut meal	20.5	10.9	9.46	6.60
Whole cottonseed	–	9.27	10.6	13.1
Protected fat[b]		–	1.38	2.62
Urea	0.91	0.92	0.98	1.00
Trace mineral supplement[a]	2.24	2.27	2.41	2.46
Diet composition, % of diet				
DM	52.0	52.0	59.0	65.0
Ash	6.40	3.00	3.00	3.50
CP	11.8	16.0	15.6	14.7
EE	1.20	3.50	5.00	6.50
NDF	52.9	33.5	29.9	27.7
Ca[c]	0.74	1.33	1.70	1.98
P[c]	0.35	0.52	0.43	0.37
NFC	25.7	44.1	46.5	47.7
NEm, Mcal/kg[c]	1.08	1.82	1.89	1.97
NEg, Mcal/kg[c]	0.53	1.19	1.25	1.32

[a]Calcium 127.9 g/kg, phosphorus 23.0 g/kg, cobalt 25.0 mg/kg, copper 420.0 mg/kg, sodium 40.0 g/kg, sulfur 14.0 g/kg, iodine 25.0 mg/kg, magnesium 15.0 g/kg, manganese 810.0 mg/kg, selenium 15.0 mg/kg, zinc 1,500.0 mg/kg, iron 0 mg/kg, vitamin A 72,000 IU/kg, vitamin D3 14,413 IU/kg, vitamin E 500 IU/kg, monensin 714.0 mg/kg, virginiamycin 714.0 mg/kg. [b]Calcium soap of fatty acids, ~86% of fatty acids from soybean and 14% of calcium. Lysoforte^{TM} eXtend inclusion was 1.7% in the premix of treated animals, in those of 1 g/1% of EE.,[c]Estimated by LRNS. [1]Pre-experimental (maintenance diet); trace mineral premix was the same as NON treatments, without LYSO inclusion.

weighing 380.9 ± 16.9 kg (mean ± SD) and approximately 24-month-old were selected for the experiment. The cattle originated from 16 different stocker ranches with an average transportation distance of 693 km (minimum 50 km and maximum 910 km) to the Agro-Pastoril Paschoal Campanelli Research Center. At the end of the weighing process, animals were immediately allocated into seven 12-hectare *Cynodon dactylon* grass paddocks equipped with feedlot bunks. A pre-experimental maintenance diet (Table 1) without feed additives was offered *ad libitum*. This diet was formulated to reestablish the ruminal environment and equalize the physiological conditions of all animals before the experiments.

All animals were ear-tagged, dewormed with an oral drench of 1 ml per 20 kg of body weight (BW) of 10% fenbendazole (Panacur, MSD Saúde Animal, São Paulo, São Paulo, Brazil), and vaccinated against bovine respiratory disease with an intranasal live and attenuated vaccine (1 ml in each nostril; Inforce, Zoetis, São Paulo, São Paulo, Brazil). Additionally, animals were vaccinated against clostridia (5-ml subcutaneous injection; Poli-Star, Valée S/A, Montes Claros, Minas Gerais, Brazil).

On 19 May, animals were submitted to a 16-h feed and water withdrawal. To ensure that all animals were weighed with the same restriction time on a subsequent day to determine initial body weight (IBW), a 15-min staggered-interval schedule for both feed and water restriction was applied. Following weighing, bullocks were blocked according to their respective IBW using a macro lottery spreadsheet with a random number function in Microsoft Excel 2011 (Microsoft Corporation, Redmond, Washington, USA). The 114 animals that presented weight variations two standard deviations above the mean or presented health issues were eliminated from the experiment.

A total of 1,760 animals were selected for Exp. 1, with 890 animals that were predominantly *Bos indicus* Nellore and 860 animals classified *as Bos indicus* × *Bos taurus* crossbred bullocks. To maintain experimental unit homogeneity, both genotypes were equally distributed in each pen (28 *Bos indicus*, predominantly Nellore, and 27 *Bos indicus* × *Bos taurus*).

In parallel to Exp. 1, a total of 96 animals, of which 64 were *Bos indicus* Nellore (NEL, 372.55 ± 20.5 kg) and 32 were *Bos indicus*

× *Bos taurus* (½ Nellore × ½ Angus, CEA, 406.42 ± 24.5 kg), with approximately 24-month-old, were similarly processed and selected for Exp. 2. Bullocks were assigned with radio frequency identification ear tags (Allflex, FDX, Joinville, Santa Catarina, Brazil), and, similar to Exp. 1, both genotypes were equally distributed in each of the four electronically monitored pens (16 NEL and 8 CEA).

2.2. Design and treatments

A randomized complete blocked design was used for Exp. 1. Bullocks were blocked in four weight groups (8 replications/treatment). For Exp. 2, a completely randomized design with a 4 × 2 factorial arrangement (96 animals, 24 animals per pen, 16 NEL, and 8 CEA) evaluated the same treatments as in Exp. 1 in addition to genotype.

On 1 June, animals from both experiments were switched to the adaptation diet. The following treatments were used: (I) no LYSO supplementation (NON); (II) LYSO supplementation starting at the growing period (GRO), from the 17th day on feed throughout the entire finishing period; (III) LYSO supplementation starting only at the finishing period (FIN), from the 34th day on feed throughout the entire finishing period; (IV) LYSO supplementation during the entire feeding period, starting at adaptation on day 0 to the end of the experimental period (ALL). Prior to the experiments, LYSO was mixed in the mineral premix supplement with a 1,000-L capacity mixer for 15 min, according to the instructions provided by the equipment manufacturer. The LYSO was dosed to provide approximately 1 g of product or 1% of dietary ether extract (DM basis). Mixed samples were sent to a commercial lab to evaluate the mixed quality (CBO Laboratory Analysis, Valinhos, SP, Brazil). This resulted in 3.91, 5.95, and 6.29 g/day of the commercial product (LYSOFORTE^{TM} eXtend, Kemin Industries, Inc., Valinhos, SP, Brazil) in adaptation, growing, and finishing diets, respectively. Monensin (Elanco Animal Health, São Paulo, Brazil) and virginiamycin (Phibro Animal Health, Guarulhos, São Paulo, Brazil) dietary concentrations during the adaptation, growing, and finishing phases were 22.77, 24.20, and 24.70 mg/kg of DM, respectively, for both of these feed additives.

2.3. Pens assignment

Animals in Exp. 1 were allocated in open dry lot pens with 0.27 cm of linear bunk space per animal, space availability of 13 m^2/animal (750 m^2; 50 m length × 15 m width) with water trough (3.0 m length × 0.8 m height × 0.25 m width). Shade (SH) was provided in all pens (2.4 m^2/animal). Sheet and cable structures of SH were manufactured with galvalume steel sheet (0.43 mm thickness × 1.08 m wide × 10 m long), 0.15 m of gap distance between sheets, tensioned with a set of eight cables (6.35 mm on top and 3.17 mm under the sheet), and held by carbon steel columns (2.6 mm thickness, 7.62 cm diameter, fixed with a 2-m height concrete base). The sheets were positioned 5 m from the ground in a north-to-south orientation, with an 18° displacement in the northeast-to-southwest direction.

In Exp. 2, animals were allocated to four dry lot pens (375 m^2, 50 m length × 7.5 m width) with a capacity of 24 steers (15.62 m^2/animal). These pens were equipped with three electronic feeding system monitors and two individual scales located at the water trough [IS, Model VW1000, Intergado Ltd., Contagem, Minas Gerais, Brazil; (19)]. Troughs were built with a dimension of 3 m length × 0.8 m height × 0.25 m width. Similar to Exp. 1, animals had access to SH, but in this case, 2.7 m^2/animal was provided to maintain the same steel sheet size according to the respective pen width.

2.4. Feeding and health management

For both studies, the experimental feeding program consisted of three diets: adaptation, growing, and finishing. The adaptation diet was fed for 16 days, the growing diet for 17 days, and the finishing diet from 52 to 73 days, according to block IBW (staggered by 7-day intervals from heavier to lighter blocks). The same adaptation and growing programs were offered to the animals in Exp. 2; however, the finishing diet was offered for 69 days.

Diets were formulated to provide nutrients for an ADG of at least 1.5 kg, according to the LRNS [http://www.nutritionmodels.com/lrns.html, accessed May 2020; (20)]. Nutrient levels and diet composition are presented in Table 1. The animals were fed twice daily at 07:30 and 14:00 h, with bunk score calls recorded daily at 06:45 h, following a modification of the method of Pritchard and Bruns (21) adapted for 1–2% of feed refusals. Feed delivery was equally divided between morning and afternoon offers. Individual pen feed refusals were weighed in a staggered manner (5 min/pen) every morning before the first feed delivery with a modified tractor-trailer (Nonino, CAR-Balança, 1,700-kg capacity, Bebedouro, São Paulo, Brazil) equipped with a ±0.10-kg precision electronic scale (Alfa Instrumentos, Samel-2CF, São Paulo). This procedure was adopted to ensure that the bunks always contained feed, considering that the difference between the first and the last pen to be fed was approximately 2.5 h.

Animals were fed using a truck-mounted mixer (Brutale, Model MTB-120CM, 16-m^3 capacity, São Carlos, São Paulo, Brazil) equipped with an electronic scale (±1 kg precision). The scale was calibrated weekly during the experimental phase. To avoid the confounding effects of feed additives, the mixer was cleaned before every treatment change, four times in the morning and four times in the afternoon. Additionally, it was re-checked for feed residues after the cleaning procedure and flushed with water when necessary. The ingredients were added to the truck-mounted mixer in the following order: corn silage, snaplage, peanut meal, citrus pulp, whole cottonseed, protected fat, soybean molasses, and mineral supplement. Following this, diets were mixed for 4 min before delivery.

One trained person checked animals for signs of disease twice daily. If needed, animals were treated with florfenicol and flunixin meglumine (intramuscular injection, 1 ml per 7.5 kg of BW, Resflor Gold®, MSD Saúde Animal, São Paulo, São Paulo, Brazil) for pulmonary issues or with tildipirosin (intramuscular injection, 1 ml per 45 kg of BW, Zuprevo®, MSD Saúde Animal, São Paulo, Brazil) when hoof anomalies were detected. If necessary, for both illnesses,

a second treatment was given, and the animal was removed from the experiment if recovery was not indicated.

2.5. Performance and carcass variables

For Exp. 1, the average DMI was calculated by the difference between offered feed and refusals. Based on that, the DMI of the large pen study (DMI_{LP}, kg/animal/day) from each large pen was given as follows:

$$DMI_{j(LP)} = \left(\frac{OF(dm_f/100) - RE\,(dm_r/100)}{NA_j} \right)$$

where OF = offered daily feed and RE = refusals were corrected daily to average DM of feed (dm_f) and refusals (dm_r), and divided by the number of animals in each pen (NAj) (j = 1…,32), respectively, of the i^{th} ordinal day from the experiment (i = 1,…,111).

On the last day of the experimental feeding period, to obtain a final body weight (FBW) measurement, animals were withheld from feed and water for 16 h in a staggered manner as described from the beginning of the experiment. Performance data such as ADG and feed conversion (FC) were calculated based on shrunken IBW and shrunken FBW using the mean DMI of the entire experimental period.

At the end of the experiment, animals were harvested at a commercial packing plant located 330 km from the feedlot. Bullocks were harvested on four separate dates (staggered weekly), according to weight blocks (heavier to lighter). Harvest weight was defined when animals reached 560 kg of shrunken FBW.

Hot carcass weight (HCW) was obtained after evisceration and removal of the kidney, pelvic, and heart fat. Dressing percentage (DP) was calculated as the ratio of HCW to shrunken FBW. As different days of feeding were necessary to reach harvest FBW, the average DP (57.03%) of all animals was also used to estimate adjusted FBW, ADG, FE, and FC. In addition, carcasses were classified by one trained packing plant employee, according to subcutaneous fat deposition (SFD), using five categories (2-, 2= scarce, absence of fat; 2+ and 3- median; 3= and 3+ uniform; 4 excessive; Farol JBS, adapted from Brazil, 2004). After 24 h of chilling, pH was measured in the longissimus muscle (CpH), between the 12th and 13th ribs, using a portable digital pH meter (model HI98163; Hanna Instruments, São Paulo, Brazil) with a puncture electrode (model V-627).

For Exp. 2, IBW was calculated as in Exp. 1. Data were analyzed as repeated measures over time to evaluate the feed additive interactions with meteorological variables. The DMI from each electronically monitored pen was given as follows:

$$DMI_{jk(EM)} = \left(\sum_{i=1}^{n} FI_{i(EM)} \right) (dm_i/100)$$

where $FI_{i(EM)}$ (kg/animal/day) is the daily amount of feed intake in the i^{th} visit in the feed bunk equipped with an electronic feeding system performed by j^{th} animal in the k^{th} ordinal day from feeding period, and dm_i (%) is the percentage of dry matter in the diet.

The live weight of animals without feed and water withdrawal (kg) was registered for every drinking event during all feeding periods. From this data basis, spline functions were adjusted as follows:

$$w(x)_{jk} = \alpha + \beta_1 x_{jk} + \gamma_1 z_1 + \gamma_2 z_2 + \gamma_3 z_3 + , \ldots , + \gamma_{19} z_{19}$$

where x is the kth ordinal day from the feeding period registered in the jth animal, consequently the average daily gain was given as follows:

$$ADG_{jk(EM)} = w(x)_{jk} - w(x)_{jk-1}$$

2.6. Chemical analyses

Dietary DM adjustments of feed ingredients (corn silage and snaplage) with variable water concentration were conducted twice daily, before feed mixing, throughout the experiment using a Koster Moisture Tester (Koster Crop Tester Inc., Model D, Medina, Ohio, USA). In a similar manner, total mixed diet and feed refusals were collected twice and once daily, respectively. For both variables, treatment composite samples (based on equal amounts of samples per pen) were generated and dried at 105°C [Tecnal, model TE-394/3-MP, Piracicaba, São Paulo, Brazil; method 930.15, (22)] for 12 h to determine DM.

For both experiments, samples of diet, ingredients, and orts were collected weekly, composited, and sampled for chemical analyses. All the samples were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground in a Wiley-type mill (1-mm screen, MA-680, Marconi Ltda, Piracicaba, São Paulo, Brazil) and analyzed for ash [method 924.05; (23)], NDF (24), CP (AOAC International, 2012), and EE (method 920.85; AOAC, 1986). The NFC was estimated according to the following equation: NFC (%) = 100% – (% NDF + % CP + % EE + % ash), according to Mertens (25).

Apparent nutrient digestibility was measured in Exp. 2. Indigestible NDF was used as an internal marker, determined by a 288-h *in-situ* incubation procedure (26). Due to the necessary adaptation period of the diet before fecal collection and the fact that during the growing feeding phase, GRO treatment was supplemented with LYSO (similar to ALL) and FIN was not supplemented (similar to NON), it was evaluated NON and ALL treatments, in which was possible to respect animal acclimatation to facilities and human presence, necessary adaptation after dietary change (12 days), and fecal collection period (5 days). Animals from NON and ALL treatments were acclimated to human presence inside pens, during the adaptation feeding period, with the objective to visualize individual animal identification. Starting on the 12^{th} day after animals were transitioned to growing diet, fecal and diet samples from all animals of NON and ALL treatments were collected during 5 consecutive days, hourly staggered, over a 10-h period (from 07:00 a.m. to 05:00 p.m.). Fecal samples were collected directly from the pen floor immediately after defecation (avoiding soil contamination) and individually identified. As a result of a successful acclimation program, it was possible to collect hourly subsamples from 47 animals (23 from NON and 24 from ALL). Composited fecal samples were generated with approximately 10%

of the wet weight from each of the hourly subsamples. The same procedure was conducted during the finishing phase, except that it was conducted during the final 5 days of feeding.

The fatty acid profile (FAP) of diets and in the *longissimus* muscle was also evaluated from NON and ALL treatments. Approximately 24-h post-mortem, samples of approximately 2.50 cm were collected from the 12th and 13th ribs of the animals in the second experiment. They were individually identified and vacuum-packed until further laboratory analyses. The FAP was performed according to the methodology described by Folch et al. (27), with the lipid fraction methylated and the methyl esters generated following techniques described by Kramer et al. (28). Qualitative and quantitative measurements of fatty acids were performed *via* gas chromatography (GC-2010 Plus autoinjector – AOC 20i; Shimadzu Scientific Instruments, Kyoto, Japan) using a 100 m × 0.25 mm diameter column of 0.02 μm thickness (Supelco SP-2560; Sigma Aldrich Pty Ltd., Castle Hill, Australia). The initial temperature was kept at 70°C for 4 min, with progressive heating (13°C/min) until the temperature reached 175°C, and this temperature was maintained for 27 min. Thereafter, 4°C/min increases were obtained until the temperature reached 215°C, and this temperature was maintained for 31 min. Hydrogen gas was used as the carrier with a 40 cm^3/s flux. Fatty acids were identified and quantified, and the peak areas were normalized using software (GC solution) with a standard (non-adecanoic acid; C19:0).

2.7. Meteorological data

Solar irradiance (R_S, W m^{-2}; CMP-22, Kipp and Zonen, Delft, Netherlands; spectral range = 0.3–3.6 μm), ultraviolet solar radiation (U_V, W m^{-2}; spectral range = 0.28–0.4 μm), air temperature (T_A, °C; range = −40 to + 70, accuracy ± 0.1°C, accuracy ± 0.1°C), the black-globe temperature in the sun (T_{Gsun}, °C; accuracy ± 0.1°C), relative humidity (R_H, %; accuracy ± 3%), wind speed (W_S, m/s; accuracy ± 0.44 m/s), and daily precipitation (P, mm/h) were all continuously recorded every minute using a portable weather station (WS-18 model 110, Nova Lynk, Auburn, CA, USA) placed near the pens. In addition to the meteorological data collected by the weather station, temperature sensors were also placed inside the pens and water troughs and attached to the roof surface of the shade structure for the characterization of the microclimate experienced by the shaded cattle. These local measurements were recorded every 5 min and included air temperature, relative humidity, black-globe temperature, the temperature of the inner surface of the roof, and water temperature.

A set of six black-globe devices was placed in two shaded pens, while three black globes were placed in three unshaded areas, positioned 2 m above the ground surface. Miniature data loggers (i-bottom DS1925L, Maxim Integrated, Sao Jose, US; size = 0.60 × 1.70 cm, height × diameter; accuracy ± 0.5°C) were inserted inside globes for measuring black-globe temperature ($T_{Gshade1}$ and $T_{Gshade2}$, °C) in the shade underneath the roof and exposed to full sun (T_{Gsun1}, T_{Gsun2}, and T_{Gsun3}, °C). Three temperature sensors (i-bottom) were attached to the inner surface of the shade roof structure to obtain the roof surface temperature (T_{RS1}, T_{RS2}, and T_{RS3}, °C). Three temperature sensors (i-bottom) were previously waxed (Sasol wax, GmbH D-20457) and placed inside the water troughs to obtain water temperature (T_{W1}, T_{W2}, T_{W3}, °C). Three temperature-humidity data loggers (HOBO® data logger, model U12-012, Onset Computer Corp., Bourne, MA), of which two were placed inside the shaded pens and one within an unshaded area, were used to obtain air temperature (T_{A1}, T_{A2}, and T_{A3}, °C) and relative humidity (R_{H1}, R_{H2}, and R_{H3}, °C). These temperature-humidity data loggers were shielded against direct solar radiation.

2.8. Heat load experienced by feedlot cattle

The principal component analyses (29–33) were used to observe dissimilarities over the days on feed concerning the meteorological conditions (T_A, H_R, R_S, U, W_s, and T_G) experienced by feedlot cattle. Principal components were obtained by computing eigenvalues (λ_i) and the respective eigenvectors $e_i' = [e_{i1}\ e_{i2}\ e_{i3}]$ of the data correlation matrix. The bi-dimensional representation of the multidimensional set was created by using scores for the first ($PCA_{1j} = e_{11}T_A + e_{12}H_R + e_{13}R_S + e_{14}U + e_{15}W_S + e_{16}T_G$) and second principal components ($PCA_{2j} = e_{21}T_A + e_{22}H_R + e_{23}R_S + e_{24}U + e_{25}W_S + e_{26}T_G$). All principal components were used according to Liu et al. (32) for the development of an environmental index, namely, the InComfort Index (InCI)-Based Membership Function Value Analysis.

$$InCI = \sum_{i=1}^{n} [R(\lambda_i)\, W(e_i)]$$

where n is the number of principal components and InCI is the weighted membership value calculated with principal components for each day linked with its meteorological conditions, thereby building a ranking with the level of thermal stress. Being $R(\lambda_i)$ given by

$$R(\lambda_i) = \frac{\lambda_i - \lambda_{i(min)}}{\lambda_{i(max)} - \lambda_{i(min)}}$$

indicating λ_i is the value of ith principal component, $\lambda_{i(min)}$ and $\lambda_{i(max)}$ are the maximum and minimum values of ith principal component, respectively:

$$W(e_i) = e_i / \sum_{i=1}^{n} e_i$$

where $W(e_i)$ is the weight of the ith principal component among all the principal components selected for evaluating the level of heat stress on animals, and e_i is the contribution rate of the ith principal component.

The values of InCI are in an interval from 0 to 1, with the lowest value representing meteorological conditions that are more comfortable for animals. Conversely, the highest InCI values, reflect meteorological conditions that negatively affect the thermal comfort of animals. Based on water intake and respiratory rate, the InCI were divided into four classes, namely, rainy days, when the mean of $0 \leq$ InCI ≤ 1, with precipitation rate above 20 mm/day; cloudy days, when the mean of $0 \leq$ InCI ≤ 0.4 (A); hot days, when the mean of $0.4 <$ InCI ≤ 0.6 (N); and very hot days, when the

mean of 0.6 < InCI ≤ (Q). Water intake (WI_{EMP}, L/animal/day) from each electronically monitored pen was calculated as follows:

$$WI_{EMP} = \sum_{i=1}^{n} \left(\frac{W_{fw} - W_{iw}}{0.997} \right)$$

where W_{fw} and W_{iw} (kg/day/animal) are the final and initial weights of the animal during water intake in the n^{th} visit of the animal in the water trough on the i^{th} ordinal day of the experiment, and 0.997 is the water constant (kg/L).

2.9. Statistical analyses

Before statistical analysis, data were checked for normality, homoscedasticity, and outliers using the PROC UNIVARIATE, evaluating Student and Pearson residuals. For the first study, feedlot performance and carcass characteristics were analyzed using the PROC GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC, USA) as a generalized randomized block design. For IBW, FBW, ADG, HCW, DP, CpH, and FAP, animals were considered the experimental unit. Treatment, block, breed, number of days in the receiving pasture, and the treatment × breed interaction were considered fixed effects. Because the treatment × block interaction was not significant ($P > 0.05$), it was removed from the model. Treatment within the pen was considered a random effect. A total of 79 animals were removed from the analysis; 59 because of health problems (pneumonia and/or foot rot; 3.35% of animals) and 20 because of divergence between the research team's notes and the packing plant sequence. For DMI and FC, the pen was considered an experimental unit. For this model, treatment, block, pen, and the treatment × block interaction were considered fixed effects, whereas treatment within a pen was considered a random effect. The Satterthwaite approximation was used to determine denominator degrees of freedom for testing fixed effects in both models. Orthogonal contrasts (NON × ALL + GRO + FIN; ALL × GRO + FIN and GRO × FIN) were performed to compare differences among treatment means, and treatment means were also compared by the PDIFF option of LSMEANS. Differences were declared significant when *P*-value was ≤0.05 and regarded as tendencies when the *P*-value was >0.05 and *P*-value was ≤0.10. Carcass classifications were evaluated by the chi-square test.

For the second study, animals were considered the experimental unit. Raw data from electronic equipment (both bunks and scales) was collected and filtered with algorithms developed by our research group (unpublished data). Performance and physiological data were analyzed as repeated measures over time. Repeated measures were analyzed using mixed model methods based on generalized least squares and a variance component estimation, which were performed by a restricted maximum likelihood (REML) algorithm with a procedure for a linear mixed model (PROC MIXED) of the Statistical Analysis System (34), according to Littell et al. (35). Treatment, breed, treatment × breed, a current day on feed nested within treatment × breed, the current weight of the animal on the respective day of evaluation, and both the duration of meals and the number of bunk visits on the evaluated day were considered fixed effects. Animal nested within treatment × breed was considered the repeated measure subject, and means were compared by the PDIFF option of LSMEANS procedure. In this study, the constructed covariance matrix demonstrated that measures taken close in time did not present the same covariance as measures far apart in time. Based on these results and because Akaike's information criterion (AIC), the AIC corrected (AICC), and the Bayesian information criterion were smaller for the autoregressive moving average (1) covariance structure, verifying the superior fit compared with other covariance structures. As NEL animals differed in IBW in comparison to CEA, it was used as a covariable. For digestibility data, treatment, breed, phase, treatment × phase, and treatment × breed were considered fixed effects, whereas pens nested within a treatment were random. The means were compared using the PDIFF option of LSMEANS. Additionally, for the FA profile, the same model described for digestibility was used, means were compared by the PDIFF option of LSMEANS, and differences were declared significant at a *P*-value of ≤0.05 and regarded as tendencies when the *P*-value was >0.05 and *P*-value was ≤0.10.

3. Results

3.1. Experiment 1 (large pen trial feedlot performance)

Performance results are presented in Table 2. No differences were detected for IBW ($P = 0.791$). The use of LYSO increased FBW ($P = 0.022$); however, no differences were detected among the LYSO phase-feeding treatments ($P = 0.191$). Regarding ADG, no LYSO supplementation effect was observed ($P = 0.122$). Furthermore, when contrasts were used to compare LYSO-supplemented animals, phase-feeding protocols improved ADG compared with ALL ($P = 0.007$). In addition, the same phase-feeding response pattern was observed when NON was compared with GRO ($P = 0.089$; regarded as a tendency) and with FIN ($P = 0.012$) by comparing the pairwise differences among the means. Feeding LYSO did not alter DMI, regardless of the protocol or feeding period ($P > 0.05$). Animals supplemented with LYSO tended to have a lower FC ($P = 0.090$), but no phase-feeding effect was observed ($P = 0.4738$). Conversely, a tendency was observed ($P = 0.060$) when NON was compared with the pairwise comparisons of the means (GRO).

Supplementing LYSO did not affect HCW ($P = 0.241$) under the experimental conditions. Similar to ADG, ALL animals tended to have a lesser HCW ($P = 0.095$) than those on the phase-feeding protocols. Likewise, animals from the FIN treatment tended ($P = 0.055$) to have heavier carcasses compared with NON. Carcass classification was not affected by LYSO supplementation ($P = 0.689$). Moreover, neither the DP (%) nor the CpH ($P = 0.611$) was affected by the LYSO supplementation ($P = 0.290$). The mean DP-adjusted performance variables did not differ (neither tendencies were observed), thus, they were removed from the "Results" and "Discussion" sections.

10.3389/fvets.2023.1041479

TABLE 2 Effects of lysolecithin-derived feed additive administration on the performance of *Bos indicus* influenced cattle in Experiment 1.

Item[b]	Treatments[a]				SEM	*P*-value[c]		
	NON	GRO	FIN	ALL		N × L	A × G + F	G × F
Performance								
IBW, kg	400.83	400.82	400.86	401.1	0.561	0.791	0.513	0.933
FBW, kg	564.77	568.38	570.18	567.00	2.206	0.021	0.191	0.357
ADG, kg/d	1.528	1.560	1.574	1.522	0.020	0.121	0.007	0.471
DMI, kg/d	11.23	11.01	11.26	11.23	0.181	0.764	0.710	0.357
DMI, %	2.31	2.27	2.29	2.29	0.040	0.532	0.841	0.803
DMI_{adap}, kg/d	10.80	10.60	10.81	10.84	0.225	0.848	0.627	0.500
DMI_{grow}, kg/d	11.42	11.40	11.59	11.59	0.159	0.575	0.639	0.404
DMI_{fin}, kg/d	11.32	11.05	11.30	11.24	0.217	0.609	0.803	0.427
FC	7.41	7.09	7.19	7.25	0.112	0.090	0.473	0.534
HCW, kg	322.50	323.79	325.03	322.48	1.481	0.241	0.095	0.342
DP, %	57.14	56.99	57.03	56.90	0.210	0.290	0.491	0.817
CpH	5.65	5.66	5.59	5.65	0.021	0.611	0.082	0.581
Carcass classification (%)								
2=	5.12	4.27	4.95	4.24				
2+	20.73	23.93	25.94	24.24				
3-	63.17	61.14	56.60	58.82				
3=	10.73	10.66	12.50	12.71				
4	0.24	0.00	0.00	0.00				

[a]Treatments main effects: N, Control (NON); G, Lysoforte supplementation starting in growing phase (GRO); F, Lysoforte supplementation starting in finishing phase (FIN); A, Lysoforte supplementation in all phases (since adaptation; ALL). [b]IBW, initial body weight; FBW, final body weight; ADG, average daily gain; DMI, dry matter intake; DMI_{adap}, dry matter intake in adaptation feeding period; DMI_{grow}, dry matter intake in growing feeding period; DMI_{fin}, dry matter intake in finishing feeding period; FC, feed convertion; HCW, hot carcass weight; DP, dressing percentage; CpH, carcass pH; Carcass classification: 2= reflects excessive lean carcass, 2+, 3-, 3+ desirable fat carcasses and 4 excessive fat carcass. [c]N × L = NON × others, A × G + F = ALL × GRO + FIN and G × F = GRO × FIN.

3.2. Experiment 2 (electronically monitored feedlot performance)

A treatment × breed interaction was observed, in which CEA animals had a higher IBW ($P < 0.001$; 406.42 vs. 372.55 for CEA and NEL, respectively); however, no differences were detected for FBW ($P = 0.223$; data not shown). Furthermore, a treatment × breed × feeding period interaction was observed for both DMI ($P = 0.001$) and ADG ($P < 0.001$; Figure 1). During the adaptation feeding period, LYSO did not influence DMI regardless of genotype ($P > 0.05$), but during the growing feeding period, CEA bullocks from ALL and GRO had greater DMI intake ($P < 0.05$) than CEA animals from NON and NEL from ALL. During the finishing period, CEA animals from ALL and GRO had greater DMI ($P < 0.05$) than NON and FIN within the same genotype. Moreover, NEL animals from FIN also had greater DMI ($P < 0.05$) than NON, GRO, and ALL cohorts within the same genotype but did not differ from CEA in the ALL and GRO protocols.

The NEL cattle from ALL had greater ADG ($P < 0.05$) than NON animals from both genotypes during the adaptation period. Similarly, during the growing feeding period, NEL from GRO had greater ADG ($P < 0.05$) than NON animals within the same genotype, whereas ALL was intermediate among the NEL cattle. The CEA animals from NON, GRO, and ALL had intermediate ADG. In the finishing feeding period, NEL from the FIN protocol had the greatest ADG ($P < 0.05$), NEL from the GRO and NON protocols (along with CEA from NON) the second greatest, CEA animals from ALL the intermediary, and NEL from ALL and CEA from GRO and FIN had the least ADG.

3.3. Experiment 2 (digestibility of nutrients and fatty acid profile of longissimus muscle)

No breed effect and breed × treatment interaction was noted for digestibility ($P > 0.05$). However, a treatment-feeding phase interaction was observed ($P = 0.004$; Figure 2). Somewhat surprisingly, no differences were observed ($P = 0.194$) for EE digestibility (81.20 vs. 79.40, NON and ALL, respectively), regardless of the feeding phase. In the finishing feeding period, for ALL protocol, a consistent increase in NDF ($P < 0.001$), CP ($P < 0.001$), and DM ($P = 0.001$) digestibilities was observed.

The FAP of experimental diets is presented in Table 3, and the effects of LYS supplementation during the whole feeding period (ALL) on the *longissimus* FAP are presented in Table 4. Also, no breed effect or breed × treatment interaction was observed for FAP

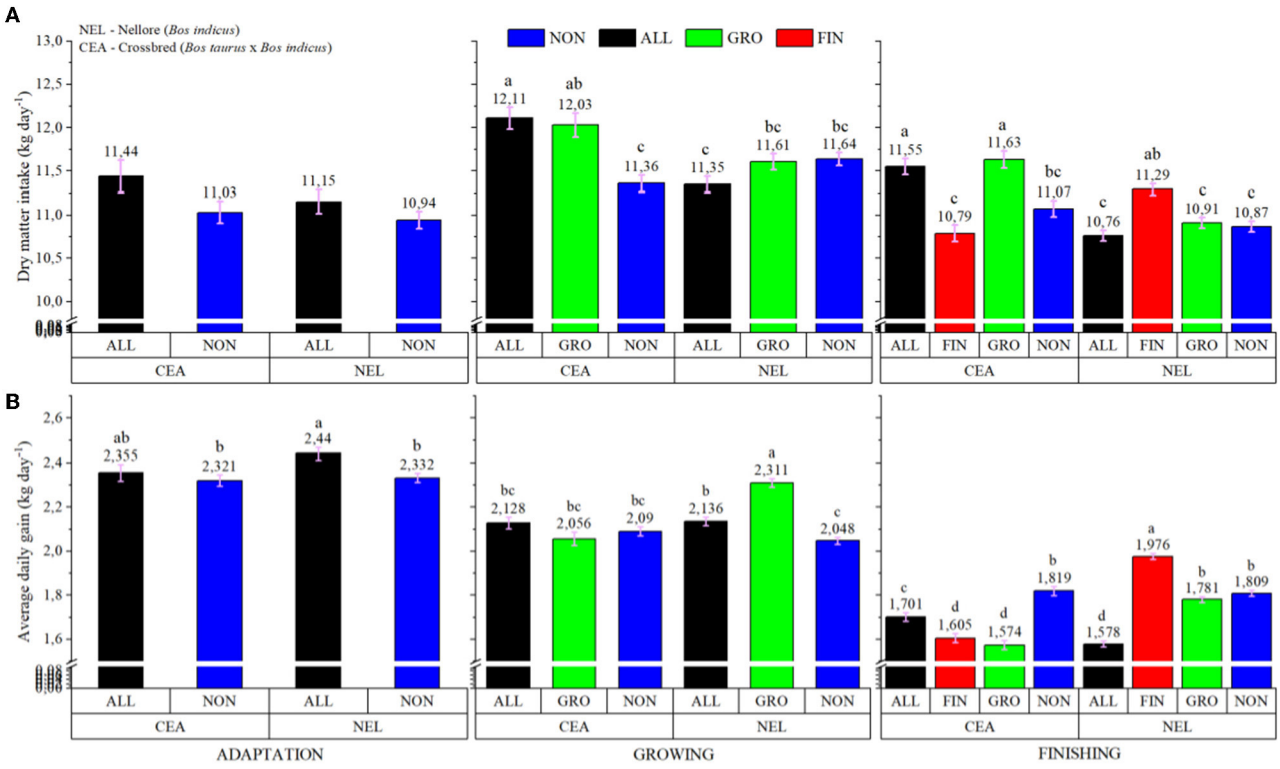


FIGURE 1
Treatment × Breed × Feeding phase interaction of dry matter intake **(A)** and average daily gain **(B)** of electronically monitored *Bos indicus* influenced bullocks. NON, Control; ALL, Lysoforte supplementation in all phases (since adaptation); GRO, Lysoforte supplementation starting at growing phase; FIN, Lysoforte supplementation starting at finishing phase. CEA = ½ Nellore × ½ Angus, NEL = Nellore. Letters within column with different superscripts differ in LSMEANS at $P \leq 0.05$.

($P > 0.05$). Supplemental LYSO increased concentrations of C8:0, C17:1, and C18:3n3 ($P = 0.014$, 0.001, and 0.048, respectively), but it decreased those of C20:0 and C24:1 ($P = 0.004$ and 0.003, respectively).

3.4. Experiment 2 (electronically monitored bullocks, breed x treatment x day classification interactions that influence DMI)

Meteorological variables are summarized in Table 5. During the experimental period, 37 days were classified as A (mild), 53 days as N (normal/hot), and 10 days as Q (very hot). Based on these classifications, a significant breed × treatment × day classification was observed ($P < 0.001$; Figure 3). There were no differences during the adaptation and growing feeding periods, but the meteorological variables played an important role during the finishing period. Regardless of genotype, DMI drastically decreased when animals experienced Q days during the finishing period. For CEA, bullocks from the GRO protocol had the greatest intake ($P < 0.05$), ALL and FIN were intermediate, and NON was the least. By comparison, NEL from FIN had the greatest DMI ($P < 0.05$), ALL was intermediate, and GRO and NON were the least.

4. Discussion

4.1. Feedlot performance

The combination of a large-pen study with a smaller-scale, electronically monitored DMI and ADG experiment led to a relevant evaluation of LYSO's mode of action throughout the different feeding phases. In Exp. 1, it was possible to assess the effects of LYSO in a commercial feedlot scenario under a rigorously monitored environment, whereas, in Exp. 2, the mechanism through which the treatments affected performance variables could be better understood within the two most common cattle genotypes fed in the tropics.

Similar to our observations in Exp.1, Zhang et al. (36) reported linear increases in FBW, ADG, and feed efficiency in supplemented Angus cattle without affecting DMI when the dose of a lysophospholipid-derived feed additive was increased from 0.5 to 0.75 g/kg of DM. Moreover, Drago (15) noted improvements in ADG and feed efficiency in LYSO-supplemented Nellore bullocks submitted to a finishing diet with 7.0% EE but not when the diet contained 3.8% EE. Using lambs, Gallo et al. (37) observed 11 and 16% improvements in ADG when the same commercial LYSO product as the one used in this study was tested in diets with 6% of EE in the form of either soybean or sunflower oil, respectively.

The aforementioned studies did not evaluate phase-feeding protocols. Based on the examination showing that animals from

10.3389/fvets.2023.1041479

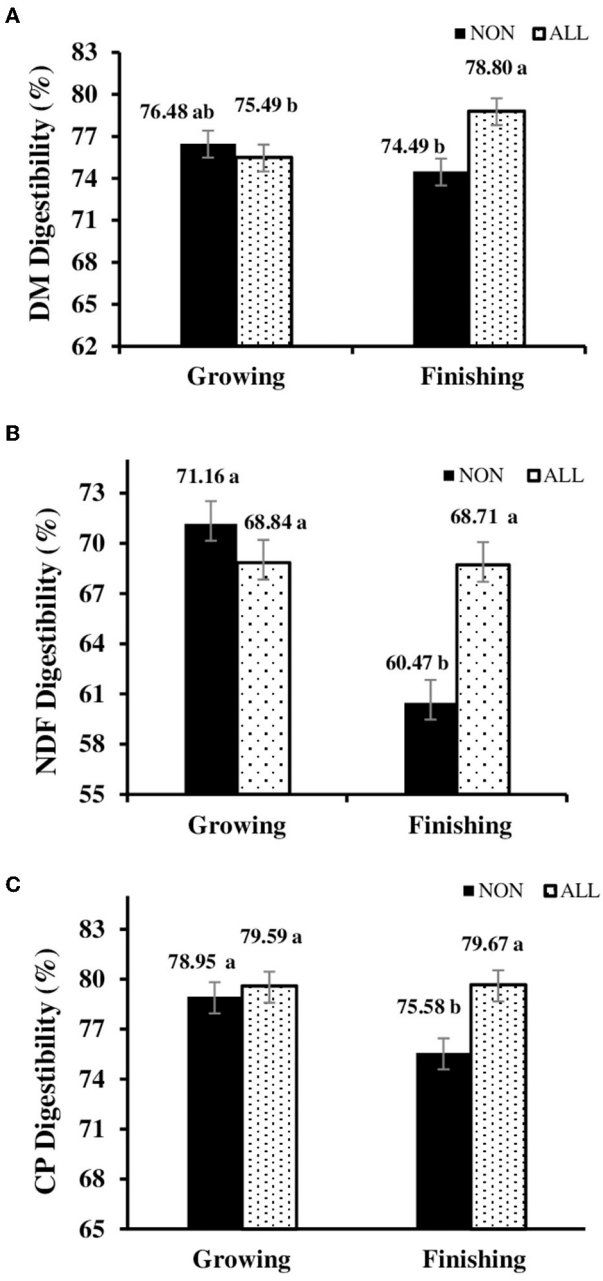


FIGURE 2
Feeding phase × digestibility interaction [**(A)** dry matter digestibility; **(B)** NDF digestibility; **(C)** crude protein digestibility] of electronically monitored *Bos indicus* influenced bullocks during growing and finishing feeding period. NON, Control; ALL, Lysoforte supplementation in all phases (since adaptation); within column, different superscripts differ in LSMEANS at $P \leq 0.05$.

TABLE 3 Fatty acid profile of growing and finishing diets of Experiment 2.

Fatty acids (g/100g)	Growing		Finishing	
	NON[a]	ALL	NON[a]	ALL
C10:0 (Capric)	0.018	0.017	0.024	0.036
C12:0 (Lauric)	0.193	0.180	0.247	0.197
C14:0 (Myristic)	0.491	0.513	0.549	0.565
C16:0 (Palmitic)	20.87	21.81	21.62	21.85
C17:0 (Margaric)	0.164	0.082	0.095	0.155
C18:0 (Stearic)	3.557	3.574	4.113	4.257
C20:0 (Arachidic)	0.323	0.303	0.291	0.272
C22:0 (Behenic)	0.243	0.269	0.256	0.247
C24:0 (Lignoceric)	0.245	0.244	0.204	0.182
C16:1 cis 9 (Palmitoleic)	0.376	0.396	0.432	0.475
C18:1:cis 9 (Oleic)	24.59	23.56	23.23	23.19
C20:1 (Eicosenoic)	0.223	0.089	0.480	0.518
C18:2:cis 9 cis 12 (Linoleic)	41.65	42.43	40.79	41.00
C18:3:n3 (Linolenic)	1.814	1.693	1.445	1.683

[a]NON, Control; ALL, Lysoforte supplementation in all phases (since adaptation).

NON and ALL protocols had similar performance, the responses observed with LYSO supplementation could be attributed to the GRO and FIN protocols, as supported by the pairwise comparisons of LSMEANS. In addition, GRO and FIN had similar performance, with the former tending to also have better FC and the latter a heavier HCW when both protocols were compared with NON. Therefore, under conditions similar to Exp. 1 (i.e., different genotypes fed in the same pen with greater *Bos indicus* influence), one may assume that starting supplementation of LYSO in growing diets is beneficial in situations when FC is an important driver, whereas the FIN protocol might be more suitable whenever heavier HCW plays an important role. As greater digestibility may be accomplished by the use of LYSO, the additional nutrient uptake might be directed accordingly to the animal's physiological status, resulting in greater ADG and carcass deposition of growing and finishing feedlot bullocks, respectively.

In Exp. 2, the effects of phase-feeding protocols according to cattle genotype were evaluated. First, it is worth noting that CEA cattle started the experiment heavier than NEL cattle. Because CEA cattle have a lighter mature weight than NEL cattle, it is expected that Angus-influenced bullocks start fat deposition at earlier days on feed (38, 39), which also results in a slower rate of gain sooner than NEL cattle.

With regard to CEA animals, DMI was statistically greater for supplemented animals during the growing and finishing feeding phases in the GRO and ALL protocols. This finding suggests that there is a persistent effect of LYSO supplementation and also that intake might be more responsive when stimulated during the initial phases of feeding programs with LYSO for this genotype. For ADG, the lower rate of gain observed for LYSO-supplemented animals during the finishing period suggests that greater fat deposition was expected, changing the composition of gain (40), driven by the higher digestibility of dietary nutrients.

For NEL animals, regardless of DMI for LYSO-supplemented bullocks, during the adaptation and growth phases, statistical differences were only noted during the finishing period. The average daily gain was greater in every phase that LYSO was introduced to the diet, but animals from the ALL protocol presented decreased ADG during the finishing feeding phase. These findings suggest that NEL animals were more physiologically

10.3389/fvets.2023.1041479

TABLE 4 Fatty acid profile of *longissimus* muscle of *Bos indicus*-influenced feedlot cattle supplement with lysolecithin-derived feed additive in Experiment 2.

Fatty acids[a] (g/100 g)	NON	ALL	SE	*P*-value
C8:0	0.004	0.007	0.008	0.014
C10:0	0.052	0.057	0.003	0.311
C12:0	0.059	0.072	0.004	0.075
C14:0	2.534	2.914	0.139	0.063
C15:0	0.243	0.264	0.013	0.285
C15:0 Iso	0.094	0.116	0.008	0.059
C15:0 Anteiso	0.116	0.121	0.011	0.772
C16:0	23.59	24.45	0.464	0.196
C17:0	0.543	0.583	0.028	0.334
C17:1	0.318	0.369	0.009	0.007
C18:0	15.50	14.50	0.623	0.266
C18:1 Trans	34.89	33.46	0.238	0.674
C18:1 Cis 9	32.91	33.65	0.607	0.231
C18:2 Cis 9 Cis 12	85.85	72.92	0.597	0.125
C18:2 Cis 9 trans 11	0.422	0.430	0.027	0.840
C18:2 Trans 10 cis 12	0.012	0.018	0.002	0.124
C18:3 n6	0.019	0.013	0.002	0.091
C18:3 n3	0.302	0.361	0.020	0.047
C20:0	0.070	0.048	0.005	0.004
C20:1	0.156	0.168	0.011	0.431
C21:0	0.046	0.051	0.004	0.440
C22:0	0.066	0.052	0.007	0.152
C23:0	0.033	0.040	0.005	0.319
C24:1	0.145	0.086	0.012	0.002
SFA	43.83	44.07	0.700	0.815
UFA	56.16	55.92	0.700	0.815

[a]SFA, Saturated fatty acids; UFA, unsaturated fatty acids. NON, Control; ALL, Lysoforte supplementation in all phases (since adaptation).

TABLE 5 Meteorological variables of Experiment 2.

Day classifications[a]	Mean	Standard deviation	Minimum	Maximum
Class A				
Air temperature, °C	20.6	2.23	14.7	23.3
Relative humidity, %	65.6	8.00	47.6	86.2
Black globe temperature, °C	23.1	3.08	14.7	27.1
Solar irradiaton, W m^{-2}	137.4	66.7	7.32	246.9
Wind speed, m s^{-1}	1.39	0.55	0.29	2.73
Wind direction, °	193.4	8.96	103.9	284.5
Class *N*				
Air temperature, °C	22.9	1.77	19.1	27.0
Relative humidity, %	61.0	6.91	45.0	72.8
Black globe temperature, °C	26.8	2.03	23.6	32.2
Solar irradiaton, W m^{-2}	195.1	44.0	108.5	33.7
Wind speed, m s^{-1}	2.20	0.65	1.21	4.22
Wind direction, °	204.0	44.4	108.4	282.0
Class Q				
Air temperature, °C	25.7	1.47	23.0	28.6
Relative humidity, %	54.2	9.52	40.1	68.4
Black globe temperature, °C	30.7	2.42	26.9	36.5
Solar irradiaton, W m^{-2}	239.8	51.4	169.9	348.2
Wind speed, m s^{-1}	2.87	0.27	1.92	4.54
Wind direction, °	234.2	30.8	168.0	277.5

[a]A, mild days; N, normal/hot days; and Q, very hot days.

responsive to the dietary digestibility increment, redirecting nutrients for a faster rate of BW gain. Nonetheless, LYSO supplementation beginning in the adaptation feeding period might also anticipate fat deposition and, consequently, change gain composition, in accordance with the ADG results from Exp. 1 and CEA bullocks of Exp. 2.

4.2. Nutrients digestibility and fatty acids profile of longissimus muscle

The absence of a breed × treatment effect for both digestibility and FAP suggests that the LYSO mode of action is independent of genotype. The digestibility of EE was not influenced by LYSO supplementation. One plausible explanation is that calcium salts of fatty acids (**CSAF**) from soybeans represented approximately one-third of total dietary EE. High-lipid digestibility in CSAF-supplemented NEL bullocks fed high-concentrate feedlot diets has been previously reported (11, 41). Similarly, Drago (15) reported greater lipid digestibility for CSAF from soybeans in feedlot NEL bullocks. In that study, LYSO did not modify lipid digestion, which suggests that the potential for LYSO to further improve EE digestion was likely decreased when animals are fed rumen-protected highly digestible fat sources. In contrast, Zhang et al. (36) observed a positive linear effect on EE digestion with a lecithin-derived feed additive (up to 8.75 g/animal/day) when rumen-protected fat was added to the diet, but the type and source of fat were not described. The authors suggested that EE digestibility was increased as a result of the effective emulsification of lecithin in reducing the size of fat globules, forming smaller micelles, and, as a consequence, increasing the surface area of lipid droplets for interaction with pancreatic enzymes. The interaction of fat sources (i.e., fatty acid profile and ruminal protection) and inclusion

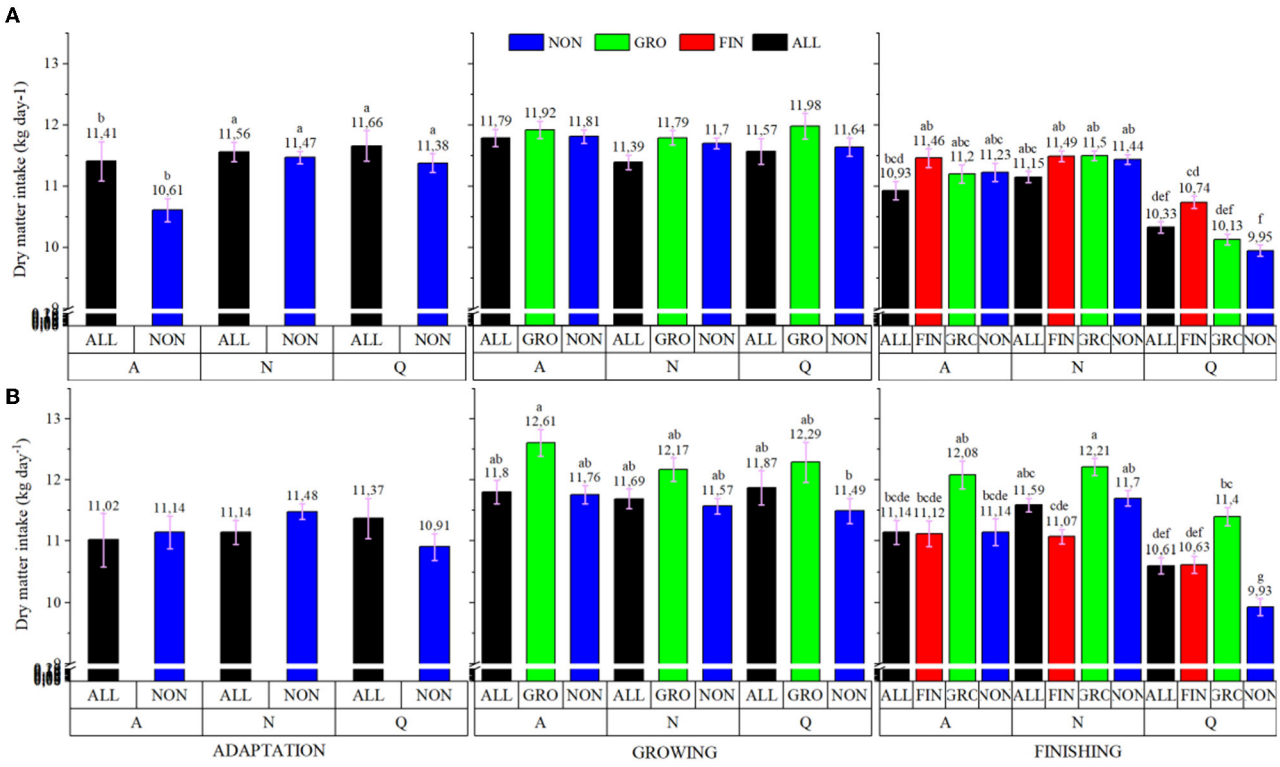


FIGURE 3
Breed × Treatment × Day classification interaction of dry matter intake of electronically monitored *Bos indicus* (**A** = Nellore; **B** = ½ Angus × ½ Nellore) bullocks during adaption, growing and finishing feeding period. NON, Control; ALL, Lysoforte supplementation in all phases (since adaptation); GRO, Lysoforte supplementation starting at growing phase; FIN, Lysoforte supplementation starting at finishing phase. A, rainy, cloudy mild days; N, normal hot days; and Q, very hot days. Within column, different superscripts differs in LSMEANS at $P \leq 0.05$.

levels with the capabilities of different lysophospholipids deserves further investigation.

Similarly to the NDF digestibility increments observed in Exp. 2, Drago (15) tested the same LYSO product supplemented in this study with three different main sources of fat (CSAF from soybean, CSAF from palm, and degummed soybean oil). The authors reported a 6.81% increment, regardless of the fat source. In addition to increased NDF digestion, Drago (15) also reported improvements in total VFA production and lower ruminal pH for the treatment with the combination of LYSO and soybean oil compared with either CSAF from soybean or palm oil, which indicates that LYSO likely improved ruminal fermentation by facilitating the emulsion and passage of fatty acids out of the rumen, decreasing the potential negative effect that unsaturated fatty acids can have on ruminal microbial function. Higher cellulolytic enzyme activities and enhanced fiber degradation in the rumen have also been noted with the use of synthetic emulsifiers (42).

Crude protein digestibility was positively affected by LYSO supplementation during the final feeding period. Increments in CP digestibility in feedlot cattle supplemented with lysophospholipids have been previously observed (15, 36). The rationale applied in nonruminant animals to explain the effect of lysolecithin-derived emulsifiers with regard to the greater number and size of membrane pores and altered fluidity and transmembrane permeability of nutrients in the intestine may be plausible for ruminants as well (36, 43), but further research is needed. Greater ruminal ammonia was observed in Drago (15) and when lysophospholipid-supplemented cows were compared with cohorts receiving monensin (44). In the later study, lysophospholipid-supplemented animals presented greater purine derivative excretion, which might indicate higher microbial protein supply, N secreted in milk, and reduced urinary N excretion. The authors suggested that dietary N was absorbed in more utilizable forms for protein synthesis in the body, which could partially explain the greater ADG of NEL bullocks fed LYSO as they have higher protein requirements (45). Therefore, the greater DM digestibility and performance observed in this study in response to supplemental LYSO seem to be related to improved CP and NDF digestibility.

The longissimus FAP of feedlot cattle supplemented with either LYSO or other emulsifiers has not previously been described. The most remarkable finding is the greater concentration of alpha-linolenic acid (C18:3n3) and a trend ($P = 0.091$) for linolenic acid (C18:3n6), which are exclusive of dietary origin (46), indicating that LYSO possibly enhanced ruminal escape of these specific fatty acids. In a biohydrogenation-induced milk fat depression trial, Rico et al. (10) concluded that the decrease in milk fat in LYSO-treated cows was not specifically related to biohydrogenation, suggesting that it was associated with substrate emulsification in both the rumen and intestines. Furthermore, the higher concentration of octanoic acid (C8:0), which is derived from branched-chain amino acids [valine,

leucine, and isoleucine; (47)], could be partially linked to greater NDF digestibility (48).

4.3. Influence of meteorological variables on DMI of LYSO-supplemented cattle

Heat stress has been extensively investigated in countries with a mature feedlot industry like the United States and Australia. In the former, it is estimated that heat stress is annually responsible for US$282 million in losses (49), while in the latter is estimated to cost a total of AU$16 million (50). Nonetheless, long-term financial losses are likely to exceed 5–10 times animal mortality because of decreased DMI and lower performance (51). Deleterious effects of heat stress on intake and digestive parameters in *Bos indicus* animals were only evaluated in calorimetric chambers (52), which may not reliably represent an open dry-lot condition as solar radiance is not measured.

As DMI was affected by heat events in the present research, we suggested that InCl would help to elucidate variation in DMI under research conditions and possibly in commercial feeding operations. Another remarkable aspect of the present methodology is the use of animal physiological variables to evaluate animal comfort, in contrast to regular heat stress induction protocols that artificially alter temperature and humidity.

During the conduct of Exp. 2, 10% of days were classified as Q. A decrease in DMI with heat stress was observed during the finishing feeding period, regardless of genotype, but effects were less in LYSO-supplemented CEA and LYSO-supplemented NEL for the FIN treatment. Thus, one might consider supplementing LYSO during conditions in which DMI might be affected by heat events. To the best of our knowledge, this is the first experiment to look at the effects of emulsifiers on heat stress amelioration in cattle. Digestibility enhancement is plausibly responsible for the improved DMI because the subcutaneous temperature was not altered by the use of LYSO (data not shown). Meneses et al. (52) observed detrimental effects on DMI and DM digestibility and a shift in nutrient digestibility from the rumen to the intestines in heat-stressed NEL heifers. These authors proposed that modifying the site of digestion in NEL animals reflected an adaptive response of the digestive tract to heat stress conditions. As LYSO may improve intestinal digestibility (CP and DM), it could partially explain the lower decrease in DMI. It should also be noted that SH availability may have possibly interfered with the DMI responses of ALL and GRO of NEL during heat events that occurred in the finishing feeding phase. Nonetheless, the mechanisms by which LYSO improved the DMI of CEA animals on very hot days need elucidation, but they might be related to a lower heat increment derived from a more efficient fiber ruminal fermentation.

5. Implications

This study's findings suggest that LYSO enhances feedlot performance when administered during the growing and/or finishing feeding phases of *Bos indicus*-influenced cattle. The emulsification increased CP, NDF, and DM digestion of the finishing diet and also increased ruminal escape of dietary fatty acids. Finally, the development of the InCl index helped to identify DMI alterations during heat events, and LYSO supplementation ameliorated decreases in DMI caused by heat stress.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Committee on Animal Use of the São Paulo State University Julio de Mesquita Filho.

Author contributions

RP: conceptualization, conduction, data curation, writing, and editing. JO: writing, data curation, and editing. GM: data curation and editing. MC: writing. LG: conceptualization and data curation. JC and RC: conceptualization, diet formulation, and conduction. PC and MN: conduction. VA and AM: conduction and data curation. VC: conceptualization, intellectual input, and conduction. LSC and LBC: analysis conduction (digestibility). DL: fatty acids profile analysis. MG: editing and revising. All authors contributed to the article and approved the submitted version.

Funding

This study received funding from Kemin Industries. The funder had the following involvement with the study: interpretation of data, the writing of the article, and the decision to submit it for publication.

Conflict of interest

MC and LG were employed by Kemin Industries. JC and RC were consultants to Nutribeef, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Vasconcelos JT, Galyean ML. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. *J Anim Sci.* (2007) 85:2772–81. doi: 10.2527/jas.2007-0261

2. NASEM. *National Academics of Sciences Engineering and Medicine. Nutrient requirements of beef cattle. 8th ed*. Washington, DC: National Academics Press (2016).

3. Pinto ACJ, Millen DD. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: the 2016 Brazilian survey. *Can J Anim Sci.* (2019) 99:392–407. doi: 10.1139/cjas-2018-0031

4. Jenkin TC. Lipid metabolism in the rumen. *J Dairy Sci.* (1993) 76:3851–63. doi: 10.3168/jds.S0022-0302(93)77727-9

5. Hall MB, Eastridge ML. Invited review: carbohydrate and fat: considerations for energy and more. *Prof Anim Sci.* (2014) 30:140–9. doi: 10.15232/S1080-7446(15)30101-7

6. Van Soest PJ. *Nutritional Ecology of the Ruminant. 2nd ed.* New York, NY: Cornell University Press (1994). p. 476.

7. Bauman DE, Lock AL. *Concepts in lipid digestion and metabolism in dairy cows.* In: *Proc. Tri-State Dairy Nutr. Conf.* (2006). P. 1–14.

8. Doreau M, Ferlay A. Digestion and utilization of fatty acids by ruminants. *Anim Feed Sci Technol*. (1993) 45:379–96. doi: 10.1016/0377-8401(94)90039-6

9. Joshi. A, Paratkar SG, Thorat BN. Modification of lecithin by physical. *chemical and enzymatic methods. Eur J Lipid Sci Technol.* (2006) 108:363–73. doi: 10.1002/ejlt.200600016

10. Rico DE, Ying Y, Harvatine KJ. Effects of Lysolecithin on milk fat synthesis and milk fatty acid profile of cows fed diets differing in fiber and unsaturated fatty acid concentration. *J Dairy Sci.* (2017) 100:9042–7. doi: 10.3168/jds.2017-12976

11. Nascimento FA, Silva N, Prados LF, Pacheco RDL, Johnson BJ, Capellozza B, et al. Calcium salts of fatty acids with varying fatty acid profiles in diets of feedlot-finished *Bos indicus*bulls: impacts on intake, digestibility, performance, and carcass and meat characteristics. *J Anim Sci.* (2020) 98:skaa382. doi: 10.1093/jas/skaa,382

12. Costa C, Rizzieri R, Melo G, Müller L, Estevan D, Pacheo R, et al. Effects of fatty acid profile of supplements on intake, performance, carcass traits, meat characteristics, and meat sensorial analysis of feedlot Bos indicus bulls offered a high-concentrate diet. *Transl Anim Sci.* (2020) 4:1–20. doi: 10.1093/tas/txaa142

13. Cooke RF, Bohnert DW, Moriel P, Hess BW, Mills RR. Effects of polyunsaturated fatty acid supplementation on ruminal in situ forage degradability, performance, and physiological responses of feeder cattle. *J Anim Sci.* (2011) 89:3677–89. doi: 10.2527/jas.2010-3515

14. Reis ME, Toledo AF, Silva AP, Poczynek M, Fioruci EA, Cantor MC, et al. Supplementation of Lysolecithin in milk replacer for Holstein dairy calves: effects on growth performance, health, and metabolites. *J Dairy Sci.* (2021) 104:5457–66. doi: 10.3168/jds.2020-19406

15. Drago FL. *Suplementação de lisolecitina em dietas com diferentes níveis e fontes de gordura para bovinos em confinamento.[master's thesis]*. Piracicaba, SP. University of São Paulo (2019).

16. Brown-Brandl T. M., Understanding heat stress in beef cattle. *Ver Bras de Zoot.* (2018) 47:e20160414. doi: 10.1590/rbz,4720160414

17. Hahn GL, Mader TL. *Heat waves in relation to thermoregulation, feeding behavior and mortality of feedlot cattle.* Bottcher RW, Hoff SJ, eds. St. Joseph, MI. (1997). p 563–71.

18. Mitlöhner FM, Galyean ML, McGlone JJ. Shade effects on performance, carcass traits, physiology, and behavior of heat-stressed feedlot heifers. *J Anim Sci.* (2002) 80:2043–50. doi: 10.2527/2002.8082043x

19. Chizzotti ML, Machado FS, Valente EEL, Pereira LGR, Campos MM, Tomich TR, et al. Validation of a system for monitoring individual feeding behavior and individual feed intake in dairy cattle. *J Dairy Sci.* (2015) 98:3438–42. doi: 10.3168/jds.2014-8925

20. Tedeschi LO, Fox DG. *The ruminant nutrition system: an applied model for predicting nutrient requirements and feed utilization in ruminants. (2nd ed.).* XanEdu, Acton, MA (2018).

21. Pritchard R, Bruns K. Controlling variation in feed intake through bunk management. *J Anim Sci.* (2003) 81:E133–8. doi: 10.2527/2003.8114_suppl_2E133x

22. AOAC International. *Official Methods of Analysis. 17th ed.* Gaithersburg, MD: AOAC International (2000).

23. AOAC. *Official Methods of Analysis. 16th ed.* Arlington, VA: Association of Analytic Chemists (1995).

24. Van Soest PJ, Robertson JB, Lewis AB. Symposium carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2

25. Mertens DR. Creating a system for meeting the fiber requirements of dairy cows. *J Dairy Sci.* (1997) 80:1463–81. doi: 10.3168/jds.S0022-0302(97)76075-2

26. Valente TAP, Detmann E, Valadares Filho SC, Cunha M, Queiroz AC, Sampaio CB. *In situ* estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. *Ver Bras de Zootec.* (2011) 40:666–75. doi: 10.1590/S1516-35982011000300027

27. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*. (1957) 226:497–509. doi: 10.1016/S0021-9258(18)64849-5

28. Kramer JK, Fellner V, Dugan ME, Sauer FD, Mossoba MM, Yurawecz MP. Evaluating acid and base cataLYSOts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids.* (1997) 32:1219–28. doi: 10.1007/s11745-997-0156-3

29. Manly BFJ. *Multivariate Statistical Methods. A primer.* Chapman and Hall, London (1986).

30. Rencher AC. *Methods of Multivariate Analysis*. United States: Wiley-Intersience (1995).

31. Liu S, Ye T, Li Z, Li J, Jamil AM, Zhou Y, et al. Identifying hub genes for heat tolerance in water buffalo (Bubalus bubalis) using transcriptome data. *Front Genet.* (2019) 209:1–13. doi: 10.3389/fgene.2019.00209

32. Liu S, Yue T, Ahmad MJ, Hu X, Zhang X, Deng T, et al. Transcriptome analysis reveals potential regulatory genes related to heat tolerance in Holstein dairy cattle. *Genes.* (2020) 11:4–12. doi: 10.3390/genes11010068

33. Oliveira SEO, de Melo Costa CC, Chiquitelli Neto M, Maia ASC. Effects of shade location and protection from direct solar radiation on the behavior of Holstein cows. *Int J Biometeorol.* (2019) 63:1465–74. doi: 10.1007/s00484-019-01747-5

34. SAS Institute Inc. *Logistic Regression Examples Using the SAS System*. Cary, NC: SAS Institute Inc (1995).

35. Littell RC, et al. *SAS for Mixed Models*. Cary, NC: SAS Institute Inc (2006).

36. Zhang M, Bai H, Zhao Y, Wang R, Li G, Zahng Y, et al. Effects of supplementation with lysophospholipids on performance, nutrient digestibility, and bacterial communities of beef cattle. *Front Vet Sci.* (2022) 9:927369. doi: 10.3389/fvets.2022.927369

37. Gallo SB, Brochado T, Brochine L, Passareli D, Costa SF, da S. Bueno IC, et al. Effect of biosurfactant added in two different oil source diets on lamb performance and ruminal and blood parameters. *Livestock Sci.* (2019) 226:66–72. doi: 10.1016/j.livsci.2019.06.006

38. Fox DG, Black JR. A system for predicting body composition and performance of growing cattle. *J Anim Sci.* (1984) 58:725. doi: 10.2527/jas1984.583725x

39. Owens FN, Dubeski P, Hanson CF. Factors that alter the growth and development of ruminants. *J Anim Sci.* (1993) 71:3138–50. doi: 10.2527/1993.71113138x

40. Owens FN, Gill DR, Secrist DS, Coleman SW. Review of some aspects of growth and development of feedlot cattle. *J Anim Sci.* (1995) 73:3152–72. doi: 10.2527/1995.73103152x

41. Cruz WFG, Schoonmaker JP, Resende FD, Siqueira GR, Rodrigues LM, Zamudio GDR, et al. Effects of maternal protein supplementation and inclusion of rumen-protected fat in the finishing diet on nutrient digestibility and expression of intestinal genes in Nellore steers. *Anim Sci J.* (2019) 90:1200–1211. doi: 10.1111/asj.13273

42. Hwang IH, Lee CH, Kim SW, Sung HG, Lee SY, Lee SS, et al. Effects of mix- tures of Tween80 and cellulolytic enzymes on nutrient digestion and cellulolytic bacterial adhesion. *Asian Australas J Anim Sci.* (2008) 21:1604–9. doi: 10.5713/ajas.2008.80333

43. Song WS, Yang Z, Hwang IH, Cho S, Choi N-J. Effect of dietary lysophospholipds (Lipidol TM) supplementation on the improvement of forage usage and growth performance in Hawoo heifers. *J Korean S Grassl Forage Sci.* (2015) 35:232–7. doi: 10.5333/KGFS.2015.35.3.232

44. Lee C, Morris DL, Copelim JE, Hettick JM, Kwon IH. Effects of lysophospholipids on short-term production, nitrogen utilization, and rumen fermentation and bacterial population in lactating dairy cows. *J Dairy Sci.* (2019) 102:3110–20. doi: 10.3168/jds.2018-15777

45. Corte BR. *Exigências Nutricionais de zebuínos e tabela de composição de* alimentos. Viçosa: UFV, DZO (2006).

46. Teixeira PD, Schoonmaker JP, Carvalho JRR, Oliveira CVR, Rodrigues AC, Santos LR, et al. Fatty acid profile and beef quality of Nellore and Angus bulls fed whole shelled corn. *Sci. Agric.* (2022) 79:1–8. doi: 10.1590/1678-992x-2020-0273

47. Wood JD, Enser M, Richardson RI, Whittington FM. Fatty acids in meat and meat products. In: *Fatty Acids in Foods and Their Health Implications*. 3rd ed. CRC Press (2007). p. 97–106. doi: 10.1201/9781420006902.ch5

48. Zhang HL, Chen Y, Xu XL, Yang YX. Effects of branched-chain amino acids on in vitro ruminal fermentation of wheat straw. *Asian Aust J Anim Sci.* (2013) 26:523–8. doi: 10.5713/ajas.2012.12539

49. St-Pierr NR, Cobanov B, Schnitkey G. Economic Losses from Heat Stress by US Livestock Industries. *J Dairy Sci.* (2003) 86:E52–77. doi: 10.3168/jds.S0022-0302(03)74040-5

50. Sackett D, Holmes P, Abbott K, Jephcott S, Barber B. *Assessing the economic cost of endemic disease on the profitability of Australian beef cattle and sheep producers - Final Report AHW.087*. Meat and Livestock Australia Limited, North Sydney NSW (2006). Available online at http://www.mla.com.au/CustomControls/PaymentGateway/ViewFile.aspx?mFDUp1AYI9VUf$+$h/ZH4CYhopVLs5O3WvlD8Tvjx4WwqIhJCS8/UdRwc9AKswn/HN3EYMKKAfsht7d1Tnt3Bqi,A==

51. Mader TL. Impact of environmental stress on feedlot cattle. *Proc Western Section Am Soc Anim Sci.* (2012) 62:335.

52. Meneses JAM, Sá OAAL, Coelho CF, Pereira RN, Batista ED, Ladeira MM, et al. Effect of heat stress on ingestive, digestive, ruminal and physiological parameters of Nellore cattle feeding low- or high-energy diets. *Livestock Sci.* (2021) 252:104676. doi: 10.1016/j.livsci.2021.104676

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 28 April 2023
DOI 10.3389/fvets.2023.1117639

OPEN ACCESS

EDITED BY
Terry Engle,
Colorado State University, United States

REVIEWED BY
Gabriel O. Ribeiro,
University of Saskatchewan, Canada
Anusorn Cherdthong,
Khon Kaen University, Thailand

*CORRESPONDENCE
Vinícius N. Gouvêa
✉ vinicius.gouvea@ag.tamu.edu

RECEIVED 06 December 2022
ACCEPTED 30 March 2023
PUBLISHED 28 April 2023

CITATION
Baggio M, Gouvêa VN, Barroso JPR, Miszura AA, Limede AC, Soares LCB, Ferraz MVC Jr, Vicente ACS, Ferreira EM, Marques RS and Pires AV (2023) Different combinations of monensin and narasin on growth performance, carcass traits, and ruminal fermentation characteristics of finishing beef cattle. *Front. Vet. Sci.* 10:1117639. doi: 10.3389/fvets.2023.1117639

Different combinations of monensin and narasin on growth performance, carcass traits, and ruminal fermentation characteristics of finishing beef cattle

Marcelo Baggio[1], Vinícius N. Gouvêa[2,3]*, José Paulo R. Barroso[1], Alexandre A. Miszura[1], Arnaldo C. Limede[1], Letícia C. B. Soares[1], Marcos Vinicius C. Ferraz Jr.[4], Ana Carolina S. Vicente[5], Evandro M. Ferreira[6], Rodrigo S. Marques[7] and Alexandre V. Pires[1,6]

[1]Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, Brazil, [2]Texas A&M AgriLife Research and Extension Center, Amarillo, TX, United States, [3]Department of Animal Science, Texas A&M University, College Station, TX, United States, [4]Department of Animal Science, Federal University of Amazonas, Parintins, Brazil, [5]Department of Animal Sciences, University of Florida, Gainesville, FL, United States, [6]Department of Animal Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, Brazil, [7]Department of Animal and Range Sciences, Montana State University, Bozeman, MT, United States

The objective of this study was to evaluate the effects of different combinations of monensin and narasin on finishing cattle. In Exp. 1, 40 rumen-cannulated Nellore steers [initial body weight (BW) = 231 ± 3.64 kg] were blocked by initial BW and assigned to one of the five treatments as follows: Control (CON): no feed additive in the basal diet during the entire feeding period; Sodium monensin (MM) at 25 mg/kg dry matter (DM) during the entire feeding period [adaptation (days 1–21) and finishing (days 22–42) periods]; Narasin (NN) at 13 mg/kg DM during the entire feeding period (adaptation and finishing periods); Sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM during the finishing period (MN); and narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM during the finishing period (NM). Steers fed MM had lower dry matter intake (DMI) during the adaptation period compared to NM ($P = 0.02$) but not compared to CON, MM, MN, or NN ($P \geq 0.12$). No differences in DMI were observed among the treatments during the finishing ($P = 0.45$) or the total feeding period ($P = 0.15$). Treatments did not affect the nutrient intake ($P \geq 0.51$) or the total apparent digestibility of nutrients ($P \geq 0.22$). In Exp. 2, 120 Nellore bulls (initial BW = 425 ± 5.4 kg) were used to evaluate the effects of the same treatments of Exp. 1 on growth performance and carcass characteristics of finishing feedlot cattle. Steers fed NM had greater DMI during the adaptation period compared to CON, MM, and MN ($P \leq 0.03$), but no differences were observed between NM and NN ($P = 0.66$) or between CON, MM, and NN ($P \geq 0.11$). No other differences between treatments were observed ($P \geq 12$). Feeding narasin at 13 mg/kg DM during the adaptation period increases the DMI compared to monensin at 25 mg/kg DM, but the feed additives evaluated herein did not affect the total tract apparent digestibility of nutrients, growth performance, or carcass characteristics of finishing cattle.

KEYWORDS

adaptation, feed additives, feedlot, intake, ionophore, rumen

1. Introduction

According to Brown et al. (1) and Pereira et al. (2), the adaptation period in which finishing cattle is transitioned from a high-roughage-based diet to a high-concentrate diet is considered the most critical period for feedlot cattle due to the changes in dry matter intake (DMI) and risks of subacute or acute acidosis caused by the increased amount of rapidly fermentable carbohydrates in the diet (3–5). The low DMI during the first 14–21 days of the adaptation period (2, 4, 6) can limit the performance of feedlot cattle. Therefore, feed additives that can modulate ruminal fermentation characteristics and increase the average daily gain (ADG) without negatively affecting the DMI would benefit the adaptation process and perhaps increase growth performance throughout the feedlot finishing phase.

Ionophores, such as sodium monensin, are largely used in the feedlot industry to improve the feed efficiency of finishing cattle. According to the surveys conducted by Samuelson et al. (7) and Pinto and Millen (8), more than 97% of feedlot nutritionists in the USA and Brazil include ionophores in finishing diets for feedlot cattle. However, according to Samuelson et al. (7), monensin is the primary ionophore included in feedlot diets in the USA. Monensin decreases DMI, especially in diets with a high proportion of forage (9). In a meta-analysis conducted by Duffield et al. (10), monensin decreased DMI by 3% and improved feed efficiency (G:F; gain to feed ratio) of finishing beef cattle by 2.5–3.5%. The positive effect of feeding monensin to ruminants is frequently attributed to the improved efficiency of energy metabolism as a result of increased propionate production in the rumen (11). Monensin can also affect meal patterns, especially during times when rumen pH is low (12). According to Erickson et al. (13), feeding monensin to finishing feedlot cattle increases the number of meals and decreases intake rate (%/h) and average meal size. However, feeding monensin reduces ruminal pH variance (13), which can contribute to preventing rumen acidosis on high-grain finishing diets (14).

Narasin is an ionophore that has been studied in high-roughage diets (15–17). According to Polizel et al. (18), feeding Narasin at 13 or 20 mg/kg DM to beef steers provided with a high-roughage diet (Tifton-85; *Cynodon dactylon* spp.) did not affect DMI and increased ruminal concentration of propionate. Feeding narasin at 13 mg/kg DM increased growth performance and benefited ruminal fermentation characteristics of steers fed a forage-based diet (19).

Therefore, we hypothesized that feeding narasin during the adaptation period would increase the DMI and growth performance compared to monensin, and the positive effects obtained during the adaptation period would carry over the entire feedlot finishing phase. The objective of this experiment was to evaluate different combinations of monensin and narasin on intake, nutrient digestibility, ruminal fermentation characteristics, growth performance, and carcass traits of finishing feedlot cattle.

2. Materials and methods

A total of two experiments were conducted at the Experimental Feedlot Cattle facilities of the Department of Animal Science and the "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP), in Piracicaba, State of São Paulo, Brazil (22°43'30" S, 47°38'51" W). All procedures using animals were approved by the Animal Care and Use Committee of the ESALQ/USP (protocol number #9763030920).

2.1. Experiment 1. Intake, digestibility, and ruminal fermentation characteristics

A total of 40 rumen-cannulated Nellore steers [*Bos indicus*; initial body weight (BW) = 231 ± 3.64 kg; age = 20 ± 1.0 months] were blocked by initial BW and allocated to 40 pens (3.5 × 8 m; 1 steer/pen), with concrete floor, fully roofed, 3.5 m of bunk space, and individual waterers (BV 009 3L, Agricola Suin, Joinville, SC, Brazil). Pens within each BW block were then randomly assigned to one of the five treatments (Figure 1) as follows: (1) Control (CON): no feed additive in the basal diet during the entire feeding period; (2) Sodium monensin (MM) at 25 mg/kg dry matter (DM) during the entire feeding period [adaptation (days 1–21) and finishing (days 22–42) periods (Rumensin 100, Elanco Brazil, São Paulo, SP, Brazil)]; (3) Narasin (NN) at 13 mg/kg DM during the entire feeding period (adaptation and finishing periods; Zimprova 100, Elanco Brazil, São Paulo, SP, Brazil); (4) Sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM during the finishing period (MN); and (5) narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM during the finishing period (NM).

The experiment lasted for 42 days. Steers were adapted to the finishing diet during the first 21 days of the experiment (adaptation period; day 1–21), using three step-up diets (Table 1) which gradually (7 days in each step) decreased the roughage source (sugarcane bagasse) from 23 to 18% (Adap. 1), to 13% (Adap. 2), and to 8% (Adap. 3) and increased concentrate accordingly. The finishing diet containing 8% of roughage (DM basis) was fed from days 22 to 42 (finishing period). The experimental diets (Table 1) were formulated to meet the nutrient requirements of finishing Nellore steers for 1.35 kg ADG as specified by NASEM (9). The experimental rations were mixed using a feed-mix wagon (Totalmix TMX25, Casale Equipamentos Ltda., São Carlos, Brazil) and delivered to the steers as a total mixed ration (TMR) once a day at 800 h. After mixing, the TMR was weighed into 100 L capacity plastic bins using an electronic scale with 50 g of readability (Welmy, W 300, Santa Bárbara d'Oeste, SP, Brazil) and manually delivered to each pen. Steers had *ad libitum* access to the experimental diets containing the treatments and water during the entire experiment (days 1–42). The amount of feed offered to each steer was adjusted daily based on the amount of feed provided on the previous day to allow 5% of refusals, that were removed daily, weighted, sampled (5% of the total amount; wet weight), and frozen (−18°C) for analysis of nutrient composition and DMI calculation. Samples of ingredients (~500 g) were collected one time each week and dried at 105°C for 24 h for DM determination and diet DM adjustments.

10.3389/fvets.2023.1117639

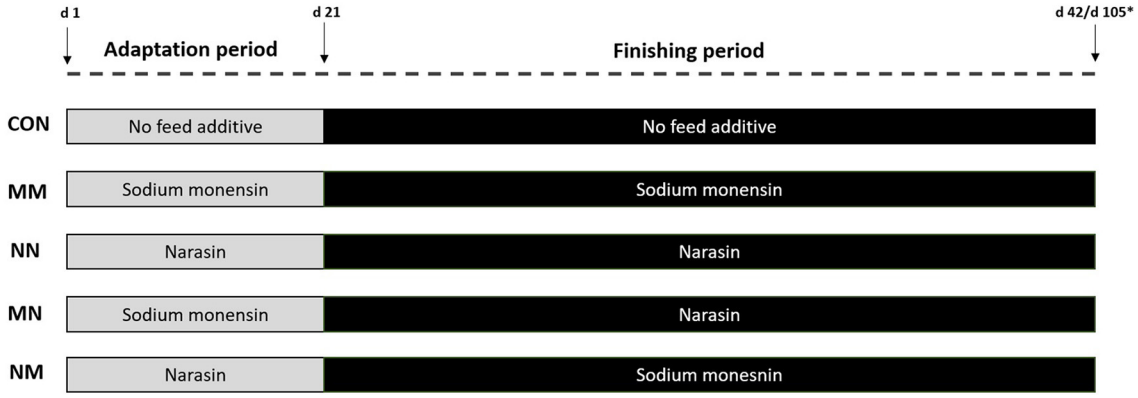


FIGURE 1
Schematic representation of the treatments. The adaptation period consisted of 21 days. During the adaptation period, steers were fed three step-up diets which gradually (7 days in each step) decreased the roughage source (sugarcane bagasse) from 23 to 18% (Adap. 1), to 13% (Adap. 2), and to 8% (Adap. 3) and increased concentrate accordingly. From days 22 to 42 (Exp. 1) or day 105 (Exp. 2), steers were fed the finishing diet containing 8% of roughage (sugarcane bagasse). Treatments were as follows: Control (CON): no feed additive in the basal diet during the entire feeding period; Sodium monensin (MM) at 25 mg/kg DM during the entire feeding period [adaptation and finishing periods (Rumensin 100, Elanco Brazil, São Paulo, SP, Brazil)]; Narasin (NN) at 13 mg/kg DM during the entire feeding period (adaptation and finishing periods; Zimprova 100, Elanco Brazil, São Paulo, SP, Brazil); Sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM during the finishing period (MN); and narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM during the finishing period (NM). *Exp. 1 lasted for 42 days and Exp. 2 lasted for 105 days.

TABLE 1 Ingredients and chemical composition [dry matter basis (DM)] of experimental diets used in Exp. 1 and 2.

Item	Experimental diets[a]			
	Adap. 1	Adap. 2	Adap. 3	Finishing[b]
Days in each diet	7	7	7	21/84
Ingredients, %				
Sugarcane bagasse	23.0	18.0	13.0	8.00
Whole cottonseed	10.0	10.0	10.0	10.0
Soybean hulls	15.0	15.0	15.0	15.0
Ground corn	44.0	49.0	56.0	62.0
Soybean meal	5.00	5.00	3.00	2.00
Urea	1.00	1.00	1.00	1.00
Mineral supplement[c]	2.00	2.00	2.00	2.00
Analyzed composition, %				
Dry matter	70.8	71.9	70.2	70.0
Organic matter	92.2	92.9	91.6	91.7
Crude protein	14.6	14.5	12.7	12.4
Neutral detergent fiber	45.4	43.4	30.5	28.0
Acid detergent fiber	26.9	25.7	17.1	16.0
Ash	7.70	7.00	8.30	8.20
Ether extract	3.30	3.20	3.10	4.40
Net energy of maintenance, Mcal/kg[d]	1.69	1.77	1.82	1.90
Net energy of gain, Mcal/kg[d]	1.07	1.15	1.23	1.30
Total digestible nutrients[d]	71.5	74.3	77.2	80.1

[a] Adap, adaptation diet; Adap. 1, fed from days 1 to 7; Adap. 2, fed from days 8 to 14; Adap. 3, fed from days 15 to 21; Finishing, finishing diet.
[b] In Exp. 1, steers were fed the finishing diet for 21 days. In Exp. 2, steers were fed the finishing diet for 84 days.
[c] Containing (DM basis): 164 g/kg Ca, 60 g/kg P, 40 g/kg S, 140 g/kg Na, 10 g/kg Mg, 780 mg/kg Mn, 3,750 mg/kg Zn, 1,010 mg/kg Cu, 60 mg/kg Co, 75 mg/kg I, and 19 mg/kg Se.
[d] Estimated using the tabular values according to NASEM (9) with no feed additive inclusion. Energy values of sugarcane bagasse were obtained from Valadares Filho et al. (20).

Ruminal content was collected from each steer 6 h after feeding on days 21 (the last day of the adaptation period), 24, 27, and 42 (end of the experiment). During the sampling days, five trained personnel were assigned to eight steers each; at least one steer of each treatment/staff, so treatments were balanced within personnel. In addition, trained personnel rotated on each sampling day to account for any variation during sampling. Steers were halter broken and used in the previous study (19) and did not require to be restrained for sampling. Sampling was completed within 40 min. Approximately 100 ml of ruminal content was manually collected from the ventral portion of the rumen and squeezed into four layers of cheesecloth as described by Polizel et al. (18). Immediately after collection, the pH of ruminal fluid was measured using a digital pH meter (Digimed-M20; Digimed Instrumentação Analítica; São Paulo, SP, Brazil). Sub-samples (~5 ml) were stored at −18°C for further analysis of short-chain fatty acids (SCFA) and ruminal ammonia nitrogen N (NH_3-N).

The SCFA concentration was determined as described by Polizel et al. (18). In brief, 1.6 ml of ruminal fluid was mixed with 0.4 mL metaphosphoric acid:formic acid (3:1) and 0.2 mL of 100 mM 2-ethyl-butyric acid (internal standard). The homogenate was centrifuged for 30 min at 15,000 × *g* at 4°C, and 1.2 ml of the supernatant was then transferred to a chromatography vial. The quantification of SCFA was performed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (7683B), a fused-silica capillary column (J &W19091F-112, Agilent Technologies, Santa Clara, CA, United States), 25 m in length, and 320 mm internal diameter, containing 0.20 M cyanopropyl polysiloxane. The data acquisition was performed using the ChemStation software (Agilent Technologies, Santa Clara, CA, United States). The concentration of NH_3-N was determined by the colorimetric method as described by Chaney and Marbach (21), adapted for a microplate reader (EON, BioTech Instruments, Winooski, VT, United States), using a 550 nm absorbance filter.

The total fecal collection was performed from days 37–41 to determine the total apparent digestibility of nutrients. The total fecal production of each steer was collected every 4 h from the concrete floor (22), weighted, sampled (10% of the total), and frozen at −18°C for further chemical analysis.

At the end of the experiment, feed ingredients and fecal samples were thawed, dried in a forced-air oven at 55°C for 72 h, and ground through a 1-mm screen using a Willey-type mill (MA-680, Marconi, Piracicaba, Brazil). All samples were analyzed for DM [method 930.15; (23)], ash [method 942.05; (23)], and nitrogen (Leco FP- 528; Leco Corp., St. Joseph, MI). The organic matter (OM) was calculated based on ash values (OM, % = 100 – ash, %). Crude protein (CP) was obtained by multiplying the total N content by 6.25. The determination of the fibrous fraction was carried out sequentially, using thermostable alpha-amylase and sodium sulfite for analysis of neutral detergent fiber (NDF) according to the methodology proposed by van Soest et al. (24) and acid detergent fiber (ADF) according to Goering and van Soest (25), using the Ankon 2000 Fiber Analyzer (Ankon Tech. Corp., Fairport, NY, United States). The NDF and ADF reports are ash corrected.

2.2. Experiment 2. Animal performance, feeding behavior, and carcass characteristics

A total of 120 Nellore bulls (*Bos indicus;* initial BW = 425 ± 5.4 kg; age = 30 ± 2.0 months) purchased from one single ranch were used in this experiment. Upon feedlot arrival, bulls were individually weighted (Id Beck 2.0, Beckhauser Balanças e Troncos, Paranavaí, Brasil), after 16 h of feed and water withdrawal, identified with ear tags, and vaccinated/dewormed as described by Gouvêa et al. (26). Bulls were blocked according to the initial BW, allocated to 40 pens as described in Exp. 1 (3.5 × 8 m; 3 bulls/pen), and then, pens within each BW block were assigned to the same treatments described in Exp. 1 (Figure 1). Experiment 2 lasted for 105 days. The experimental diets and adaptation protocol were the same as described in Exp. 1 (Table 1). The finishing diets were fed from days 22 to 105. Bulls had *ad libitum* access to water and to the diets containing the treatments throughout the experiment. Feeding management, diet, and refusal sampling were as described in Exp. 1.

Bulls were individually weighed after 16 h of fasting (feed and water) at the beginning of the experiment (day 1) and at the end of the trial (day 105). Full BW was collected at the end of the adaptation period (day 21) and discounted by 4% as ruminal fill (9) to calculate shrunk BW at the end of the adaptation period. The ADG, DMI, and G:F were calculated for each experimental period. At the end of the experiment (day 105), bulls were transported to a commercial packing plant and slaughtered on the following day as described by Gouvêa et al. (26). In brief, the hot carcass weight (HCW) was obtained after the removal of the hide, head, feet, tail, kidneys, and visceral fat. The dressing percentage was calculated using the HCW obtained after slaughter, divided by the final shrunk weight. *Longissimus muscle* (**LM**) area and subcutaneous fat thickness were measured between the 12 and 13th rib from each carcass after a 24-h chill at 2°C as described by Toseti et al. (27), using a digital camera attached to a fixed distance (10 cm) of a 15 × 20 cm rectangular steel base. The images obtained by the digital camera were interpreted by one experienced technician using the AutoCAD® software.

2.3. Statistical analysis

Both experiments were analyzed using the MIXED procedure of SAS 9.4 software (SAS Inst. Inc., Cary, NC), as a randomized complete block design with five treatments and initial BW used as the blocking factor. The steer was the experiment unit in Exp. 1, and the pen served as the experimental unit in Exp. 2. The Kenward Roger approximation was used to determine the correct denominator degrees of freedom for testing fixed effects. Each experimental period (adaptation, finishing, and total feeding period) was analyzed separately. In Exp. 1, the statistical model used to analyze intake and nutrient digestibility was: $yij = \mu + Ti + Bj + eij$, where y = dependent variable, μ = overall mean, Ti = fixed effect of treatment, Bi = random effect of block, and eij = the residual error. The SCFA, pH, and NH_3-N in Exp. 1 were analyzed as repeated measurements over time using the

MIXED procedure following the statistical model: yijk = μ+ Ti + Bj + eij + Pk + TiPk + eijk, where μ = overall mean, Ti = the fixed effect of treatment; Bj = random block effect, eij = subject level random error, Pk = fixed effect of time, TiPk = fixed effect of treatment × time interaction, and eijk = the residual error. The covariance matrix used was the compound symmetry (CS) and was selected using the Bayesian information fit criteria (smaller is better), after adjusting models with the AR(1), ARH(1), ANTE(1), CS, CSH, and UN covariance. The subject was treatment (pen). In Exp. 2, the statistical model used to analyze growth performance and carcass data was as follows: yij = μ + Ti + bj + eij, where μ = overall mean, Ti = fixed effect of treatments, bi = random effect of a block, and eij = the residual error.

Data from all experiments were reported as least-square means. Effects were declared significant at $P < 0.05$. The tendency was discussed when $P > 0.05$ and ≤ 0.10. When a significant treatment effect was observed, a *post-hoc* analysis using the Tukey test was used to identify significant differences among the treatment's least square means. When a significant treatment × time interaction was observed in Exp. 2 for SCFA, NH_3-N, or pH, treatments were compared within each time point using the Tukey test.

3. Results

3.1. Experiment 1

Feed additives affected the DMI during the adaptation period ($P = 0.02$; Table 2). Steers fed MM had lower DMI during the adaptation period compared to NM ($P = 0.02$; 5.76 vs. 6.92 kg/day, respectively) but not compared to CON, MM, MN, or NN ($P \geq 0.12$). No differences in DMI were observed between the treatments during the finishing period ($P = 0.45$) or the total feeding period ($P = 0.15$).

Treatments did not affect the nutrient intake ($P \geq 0.51$) and the total tract apparent digestibility of nutrients ($P \geq 0.22$; Table 3).

No treatment × day interaction was observed for any of the ruminal fermentation characteristics evaluated in the present study ($P \geq 0.14$; Table 4). Treatments tended to affect the ruminal concentration of NH_3-N ($P = 0.06$). Steers fed NN tended to have greater NH_3-N concentration compared to CON ($P = 0.15$; 5.81 vs. 4.11 mg/dL, respectively) but not compared with MM, MN, or NM ($P \geq 0.20$). No other treatment effects were observed on the ruminal fermentation characteristics ($P \geq 0.61$).

An effect of the day ($P \leq 0.01$) was observed for the total concentration of SCFA, molar proportion of acetate, propionate, and ruminal pH (Tables 4, 5). The sampling day tended ($P = 0.06$) to affect the molar proportion of butyrate (Tables 4, 5). Overall, ruminal pH, molar proportion of acetate and butyrate, and acetate:propionate ratio decreased over the sampling days ($P < 0.05$), and the total ruminal concentration of SCFA and molar proportion of propionate increased ($P < 0.05$) over the sampling days (Table 5).

3.2. Experiment 2

Feed additives affected the DMI during the adaptation period ($P < 0.001$; Table 6). Steers fed NM had greater DMI during the adaptation period compared to CON, MM, and MN ($P \leq 0.03$; 10.4 vs. 9.73, 9.57, and 9.43 kg/day, respectively), but no differences were observed between NM and NN ($P = 0.66$) or between CON, MM, and NN ($P \geq 0.11$). Treatments also tended to affect the DMI during the total feeding period ($P = 0.07$). Steers fed NN tended to have greater DMI compared to MM ($P = 0.09$; 10.4 vs. 9.83 kg/day) but not compared to CON, MN, and NM ($P \geq 0.16$).

No other differences between treatments were observed for any of the growth performance and carcass characteristics evaluated in the present study ($P \geq 12$), except for 12th-rib fat ($P = 0.04$). Steers fed MM had greater 12th-rib fat compared to CON ($P = 0.02$), with no differences between the other treatments ($P \geq 0.16$).

4. Discussion

Feed additives such as ionophores, also known as non-nutritional ingredients, are fed to feedlot cattle to increase feed efficiency (10), decreasing the cost of production and improving the potential profit of the feedlot operation. The most common response to monensin inclusion in beef cattle diets is the

TABLE 2 Effects of feed additives on dry matter intake of finishing beef cattle—Exp. 1.

Item	Treatments[a]					SEM[b]	*P*-value
	CON	MM	MN	NM	NN		
Pens (steers)	8 (8)	8 (8)	8 (8)	8 (8)	8 (8)	-	-
Dry matter intake, kg/day							
Adaptation period (days 1–21)	6.65[cd]	5.76[d]	6.06[cd]	6.92[c]	6.57[cd]	0.26	0.02
Finishing period (days 22–42)	7.85	6.95	7.57	7.77	7.43	0.36	0.45
Total feeding period (days 1–42)	7.25	6.35	6.87	7.34	7.00	0.28	0.15

[a]CON, control; no feed additive; MM, sodium monensin at 25 mg/kg DM during the adaptation and finishing periods (Rumensin, Elanco Saude Animal, São Paulo, SP, Brazil); MN, sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM (Zimprova, Elanco Saude Animal, São Paulo, SP, Brazil) during the finishing period; NM, narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM of during the finishing period; NN, narasin at 13 mg/kg DM during the adaptation and finishing periods.
[b]SEM, standard error of the mean.
[cd]Means that do not have common superscript letters are different (Tukey's test; $P < 0.05$).

10.3389/fvets.2023.1117639

TABLE 3 Effect of feed additives on intake and apparent digestibility of nutrients of finishing beef cattle—Exp. 1; days 37–42.

Item	Treatments[a]					SEM[b]	*P*-value
	CON	MM	MN	NM	NN		
Pens (steers)	8 (8)	8 (8)	8 (8)	8 (8)	8 (8)	-	-
Intake, kg/day							
Dry matter	7.03	6.33	7.03	6.51	6.34	0.49	0.71
Organic matter	6.34	5.86	6.47	6.03	5.83	0.45	0.81
Neutral detergent fiber	1.90	1.74	2.08	1.80	1.92	0.14	0.51
Acid detergent fiber	1.07	1.02	1.19	1.05	1.10	0.08	0.67
Apparent digestibility, %							
Dry matter	67.5	66.7	68.7	67.0	63.5	1.67	0.27
Organic matter	68.9	69.2	70.3	69.6	66.0	1.57	0.35
Neutral detergent fiber	52.9	53.1	59.1	52.1	54.2	2.22	0.22
Acid detergent fiber	51.0	54.2	57.7	54.2	53.9	2.36	0.46

[a]CON, control; no feed additive; MM, sodium monensin at 25 mg/kg DM during the adaptation and finishing periods (Rumensin, Elanco Saude Animal, São Paulo, SP, Brazil); MN, sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM (Zimprova, Elanco Saude Animal, São Paulo, SP, Brazil) during the finishing period; NM, narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM of during the finishing period; NN, narasin at 13 mg/kg DM during the adaptation and finishing periods.
[b]SEM, standard error of the mean.

TABLE 4 Effect of feed additives on the ruminal concentration of short-chain fatty acids (SCFAs), pH, and ammonia nitrogen (NH_3-N) of finishing beef cattle—Exp. 1.

Item	Treatments[a]					SEM[b]	*P*-value[c]		
	CON	MM	MN	NM	NN		Treat	Day	Treat × Day
Pens (steers)	8 (8)	8 (8)	8 (8)	8 (8)	8 (8)	-	-	-	-
Total SCFA, mmol/L	119	112	125	117	119	8.91	0.86	<0.001	0.90
SCFA, mol/100 mol									
Acetate	58.9	58.9	57.6	57.9	59.1	1.80	0.96	<0.001	0.34
Propionate	27.1	26.6	26.9	27.5	26.7	1.61	0.92	<0.001	0.15
Butyrate	9.77	10.8	11.7	10.7	10.7	0.71	0.35	0.06	0.22
Acetate:propionate ratio	2.31	2.31	2.24	2.27	2.48	0.06	0.92	<0.01	0.14
Ruminal pH	6.20	6.08	6.00	6.08	6.10	0.11	0.79	<0.01	0.43
NH_3-N mg/dL	4.11[e]	5.21[de]	5.75[de]	4.11[de]	5.81[d]	0.55	0.06	0.23	0.72

[a]CON, control; no feed additive; MM, sodium monensin at 25 mg/kg DM during the adaptation and finishing periods (Rumensin, Elanco Saude Animal, São Paulo, SP, Brazil); MN, sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM (Zimprova, Elanco Saude Animal, São Paulo, SP, Brazil) during the finishing period; NM, narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM of during the finishing period; NN, narasin at 13 mg/kg DM during the adaptation and finishing periods.
[b]SEM, standard error of the mean.
[c]Treat, effect of treatment; day, effect of day (days 21, 24, 27, and 42); Treat × Day = interaction between treatment and day. [de]Means that do not have common superscript letters are different (Tukey's test; $P < 0.05$).

increased G:F by improving or maintaining ADG and reducing DMI (10, 28, 29).

Monensin is one of the most used feed additives in finishing diets for beef cattle (7, 8). It selectively inhibits gram-positive bacteria (11), increasing the efficiency of energy metabolism (30) and nitrogen metabolism (31), and it is also used to control bloat (32), probably due to reduced feed intake variation (33) and meal size and frequency of meals (34). According to Duffield et al. (10), the average concentration of monensin in feed across 40 peer-reviewed manuscripts and 24 trial reports published from 1972 to 2003 was 28.1 mg/kg DM. Monensin decreases DMI, especially in diets with a high proportion of forage (9) due to the increased molar proportion of propionate and decreased molar proportion of acetate and butyrate (35).

Narasin is an ionophore produced by *Streptomyces aureofaciens*. It has been evaluated for pigs (36) and chicken (37), and more recently in beef cattle diets (15, 16, 18). According to these last authors, narasin fed at 13 mg/kg DM has the potential to increase ADG and improve ruminal fermentation characteristics, especially increasing the molar proportion of propionate and decreasing the molar proportion of acetate and acetate:propionate ratio, without negatively affecting DMI of beef cattle fed high-roughage diets. This characteristic would also benefit finishing cattle, especially during the adaptation period, in

TABLE 5 Effect of sampling days on the ruminal concentration of short-chain fatty acids (SCFAs) and pH of finishing beef cattle—Exp. 1.

Item	Sampling days[a]				SEM[b]	*P*-value
	21	24	27	42		
Total SCFA, mmol/L	117^{d}	88.2^{e}	137^{c}	133^{cd}	6.45	<0.001
SCFA, mol/100 mol						
Acetate	60.6^{c}	59.5^{c}	57.1^{d}	56.8^{d}	0.974	<0.001
Propionate	24.5^{e}	25.5^{de}	27.6^{cd}	29.4^{c}	0.998	<0.001
Butyrate	11.1^{cd}	10.6^{cd}	11.5^{c}	9.73^{d}	0.60	0.06
Acetate:propionate ratio	2.61^{c}	2.47^{cd}	2.19^{d}	2.03^{d}	0.11	<0.01
Ruminal pH	6.14^{c}	6.20^{c}	5.98^{d}	6.03^{d}	0.065	<0.01

[a]Ruminal content was collected from each steer 6 h after feeding on days 21 (the last day of the adaptation period), 24, 27, and 42 (end of the experiment).
[b]SEM, standard error of the mean.
[cde]Means that do not have common superscript letters are different (Tukey test; $P < 0.05$).

TABLE 6 Effect of feed additives on growth performance and carcass characteristics of finishing beef cattle—Exp. 2.

Item	Treatments[a]					SEM[b]	*P*-value
	CON	MM	MN	NM	NN		
Pens (steers)	8 (24)	8 (24)	8 (24)	8 (24)	8 (24)	-	-
Growth performance							
Body weight, kg[c]							
Day 1	424	424	425	425	425	10.6	0.41
Day 21	437	442	441	442	439	10.1	0.73
Day 105	570	571	576	578	569	13.2	0.85
Dry matter intake, kg/day							
Adaptation period	9.73^{ef}	9.57^{ef}	9.43^{f}	10.4^{d}	10.1^{de}	0.221	<0.001
Finishing period	10.1	9.90	10.3	10.4	10.5	0.260	0.15
Total feeding period	10.0^{de}	9.83^{e}	10.1^{de}	10.4^{d}	10.4^{a}	0.244	0.07
Average daily gain, kg/day							
Adaptation period	0.590	0.819	0.761	0.821	0.701	0.150	0.78
Finishing period	1.59	1.54	1.61	1.62	1.54	0.069	0.88
Total feeding period	1.39	1.40	1.44	1.46	1.38	0.066	0.87
Feed efficiency (gain:feed ratio)							
Adaptation period (days 1–21)	0.060	0.084	0.080	0.079	0.070	0.015	0.79
Finishing period (days 22–105)	0.156	0.156	0.158	0.154	0.149	0.006	0.87
Total feeding period (days 1–105)	0.140	0.141	0.144	0.140	0.133	0.006	0.76
Carcass characteristics							
Dressing, %	57.3	58.3	58.5	58.4	57.5	0.51	0.12
Hot carcass weight, kg	328	334	336	333	332	4.00	0.70
Longissimus muscle area, cm^2	74.8	76.7	75.3	73.2	75.9	1.69	0.64
12th-rib fat, mm	4.69^{e}	6.58^{d}	5.78^{de}	6.05^{de}	5.83^{d}	0.45	0.04

[a]CON, control; no feed additive; MM, sodium monensin at 25 mg/kg DM during the adaptation and finishing periods (Rumensin, Elanco Saude Animal, São Paulo, SP, Brazil); MN, sodium monensin at 25 mg/kg DM during the adaptation period and narasin at 13 mg/kg DM (Zimprova, Elanco Saude Animal, São Paulo, SP, Brazil) during the finishing period; NM, narasin at 13 mg/kg DM during the adaptation period and sodium monensin at 25 mg/kg DM of during the finishing period; NN, narasin at 13 mg/kg DM during the adaptation and finishing periods.
[b]SEM, standard error of the mean.
[c]Shrunk body weight on days 1 and 105 (after 16 h of feed and water withdrawal). Body weight on day 21 was discounted by 4% from the full BW as ruminal fill.
[def]Means that do not have common superscript letters are different (Tukey's test; $P < 0.05$).

which finishing cattle is transitioned from high-roughage-based diets to high-concentrate diets, and the low DMI during the first 1–21 days of the adaptation period (2, 4, 6) could limit the growth performance.

In agreement with our hypothesis, steers fed narasin during the adaptation period had greater DMI compared to monensin. In Exp. 2, this difference tended to carry over the finishing period, but no differences in ADG or feed efficiency were detected between the treatments. Apparently, any difference in feed intake during the adaptation period due to feeding monensin will be compensated during the finishing phase, so no differences in the growth performance were detected at the end of the total feeding period.

The lack of treatment effect on growth performance and carcass characteristics in the present study is in agreement with Stackhouse-Lawson et al. (38) and Gouvêa et al. (39), who also did not observe differences in growth performance and carcass characteristics when monensin was fed to finishing cattle. The inclusion of 0, 22, 33, and 44 mg monensin/kg diet DM for finishing beef cattle also did not affect the DMI, rumen pH, SCFA concentrations, and H_2S gas (40). Bell et al. (41) also did not observe differences in nutrient digestibility of beef steers receiving a forage-based diet with or without monensin. On the contrary, in a meta-analysis using 40 peer-reviewed manuscripts and 24 additional trial reports, Duffield et al. (10) concluded that monensin decreased DMI by 3% and improved G:F of finishing beef cattle by 2.5–3.5%. According to Yang and Russell (42), monensin can inhibit amino acid-fermenting ruminal bacteria, decreasing ruminal amino acid deamination and ammonia production in the rumen.

Contrary to Polizel et al. (18) and Limede et al. (19), narasin supplementation did not increase the ruminal concentration of propionate or the total concentration of SCFA in the current study. The lack of treatment effect on ruminal fermentation characteristics and nutrient digestibility agrees with the lack of treatment effect on growth performance in the current experiment.

The increase in the molar proportion of propionate and decrease in the molar proportion of acetate and butyrate throughout the days on feed is probably a result of rumen microbial change due to an increase in the amount of starch fermented in the rumen, as a combined result of dietary changes during the adaptation period and increased DMI following changes in BW.

According to Clary et al. (43), a diminished response to ionophores is observed when fat is fed in high-concentrate diets. However, according to these authors, supplementing monensin in finishing diets containing tallow (4% DM) did not affect feed efficiency but did increase the feed efficiency by 4% in diets with no tallow. According to Zinn and Borques (44), the growth performance response to monensin supplementation is reduced in fat-supplemented finishing diets. In the current study, although no supplemental fat was included in the diet, the inclusion of whole cottonseed contributed to an increase in the total fat content of the diet, which can be related to the lack of response to ionophore supplementation on the growth performance. According to Clary et al. (43), the interaction between fat and ionophores may be related to the similar effects of these two ingredients on ruminal fermentation (45) or to the decreased solubility of ionophores in lipids (43). More research is needed to better understand the nutritional and management factors that can impair monensin in finishing diets.

The lack of treatment effect on growth performance and ruminal fermentation characteristics in the current experiment is a good example of the importance of using a negative control treatment (without feed additives) when comparing different feed additives. Monensin is frequently used as the control treatment to evaluate alternative feed additives (46, 47), which makes it difficult to accurately account for the benefits (or the lack of benefits) of alternative feed additives on animal performance or ruminal fermentation characteristics.

5. Conclusion

Feeding narasin at 13 mg/kg DM during the adaptation period increases the dry matter intake compared to monensin at 25 mg/kg DM; however, no effects of feed additives were observed on nutrient digestibility, growth performance, or carcass characteristics of finishing cattle.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Animal Care and Use Committee of the Luiz de Queiroz College of Agriculture, University of São Paulo.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Brown MS, Ponce CH, Pulikanti R. Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. *J Anim Sci.* (2006) 84(Suppl.):25–33. doi: 10.2527/2006.8413_supplE25x

2. Pereira MCS, Dellaqua JVT, Sousa OA, Santi PF, Felizari LD, Reis BQ, et al. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of Nellore cattle submitted to strategic diets prior the adaptation period. *Livest Sci.* (2020) 234:103985. doi: 10.1016/j.livsci.2020.103985

3. Bevans DW, Beauchemin KA, Schwartzkopf-Genswein KS, Mckinnon JJ, Mcallister TA. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. *J Anim Sci.* (2005) 83:1116–32. doi: 10.2527/2005.8351116x

4. Huls TJ, Lueble MK, Watson AK, Meyer NF, Griffin WA, Klopfenstein TJ, et al. Using Sweet Bran instead of forage during grain adaptation in finishing feedlot cattle. *J Anim Sci.* (2016) 94:1149–58. doi: 10.2527/jas.2015-0008

5. Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: A review. *J Anim Sci.* (1998) 76:275–86. doi: 10.2527/1998.761275x

6. Galyean ML, Malcolm KJ, Duff GC. Performance of feedlot steers fed diets containing laidlomycin propionate or monensin plus tylosin, and effects of laidlomycin propionate concentration on intake patterns and ruminal fermentation in beef steers during adaptation to a high-concentrate diet. *J Anim Sci.* (1992) 70:2950–8. doi: 10.2527/1992.70102950x

7. Samuelson KL, Hubbert ME, Galyean ML, Löest CA. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico state and Texas tech university survey. *J Anim Sci.* (2016) 94:2648–63. doi: 10.2527/jas.2016-0282

8. Pinto ACJ, Millen DD. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists : The 2016 Brazilian. *Can J Anim Sci.* (2019) 99:392–407. doi: 10.1139/cjas-2018-0031

9. NASEM. *National Academies of Sciences, Engineering, and Medicine (NASEM). Nutrient Requirements of Beef Cattle*. 8th ed. Washington, DC: National Academies of Sciences, Engineering, and Medicine (2016).

10. Duffield TF, Merrill JK, Bagg RN, Duffi TF. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. *J Anim Sci.* (2012) 90:4583–92. doi: 10.2527/jas.2011-5018

11. Russel JB, Strobel HJ. Effect of lonophores on ruminal fermentation. *Appl Environ Microbiol.* (1989) 55:1–6. doi: 10.1128/aem.55.1.1-6.1989

12. Lunn DE, Mutsvangwa T, Odongo NE, Duffield TF, Bagg R, Dick P, et al. Effect of monensin on meal frequency during sub-acute ruminal acidosis in dairy cows. *Can J Anim Sci.* (2005) 85:247–9. doi: 10.4141/A04-033

13. Erickson GE, Milton CT, Fanning KC, Cooper RJ, Swingle RS, Parrott JC, et al. Interaction between bunk management and monensin concentration on finishing performance, feeding behavior, and ruminal metabolism during an acidosis challenge with feedlot cattle. *J Anim Sci.* (2003) 81:2869–79. doi: 10.2527/2003.81112869x

14. Nagaraja TG, Titgemeyer EC. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. *J Dairy Sci.* (2007) 90:E17–38. doi: 10.3168/jds.2006-478

15. Miszura A, Polizel D, Ferraz M, Barroso J, Gobato L, Martins A, et al. Effects of feed additives on rumen parameters of steers fed a high-forage diet. *J Anim Sci.* (2018) 96:441–441. doi: 10.1093/jas/sky404.964

16. Silva R, Ferraz Junior M, Gouvêa V, Polizel D, Santos M, Miszura A, et al. Effect of narasin in mineral mix to Nellore heifers fed with high forage. *J Anim Sci.* (2015) 93:118. doi: 10.1093/tas/txad027

17. Polizel DM, Marques SS, Westphalen MF, Gouvea VN, Ferraz Júnior MVdC, Miszura AA, et al. Narasin inclusion for feedlot lambs fed a diet containing high amounts of ground flint corn. *Sci Agric.* (2020) 78:1–8. doi: 10.1590/1678-992x-2020-0010

18. Polizel D, Cappellozza B, Hoe F, Lopes C, Barroso J, Miszura A, et al. Effects of narasin supplementation on dry matter intake and rumen fermentation characteristics of *Bos indicus* steers fed a high-forage diet. *Transl Anim Sci.* (2020) 4:118–28. doi: 10.1093/tas/txz164

19. Limede AC, Marques RS, Polizel DM, Cappellozza BI, Miszura AA, Barroso JPR, et al. Effects of supplementation with narasin, salinomycin, or flavomycin on performance and ruminal fermentation characteristics of *Bos indicus* Nellore cattle fed with forage-based diets. *J Anim Sci.* (2021) 99:skab005. doi: 10.1093/jas/skab005

20. Valadares Filho SC, Machado PAS, Chizotti ML, Amaral HF. *Tabelas Brasileiras de Composicao de Alimentos para Ruminantes*. 3rd ed. Viçosa: Federal University of Vicosa (2015).

21. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. *Clin Chem.* (1962) 8:130–2. doi: 10.1093/clinchem/8.2.130

22. Gouvêa VNd, Biehl MV, Ferraz Junior MVdC, Moreira EM, Faleiro Neto JA, Westphalen MF, et al. Effects of soybean oil or various levels of whole cottonseed on intake, digestibility, feeding behavior, and ruminal fermentation characteristics of finishing beef cattle. *Livest Sci.* (2021) 244:104390. doi: 10.1016/j.livsci.2020.104390

23. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*. 14th ed. Arlington, VA: Association of Official Analytical Chemists (1986).

24. van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci.* (1991) 74:3583–97. doi: 10.3168/jds.S0022-0302(91)78551-2

25. Goering HK, van Soest PJ. Forage fiber analysis. *Agri Handb.* (1970) 2:1–19. doi: 10.1111/j.1438-8677.1970.tb00621.x

26. Gouvêa VNd, Biehl MV, Andrade TS, Ferraz Junior MVdC, Ferreira EM, Polizel DM, et al. Effects of soybean oil or various levels of whole cottonseed on growth performance, carcass traits, and meat quality of finishing bulls. *Livest Sci.* (2020) 232:103934. doi: 10.1016/j.livsci.2020.103934

27. Toseti LB, Goulart RS, Gouvêa VN, Acedo TS, Vasconcellos GSFM, Pires A, et al. Effects of a blend of essential oils and exogenous α-amylase in diets containing different roughage sources for finishing beef cattle. *Anim Feed Sci Technol.* (2020) 269:114643. doi: 10.1016/j.anifeedsci.2020.114643

28. Bretschneider G, Elizalde JC, Pérez FA. The effect of feeding antibiotic growth promoters on the performance of beef cattle consuming forage-based diets: A review. *Livest Sci.* (2008) 12:17. doi: 10.1016/j.livsci.2007.12.017

29. Tedeschi LO, Fox DG, Tylutki TP. Potential environmental benefits of ionophores in ruuminant diets. *J Environ Qual.* (2003) 32:1591–602. doi: 10.2134/jeq2003.1591

30. McGuffey RK, Richardson LF, Wilkinson JID. Ionophores for dairy cattle: Current status and future outlook. *J Dairy Sci.* (2001) 84:E194–203. doi: 10.3168/jds.S0022-0302(01)70218-4

31. Spears JW. Ionophores and nutrient digestion and absorption in ruminants. *J Nutr.* (1990) 120:632–8. doi: 10.1093/jn/120.6.632

32. Bartley EE, Nagaraja TG, Pressman ES, Dayton AD, Katz MP, Fina LR. Effects of lasalocid or monensin on legume or grain (feedlot) bloat4. *J Anim Sci.* (1983) 56:1400–6. doi: 10.2527/jas1983.5661400x

33. Stock RA, Laudert SB, Stroup WW, Larson EM, Parrott JC, Britton RA. Effect of monensin and monensin and tylosin combination on feed intake variation of feedlot steers. *J Anim Sci.* (1995) 73:39–44. doi: 10.2527/1995.73139x

34. González LA, Manteca X, Calsamiglia S, Schwartzkopf-Genswein KS, Ferret A. Ruminal acidosis in feedlot cattle: Interplay between feed ingredients, rumen function and feeding behavior (a review). *Anim Feed Sci Technol.* (2012) 172:66–79. doi: 10.1016/j.anifeedsci.2011.12.009

35. Ellis JL, Dijkstra J, Bannink A, Kebreab E, Hook SE, Archibeque S, et al. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. *J Anim Sci.* (2012) 90:2717–26. doi: 10.2527/jas.2011-3966

36. Kerr BJ, Trabue SL, Andersen DS. Narasin effects on energy, nutrient, and fiber digestibility in corn-soybean meal or corn-soybean meal-dried distillers grains with solubles diets fed to 16-, 92-, and 141-kg pigs. *J Anim Sci.* (2017) 95:4030–6. doi: 10.2527/jas2017.1732

37. Ruff MD, Reid WM, Rahn AP, McDoulgard LR. Anticoccidial activity of narasin in broiler chickens reared in floor pens. *Poult Sci.* (1980) 59:2008–13. doi: 10.3382/ps.0592008

38. Stackhouse-Lawson KR, Calvo MS, Place SE, Armitage TL, Pan Y, Zhao Y, et al. Growth promoting technologies reduce greenhouse gas, alcohol, and ammonia emissions from feedlot cattle. *J Anim Sci.* (2013) 91:5438–47. doi: 10.2527/jas.2011-4885

39. Gouvêa V, Duff G, Sowers C, Barnes M. Effects of supplemental phytomolecules on growth performance, carcass characteristics and liver abnormalities of finishing beef steers. *J Appl Anim Res.* (2021) 49:324–9. doi: 10.1080/09712119.2021.1960841

40. Felix TL, Pyatt NA, Loerch SC. Effects of monensin supplementation on ruminal metabolism of feedlot cattle fed diets containing dried distillers grains. *J Anim Sci.* (2012) 90:3905–13. doi: 10.2527/jas.2011-5059

41. Bell NL, Anderson RC, Callaway TR, Franco MO, Sawyer JE, Wickersham TA. Effect of monensin inclusion on intake, digestion, and ruminal fermentation parameters by *Bos taurus indicus* and *Bos taurus taurus* steers consuming bermudagrass hay. *J Anim Sci.* (2017) 95:2736–46. doi: 10.2527/jas.2016.1011

42. Yang CM, Russell JB. The effect of monensin supplementation on ruminal ammonia accumulation *in vivo* and the numbers of amino acid-fermenting bacteria. *J Anim Sci.* (1993) 71:3470–6. doi: 10.2527/1993.71123470x

43. Clary EM, Brandt RT, Harmon DL, Nagaraja TG. Supplemental fat and ionophores in finishing diets: Feedlot performance and ruminal digesta kinetics in steers. *J Anim Sci.* (1993) 71:3115–23. doi: 10.2527/1993.71113115x

44. Zinn RA, Borques JL. Influence of sodium bicarbonate and monensin on utilization of a fat-supplemented, high-energy growing-finishing diet by feedlot steers. *J Anim Sci.* (1993) 71:18–25. doi: 10.2527/1993.71118x

45. Chalupa W, Rickabaugh B, Kronfeld D, David Sklan S. Rumen fermentation *in vitro* as influenced by long chain fatty acids. *J Dairy Sci.* (1984) 67:1439–44. doi: 10.3168/jds.S0022-0302(84)81459-9

46. Meschiatti MAP, Gouvêa VN, Pellarin LA, Batalha CDA, Biehl MV, Acedo TS, et al. Feeding the combination of essential oils and exogenous α-amylase increases performance and carcass production of finishing beef cattle. *J Anim Sci.* (2019) 97:456–71. doi: 10.1093/jas/sky415

47. Mobiglia AM, Camilo FR, Couto VRM, Castro FGF, Drouillard JS, Gouvêa VN, et al. Effects of grain adaptation programs and antimicrobial feed additives on performance and nutrient digestibility of *Bos indicus* cattle fed whole shelled corn. *Transl Anim Sci.* (2021) 5:txab119. doi: 10.1093/tas/txab119

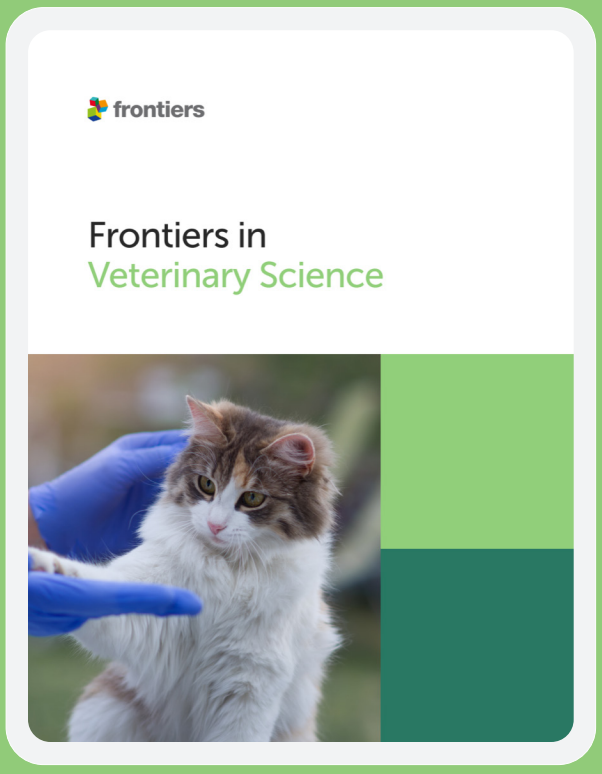
Frontiers in Veterinary Science

Transforms how we investigate and improve animal health

The third most-cited veterinary science journal, bridging animal and human health with a comparative approach to medical challenges. It explores innovative biotechnology and therapy for improved health outcomes.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact