Animal Feed Science and Technology 329 (2025) 116489

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Feeding a clay mineral-based feed additive improves performance, eating behavior and liver health by feedlot beef cattle fed a high-concentrate diet

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A R T I C L E I N F O

Keywords:
Endotoxin
Fumonisin
Hepatocyte
Cirrhosis
Nellore

A B S T R A C T

Feedlot cattle are continuously exposed to liver health challenges during the finishing period, either due to high-energy diet intake or mycotoxin presence, which can cause liver damage, impairing hepatic metabolism and overall performance. This study evaluated the impacts of adding clay mineral-based feed additive with plant extracts (CMFA) on performance, eating behavior, carcass traits, blood metabolites, and liver health of feedlot Nellore cattle. The trial was a completely randomized design, animal was considered an experimental unit. Forty-eight 22-month-old Nellore bulls (402.50 kg ± 4.95 kg) were fed in a collective pen, containing six electronic bunks with automatic selective doors for individual feeding and feed intake monitoring according to two treatments: Control (without CMFA) and CMFA (1.5 g/kg of dry matter [DM]). The study lasted 96 days. CMFA-fed animals had greater final body weight ($P = 0.04$), average daily gain ($P = 0.05$), final hot carcass weight (HCW; $P = 0.05$), and HCW daily gain ($P = 0.05$) compared to the control group. In addition, feeding CMFA altered the feeding behavior, reducing total feeding time and the number of meals, whereas increasing the DM intake rate. CMFA-fed cattle demonstrated lower aspartate aminotransferase serum concentrations and reduced histopathological scores of inflammations and liver injury, including inflammatory infiltrate, hepatitis, focal necrosis, bile stasis, and bile duct hyperplasia, demonstrating better hepatic tissue integrity. Thus, adding a clay mineral-based feed additive to a high-concentrate diets can improve performance by mitigating inflammation and improving liver health of feedlot Nellore cattle.

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https://doi.org/10.1016/j.anifeedsci.2025.116489
Received 14 April 2025; Received in revised form 30 August 2025; Accepted 1 September 2025
Available online 2 September 2025

1. Introduction

An increased proportion of concentrate in finishing diets promotes greater energy intake and propionate production in the rumen (Zinn et al., 1994; Zinn and Plascencia, 1996; Salinas-Chavira et al., 2013). However, higher grain inclusion occurs at the expense of forage levels, resulting in a fermentative challenge for the rumen and gastrointestinal tract (GIT) (Fox and Tedeschi, 2002; Bevans et al., 2005). Although feed additives can help modulate ruminal fermentation and mitigate abrupt pH declines (Nagaraja et al., 1981; Silvestre et al., 2023), increased grain intake combined with reduced chewing activity promotes the occurrence of subacute ruminal acidosis (SARA) (González et al., 2012; Holtshausen et al., 2013; Chibisa et al., 2016), bacterial death (Monteiro and Faciola, 2020), and increased intestinal permeability (Emmanuel et al., 2007; Zebeli and Metzler-Zebeli, 2012; Plaizier et al., 2022).

Metabolic disorders resulting from high-grain diets have been widely studied in the context of rumen fermentation and health (Nagaraja and Titgemeyer, 2007; Brown et al., 2006; Estevam et al., 2024; Linder et al., 2024), as well as in the etiology of liver abscesses and their association with ruminal acidosis (Nagaraja and Lechtenberg, 2007). However, a new research frontier is emerging that focuses on the gut–liver axis and its relevance to animal health and productivity (Neubauer et al., 2020; Sanz-Fernandez et al., 2020; Abeyta et al., 2023a, 2023b). Bacterial death triggered by GIT pH changes may lead to the release and translocation of microbial endotoxins from the gastrointestinal tract into systemic circulation. The liver is one of the primary affected organs, as it is responsible for metabolizing most of the molecules absorbed across the ruminal and intestinal epithelium (Plaizier et al., 2008; Monteiro and Faciola, 2020). Endotoxins such as lipopolysaccharide (LPS), released from lysed Gram-negative bacteria, have high inflammatory potential and directly impair liver function (Soppert et al., 2023), causing histopathological damage, reducing metabolic capacity, and increasing serum levels of liver-associated enzymes (Slámová et al., 2011; Lim et al., 2021; Zhou et al., 2023).

In addition to the challenge posed by endotoxins, feedlot cattle are frequently exposed to a wide range of mycotoxins present in commonly used ingredients in finishing diets (Custodio et al., 2019; Pires et al., 2025). These mycotoxins can impair ruminal, intestinal, and hepatic metabolism, as well as overall animal health, often resulting in subclinical performance losses (Gallo et al., 2015; Custodio et al., 2020; Jennings et al., 2020; Bierworth et al., 2024). To mitigate the harmful effects of endotoxins and mycotoxins, clay-based minerals have been investigated due to their ability to bind toxins and pathogenic microorganisms (Slámová et al., 2011). The inclusion of clay minerals combined with plant extracts in high-concentrate diets has proven effective in reducing lactate and biogenic a FDA (2001) mine concentrations, preserving normobiotic microbial populations, and lowering serum levels of liver-damage associated enzymes (Humer et al., 2019; Pacífico et al., 2021).

Most findings on the inclusion of clay mineral-based feed additives (CMFA) in high-concentrate diets have been obtained from studies with dairy cows (Humer et al., 2019; Pacífico et al., 2021). Although concentrate levels evaluated in those studies were high, they remain lower than those typically used in commercial feedlot systems, suggesting that the fermentative challenge faced by feedlot cattle is even greater. Therefore, this study was based on the hypothesis that CMFA supplementation in high-concentrate diets would improve productive performance, carcass traits, and liver health in feedlot cattle. Consequently, this experiment was designed to evaluate the effects of CMFA supplementation on performance, feeding behavior, carcass characteristics, blood metabolites, and liver integrity in feedlot Nellore cattle.

2. Material and methods

2.1. Experimental design and treatments

The trial was carried out at the DSM Nutritional Products Innovation Applied Science Beef Center located in Rio Brilhante, MS, Brazil. A total of forty-eight 22-month-old commercial Nellore bulls with initial body weight (BW) of 402.50 kg ± 4.95 kg were purchased from a local farm. The animals were randomly allocated in a collective pen (720 m^2, 15 m^2 per animal of pen space), containing 6 electronic bunks with automatic selective doors for individual feeding and monitoring feed intake (AF-1000 Master Gate, Betim, Brazil), one feeder to 8 animals, according to one of two treatments: Control (no addition of CFMA) and CMFA (1.5 g/kg dry matter [DM], Mycofix, DSM-Firmenich, Herzogenburg, Austria). The CMFA dosage was based on the recommendations of Gallo et al. (2020). The additive consists primarily of purified bentonite, authorized by the European Union, a mineral adsorbent with high affinity for aflatoxins, ergot alkaloids, and endotoxins (EFSA, 2011). Additionally, it includes a blend of natural bioactive components with antioxidant and hepatoprotective properties (Pacífico et al., 2021), such as seaweed meal *(Ascophyllum nodosum)*, rich in polyphenols and polysaccharides that contribute to immune modulation and oxidative stress reduction (Frazzini et al., 2024), and thistle seed expeller (*Silybum marianum)*, a source of silymarin, known for its hepatoprotective and anti-inflammatory effects (Tedesco and Guerrini, 2023). The exact proportions of the mixture are confidential, but its safety and efficacy have been demonstrated in previous studies.

2.2. Description of feeding and management

At the beginning of the study, all animals were dewormed and vaccinated (Albendathor, JA Saúde Animal, Brazil, and Ectic Pour-On, Fabiani Saúde Animal, Brazil). The bulls underwent a 15-day acclimation period in which they received the experimental diet before the trial started. Each animal was identified using an ear tag containing a unique passive transponder, which enabled unrestricted access to the feed bunk.

The trial lasted 96 days, including a 14-day step-up dietary adaptation phase, during which diets containing 64.90 % (Adaptation

01) and 71.45 % (Adaptation 02) concentrate were provided for 7 days each. This was followed by an 82-day finishing phase with a diet containing 76.50 % concentrate. Cattle were fed ad libitum twice daily, at 10:00 h (60 % of total feed) and 16:00 h (40 % of total feed), with unrestricted access to water. To facilitate ad libitum intake, the feeding protocol was designed to ensure 5–10 % feed refusals based on the initial feed weight. The experimental diets provided during the study were formulated using the Large Ruminant Nutrition System (LRNS; Fox et al., 2004), as detailed in Table 1.

2.3. Diet sampling and chemical analysis

Concentrate ingredients used in the experimental diets were analyzed weekly for dry matter (DM) content, while corn silage samples were analyzed daily by oven drying at 105°C for 24 h using a forced-air oven (AOAC, 1990). The DM content of the diets was adjusted based on observed fluctuations in ingredient DM, and monitored daily using the AOAC (1990) method, targeting 65 % DM.

Throughout the experimental period, daily samples of the offered diets were collected and frozen at –4°C to prepare composite samples. For the adaptation diets, two composite samples were prepared for each diet. For the finishing diets, daily samples were pooled monthly for chemical analyses and biweekly for mycotoxin analysis.

Particle size distribution of the composite samples was determined using the Penn State Particle Separator (Nasco, Fort Atkinson, WI, USA) as described by Heinrichs and Kononoff (1996). All composite samples were dried at 55°C in a forced-air oven until constant weight (AOAC, 1990) and ground using a hammer mill with a 1-mm screen (AOAC, 1997), for subsequent laboratory analysis.

2.3.1. Chemical composition

Monthly composite samples were analyzed for chemical composition (Table 1). Crude protein was determined according to AOAC (1997; method 990.02), neutral detergent fiber (NDF) using α-amylase, as described by Van Soest et al. (1991), and ether extract according to AOAC (1990; method 920.39). Net energy for gain was estimated using the LRNS software (Fox et al., 2004).

2.3.2. Mycotoxin analysis

Biweekly composite samples were prepared based on the frequency of new batches of ground corn, aiming to monitor potential variation in contamination. Mycotoxin analysis was performed using liquid chromatography coupled with tandem mass spectrometry via electrospray ionization (LC/ESI-MS/MS) at the Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna, Austria, following the protocols described by Sulyok et al. (2020) and Steiner et al. (2020). For the quantification of multiple metabolites, 5 ± 0.01 g of each total mixed ration (TMR) sample were used. The analytical method was validated for various complex feed matrices, including forages, grains, and TMRs, allowing for the detection of metabolites even when conjugated to carbohydrates. The detected mycotoxins are presented in Table 2, including compounds with known effects reported in the literature as well as emerging mycotoxins, grouped by their respective chemical classes

2.4. Feedlot performance, eating behavior, and carcass traits

At both the beginning and end of the study, all animals were deprived of feed and water for 14 h before the BW assessment. Additionally, the cattle were weighed on day 28 without fasting, with a 4 % shrink correction. Performance metrics were evaluated, including final BW, average daily gain (ADG), dry-matter intake (DMI) presented in kg and percentage of BW, gain-to-feed (G: F) ratio,

Table 1
Feed ingredients and chemical composition of the experimental diets fed to Nellore yearling bulls during the adaptation and finishing periods.

Item	Proportion of concentrate		
	64.90	71.45	76.50
Ingredients, % of DM[a]			
Corn silage	35.10	28.55	23.50
Corn grain fine grind	45.00	53.00	58.00
Cottonseed press cake	8.00	8.50	11.50
Soybean meal	8.00	6.20	3.10
Mineral[b]	3.90	3.75	3.90
Nutrient content[c], % of DM			
DM, %	52.70 ± 5.19	56.00 ± 4.78	65.60 ± 8.14
Crude protein	14.78 ± 0.44	13.64 ± 0.63	12.77 ± 0.76
Neutral detergent fiber	31.87 ± 2.88	26.91 ± 1.35	24.41 ± 2.91
Physically effective neutral detergent fiber	27.07 ± 2.44	20.46 ± 1.03	19.41 ± 2.31
Ether extract	1.91 ± 0.20	3.29 ± 0.35	3.39 ± 0.57
Net energy for gain[d], Mcal/kg	1.11	1.15	1.17

[a] Dry matter.
[b] Mineral contained 25.00 % of urea as a N source, as well as Ca: 11.00 %; K: 2.50 %; Mg: 1.50 %; Na: 4.20 %; P: 1.20 %; S: 2.70 %; Co: 6.00 mg/kg; Cu: 400.00 mg/kg; Cr: 5.00 mg/kg; F: 120.00 mg/kg; I: 20.00 mg/kg; Mn: 800.00 mg/kg; Se: 5.00 mg/kg; Zn: 1500.00 mg/kg; Vit. A: 125000.00 IU/kg; Vit D3: 12500.00 IU/kg; Vit E: 1300.00 IU/kg; Biotin: 67.00 mg/kg; D-Lemonene: 855.00 mg/kg; and *Saccharomyces cerevisiae*: 2.0 × 10^9 CFU/kg.
[c] Nutrient content is expressed as means ± SD (n = 3).
[d] Estimate by equations according to Large Ruminant Nutrition System (Fox et al., 2004).

Table 2
Mycotoxin content of the experimental diets during the feeding period (96 days).

Concentration µg/kg DM		Diet		
		Adaptation 01 ($n = 2$)	Adaptation 02 ($n = 2$)	Finish ($n = 6$)
Total Fumonisins	Pos. samples[a]	50	50	50
	Average[2]	34.1	115.1	76.6
	Maximum[3]	34.1	115.1	178.8
Ergot Alkaloides	Pos. samples	50	0	83.3
	Average	0.18	-	1.9
	Maximum	0.18	-	4.4
Kojic acid	Pos. samples	0	0	83.3
	Average	-	-	507.1
	Maximum	-	-	1065.8
Siccanol	Pos. samples	100	50	33.3
	Average	838.3	240.0	206.8
	Maximum	1271.8	240.0	211.4
Enniantins and Beauvericin	Pos. samples	100	100	83.3
	Average	2.2	2.4	2.4
	Maximum	3.48	3.2	3.4
Aspergillus toxins	Pos. samples	100	100	83.3
	Average	3.66	144.9	240.6
	Maximum	6.79	284.0	692.9
Penicillium toxins	Pos. samples	100	100	83.3
	Average	176.9	153.1	134.1
	Maximum	330.1	266.9	308.8
Fusarium metabolites	Pos. samples	100	100	100
	Average	788.1	133.8	149.2
	Maximum	1324.,6	236.1	415.9
Alternaria toxins	Pos. samples	100	100	83.3
	Average	34.1	82.0	46.2
	Maximum	36.6	135.2	110.1

[a] Percentage of positive tested samples; [2]Average of positive tested samples; [3]Maximum concentrations found in positive tested samples

and DMI variation, which were calculated between days 1–28, and days 1–96. The DMI was determined daily by weighing the diet provided and refusals before the next feeding delivery day. The ADG was calculated by the difference between the initial and final BW, divided by the number of feeding days for each period. The G:F ratio was measured individually as ADG divided by DMI.

The experimental diets were offered through Intergado electronic feed bunks (AF-1000 Master Gate, Betim, Brazil) which measure individual feeding behavior and feed intake daily, as described by Chapinal et al. (2007), Chizzotti et al. (2015), and Oliveira et al. (2018). Data on eating behavior were recorded individually using ID ear tags, including the time spent eating (expressed in minutes), and meals per day. A meal was defined as the uninterrupted period during which cattle consumed feed at the bunk. The average meal length, expressed in minutes, was obtained by dividing the time spent eating by meals per day. The average DMI per meal was determined by dividing the DMI by the number of meals. The time spent eating data was used to compute the eating rate of DM (time spent eating/DMI), expressed in minutes per kilogram of DM, as described by Pereira et al. (2015). Animals had ad libitum access to water from conventional drink troughs.

The 12th rib fat thickness, *Biceps femoris* fat thickness, Longissimus muscle area, and marbling were evaluated using an Aloka SSD-1100 Flexus RTU unit (Aloka Co. Ltd., Tokyo, Japan) with 17.2-cm, 3.5-MHz probe access at the end of the study according to Perkins et al. (1992). Final BW was measured at the feedlot before harvest. After the finishing period, the cattle were transported 226 km to a commercial slaughter plant. Hot carcass weight (HCW) was recorded following the removal of kidney pelvic fat. The dressing percentage was obtained by dividing HCW by the final BW. The initial dressing percentage was considered 50 %, as described by Costa et al. (2020). The HCW daily gain, the proportion of live BW gain captured as HCW (carcass transfer), and the carcass gain-to-feed ratio were calculated according to Pereira et al. (2020):

$$HCW\ daily\ gain = (Final\ HCW - Initial\ HCW)/Days\ on\ feed \quad [1]$$

$$Carcass\ trasnfer = ((Final\ HCW - Initial\ HCW)/Days\ on\ feed \times 100)/ADG \quad [2]$$

$$Carcass\ gain\ to\ feed\ ratio = Total\ DMI/((Final\ HCW - Initial\ HCW)/15) \quad [3]$$

2.5. Blood metabolites, and liver health and function

Blood samples were collected one day before the start of the trial and at day 96 from the tail vein into two 10 mL evacuated sterile sampling tubes containing a clot activator and silica (BD Vacutainer, Franklin Lakes, NJ, USA) for serum analysis. The serum samples were allowed to clot at room temperature before undergoing centrifugation. All samples were centrifuged at 2500 × g for 15 min and subsequently stored at −20 °C until analysis. Serum samples were sent to the College of Veterinary Medicine and Animal Science (Botucatu, São Paulo, Brazil) for analysis.

The level of total plasma proteins was measured using a biuret colorimetric test (Total Proteins Monoreagent K031, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), cholesterol was measured by colorimetric enzymatic test (Colesterol Monoreagente K083, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), urea was measured by fixed-time kinetic determination (Ureia UV K056, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), creatinine was measured by time-kinetic colorimetric test fixed with the formation of alkaline pictrate (Creatinina K016, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), and albumin was measured by colorimetric test using bromocresol green (Albumina Monoreagente K040, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil). Serum levels of liver enzymes were measured by determination UV kinetics, aspartate aminotransferase (AST; Transaminase AST Cinética K048, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), gamma-glutamyl aminotransferase (GGT; Gama GT Cinético K080, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), alanine aminotransferase (ALT; Transaminase ALT Cinética K049, Bioclin, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil).

At slaughter, livers were assessed for the presence of abscesses and were classified (Brink et al., 1990). During evisceration, a fragment of each liver was collected and placed into 10 % formaldehyde, and the fragments were fixed, processed, and stained using the hematoxylin-eosin technique according to Luna (1968). The slides were subsequently prepared for analysis using a Leica Qwin Image Analyzer with a Leica electron light microscope and evaluated by a trained veterinarian. Liver injury was categorized based on the incidence of lesions following the classification system established by Vince et al. (2014), employing a scale of 0–3, where 0 indicates no injury, 1 signifies minor injury, 2 denotes moderate injury, and 3 represents severe injury. For each liver, individual histopathological characteristics were scored according to this scale. The mean score for each specific lesion characteristic was then calculated for each treatment group. Additionally, a 'Total Injury Incidents' score was determined for each animal by summing the scores of all individual lesion characteristics assessed. These individual sums were then averaged per treatment group to represent the overall liver injury incidence.

Table 3
Impacts of adding clay mineral-based feed additive in high-concentrate diets on feedlot performance and carcass traits of Nellore cattle.

Item	Control	CMFA[1]	SEM[11]	*P*-value
Feedlot performance				
Initial BW[2], kg	401.60	403.40	4.95	0.80
1–28 days				
Final BW, kg	444.30	450.00	2.20	0.08
Average daily gain, kg	1.49	1.69	0.08	0.08
Dry matter intake, kg	10.83	11.08	0.21	0.40
Dry matter intake, % of BW	2.56	2.60	0.05	0.52
G:F[3] ratio, kg/kg	0.136	0.154	0.007	0.07
1–96 days				
Final BW, kg	545.00	561.00	5.32	0.04
Average daily gain, kg	1.50	1.66	0.06	0.05
Dry matter intake, kg	11.42	11.89	0.22	0.14
Dry matter intake, % of BW	2.41	2.47	0.04	0.29
G:F ratio, kg/kg	0.131	0.140	0.004	0.11
Dry matter intake variation, kg	1.90	1.88	0.05	0.77
Carcass traits				
Initial HCW[4,5], kg	208.00	209.00	2.35	0.80
Final HCW, kg	308.00	317.00	3.29	0.05
HCW daily gain[6], kg	1.05	1.15	0.03	0.05
Final dressing percentage, %	56.41	56.58	0.27	0.66
Carcass transfer[7], %	70.77	69.14	1.34	0.39
Carcass G:F ratio[8], kg/kg	157.00	148.00	3.68	0.12
Final 12th rib fat, mm	8.01	7.65	0.37	0.49
Final BF[9] fat thickness, mm	4.89	5.15	0.21	0.40
Final LM[10] area, cm^2	89.79	92.15	2.07	0.42
Final marbling, %	2.77	2.87	0.12	0.54

[1] Clay mineral-based feed additive 1.5 g/ kg DM (Mycofix®; DSM-Firmenich, Herzogenburg, Austria)
[2] Body weight.
[3] Gain to feed.
[4] Hot carcass weight.
[5] Initial dressing percentage was estimated according to Costa et al. (2020).
[6] HCW daily gain= (Final HCW–Initial HCW)/Days on feed.
[7] Carcass transfer = ((Final HCW–Initial HCW)/Days on feed × 100)/ADG.
[8] Carcass gain to feed ratio = Total DMI/((Final HCW–Initial HCW)/15).
[9] Biceps femoris.
[10] Longissimus muscle.
[11] Values presented as mean ± standard error of the mean (SEM). 95 % Confidence Intervals (95 % CI) for the means were calculated using a critical t-value of 2.069 (for 23 degrees of freedom, n = 24 per treatment).

2.6. Statistical analysis

The trial was a completely randomized design, with animals designated as the experimental unit (n = 48), and each treatment was replicated 24 times. The statistical model included animals as a random effect. Tests for normality were confirmed before analyzing data. Normality was assessed using SAS 9.4 (SAS Inst., Inc., Cary, NC) PROC UNIVARIATE procedure, where normal distribution was considered based on a Shapiro-Wilk score. Data were analyzed by ANOVA using the MIXED procedure of SAS. For all statistical approaches, differences were considered significant when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$. Effects sizes for all outcomes were quantified using Cohens'd.

To interpret the results, individual P-values for each dependent variable were presented without adjustment for multiple comparisons across the family of outcomes. This decision was based on the recognition that many of the assessed parameters (e.g., various blood metabolites, histopathological scores, and performance variables) represent distinct, yet biologically interconnected, aspects of the animal's response, where the primary objective is to identify potential effects on each specific physiological marker. Presenting individual P-values, in conjunction with Cohen's d, provides insight into both the statistical significance and the practical magnitude of the observed differences. Readers are encouraged to interpret these findings considering the increased likelihood of Type I errors when multiple tests are performed.

3. Results

3.1. Feedlot performance, carcass traits, and eating behavior

Initial BW, DMI in kg, and expressed as a percentage of BW, from 1 to 28 days on feeding, also DMI in kg, and expressed as a percentage of BW, G:F ratio, and DMI variation from 1 to 96 days on feeding were not affected ($P > 0.10$; Table 3) by adding CMFA. However, Nellore bulls fed CMFA showed a tendency to have greater final BW ($P = 0.08$) with a medium effect size according to Cohen's d ($d = 0.53$), ADG ($P = 0.08$; $d = 0.51$), and G:F ratio ($P = 0.07$; $d = 0.52$) from 1 to 28 days on feeding. The moderate magnitude of effects between treatments, as indicated by Cohen's d, suggests that despite the lack of statistical significance ($P > 0.05$), the biological relevance of the improvements in BW, ADG, and G:F ratio during the initial period may be meaningful.

Cattle fed CMFA had greater final BW ($P = 0.04$; $d = 0.61$), and ADG ($P = 0.05$; $d = 0.54$) when compared to the control group. Specifically, the overall ADG for the CMFA group averaged 1.66 kg/day (95 % CI: 1.54–1.78 kg/day), compared to 1.50 kg/day (95 % CI: 1.38–1.62 kg/day) for the Control group. Carcass characteristics are summarized in Table 3. Initial HCW, final dressing percentage, carcass transfer, carcass G:F ratio, 12th rib fat, *Biceps femoris* fat thickness, Longissimus muscle, and marbling were not affected ($P > 0.11$) by adding clay-minerals feed additive. Nevertheless, cattle fed CMFA had greater final HCW ($P = 0.05$; $d = 0.56$) and HCW daily gain ($P = 0.05$; $d = 0.68$) when compared to the control group. The average HCW for the CMFA was 317 kg (95 % CI: 310.19 – 323.81 kg), whereas the Control averaged 308 kg (95 % CI: 301.19 – 413.81 kg). Adding CMFA did not affect meals per day ($P = 0.85$; Table 4). Nellore bulls fed CMFA had less time eating ($P < 0.01$), meals per day ($P = 0.03$), and had a faster eating rate of DM ($P < 0.01$) when compared to the control group. However, cattle in the control group had less DMI per meal ($P = 0.01$) compared to those fed CMFA.

3.2. Blood metabolites, and liver health

Urea, creatinine, albumin, total protein, cholesterol, ALT, and GGT were not affected ($P > 0.17$; Table 5) by adding CFMA. However, Nellore bulls fed CMFA had lower AST concentration ($P = 0.02$; $d = -0.84$), compared to the control group. AST levels were 79.42 UI/L (95 % CI: 71.43 – 87.41 UI/L) for the CMFA, significantly lower than the 95.34 UI/L (95 % CI: 87.35 – 103.33 UI/L) observed in Control. No liver abscess was found in the animals evaluated in this study. Fibrosis and steatosis/vacuolation were not affected ($P > 0.25$; Table 5) by adding a clay-minerals feed additive. Cattle fed CMFA showed a lower incidence of liver lesion (Fig. 1), including reduced inflammatory infiltrate ($P = 0.05$; $d = -0.56$), hepatitis ($P = 0.04$; $d = -1.46$), focal necrosis ($P = 0.01$; $d = -1.49$), bile stasis ($P = 0.03$; $d = -1.35$), bile duct hyperplasia ($P = 0.03$, $d = -0.90$), and total injury incidents ($P < 0.01$; $d = -1.23$), compared with the control group.

To provide further clarity on these key findings, for hepatitis, the CMFA group averaged 0.00 (95 % CI: 0–0.31) versus 1.08 (95 %

Table 4
Impacts of adding clay mineral-based feed additive in high-concentrate diets on feeding behavior of Nellore cattle.

Item	Control	CMFA[1]	SEM[3]	*P*-value
Time spent eating, min	77.75	61.26	3.90	< 0.01
Meal length, min	1.43	1.15	0.08	0.03
Meals per day, n	59.29	58.75	2.02	0.85
Dry matter intake per meal, kg	0.221	0.251	0.008	0.01
Eating rate of DM[2], min/kg of DM	7.17	5.27	0.40	< 0.01

[1] Clay mineral-based feed additive 1.5 g/ kg DM (Mycofix®; DSM-Firmenich, Herzogenburg, Austria)
[2] Dry matter.
[3] Values presented as mean ± standard error of the mean (SEM). 95 % Confidence Intervals (95 % CI) for the means were calculated using a critical t-value of 2.069 (for 23 degrees of freedom, n = 24 per treatment).

Table 5
Impacts of adding clay mineral-based feed additive in high-concentrate diets on blood metabolites and liver injury of Nellore cattle.

Item	Control	CMFA[1]	SEM[3]	*P*-value	*Cohen's d*
Blood metabolites					
Urea, mg/dL	35.65	32.54	1.58	0.18	−0.40
Creatinine, mg/dL	2.04	2.01	0.08	0.78	−0.08
Albumin, mg/dL	2.37	2.33	0.56	0.62	−0.02
Total proteins, mg/dL	6.29	6.17	1.09	0.44	−0.02
Cholesterol, mg/dL	174.44	154.64	9.60	0.18	−0.42
Alanine aminotransferase, U/L	29.00	27.28	1.18	0.32	−0.30
Aspartate aminotransferase, U/L	95.34	79.42	3.87	0.02	−0.84
Gamma-glutamyl aminotransferase, U/L	17.92	19.84	1.35	0.37	0.29
Liver injury[2]					
Inflammatory infiltrate	1.08	0.75	0.12	0.05	−0.56
Portal inflammation	1.00	0.75	0.10	0.09	−0.51
Hepatitis	1.08	0	0.15	0.04	−1.46
Focal necrosis	0.58	0	0.08	0.01	−1.49
Confluent necrosis	0.25	0	0.05	0.07	−1.00
Fibrosis	0.67	0	0.19	0.26	−0.72
Steatosis/Vacuolation	1.58	1.42	0.19	0.55	−0.17
Bile stasis	0.33	0	0.05	0.03	−1.35
Bile duct hyperplasia	0.83	0.08	0.17	0.03	−0.90
Cirrhosis	0.25	0	0.05	0.07	−1.02
Total injury incidents	8.42	4.25	0.69	< 0.01	−1.23

[1] Clay mineral-based feed additive 1.5 g/ kg DM (Mycofix®; DSM-Firmenich, Herzogenburg, Austria)
[2] Liver lesion were scored on a 0 – 3 scale (0 = no injury, 1 = mild, 2 = moderate, 3 = severe), based on the criteria described by Vince et al. (2014).
[3] Values presented as mean ± standard error of the mean (SEM). 95 % Confidence Intervals (95 % CI) for the means were calculated using a critical t-value of 2.069 (for 23 degrees of freedom, n = 24 per treatment).

CI: 0.92–1.24) for the Control group. Focal necrosis was also absent in the CMFA group (0.00; 95 % CI: 0–0.17) compared to the Control group (0.58; 95 % CI: 0.41–0.75). The total injury incidents were 4.25 (95 % CI: 2.87–5.63) in the CMFA group, significantly lower than 8.42 (95 % CI: 7.04–9.80) in the Control group. Additionally, a tendency for lower portal inflammation (P = 0.09; d=−0.51), confluent necrosis (P = 0.07; d=−1.00), and cirrhosis (P = 0.07; d=−1.02) was in CMFA-fed cattle. The absence of lesions (mean score of zero) in the CMFA group for confluent necrosis (0.00; 95 % CI: 0–0.10), bile stasis (0.00; 95 % CI: 0–0.10), and cirrhosis (0.00; 95 % CI: 0–0.10) further supports the beneficial effects of CMFA.

4. Discussion

We hypothesized that the inclusion of a clay mineral feed additive improves overall performance, carcass characteristics, and liver health of feedlot Nellore cattle fed a high-concentrate diet. This hypothesis was based on the premise that clay minerals can chelate toxins in the intestinal lumen (Pacífico et al., 2021), while the plant extracts that compose the additive may assist in liver protection and recovery, preventing and reversing the negative impacts of disorders associated with high concentrate feeding.During the feedlot period, cattle face significant metabolic challenges that can impair performance and carcass traits (Brown et al., 2006; Estevam et al., 2020; Pereira et al., 2020). The transition from a forage-rich diet to a high-concentrate diet increases the risk of metabolic disorders, as it not only alters ruminal fermentation patterns and increases the risk of SARA, but also favors increased passage of undigested starch to the GIT, especially when using flint corn, which has lower ruminal degradability (Philippeau et al., 1999; Neubauer et al., 2020). This may alter intestinal fermentation, promote dysbiosis, and increase the release of endotoxins resulting from bacterial lysis, intensifying hepatic exposure to inflammatory agents and stress inducers (Gozho et al., 2006; Sanz-Fernandez et al., 2020).

Mycotoxin ingestion represents an additional risk factor for animal health and productivity, potentially acting as cofactors in the development of metabolic disorders associated with GIT acidification, compromising intestinal epithelial integrity and favoring the translocation of toxins and pathogenic microorganisms into the bloodstream (Baines et al., 2011; Dong et al., 2011). Additionally, mycotoxins impair ruminal and intestinal microbiota (Santos and Fink-Gremmels, 2014; Gallo et al., 2015), reduce nutrient digestibility (Shi et al., 2022) and short-chain fatty acid production (Sarich et al., 2022), and induce hepatic damage and immune suppression (Garret et al., 1968; Zain, 2011; Custodio et al., 2020).

In the present study, feed mycotoxin contamination was monitored periodically. However, low and intermittent contamination levels were detected. Fumonisin was the most frequently identified mycotoxin; nevertheless, concentrations were below the maximum limits established by the FDA (2001); and the European Comission (2006), as well as reference values proposed by Osweiler et al. (1993) and Jennings et al. (2020), indicating that levels below 120 mg/kg DM do not impair animal performance even under prolonged exposure. Ergot alkaloids, known to reduce DMI, ADG, and induce hyperthermia at concentrations above 0.75 mg/kg DM (Sarich et al., 2023; Bierworth et al., 2024), were also detected; however, their presence was limited to low levels and not consistent throughout the experimental period. Furthermore, some diet samples contained secondary metabolites and emerging mycotoxins such as kojic acid, sicanol, enniatins, and beauvericin; however, the toxic effects of these compounds are not fully elucidated, with data restricted to in vitro cell culture studies suggesting these substances may exert antimicrobial activity, induce oxidative stress, and

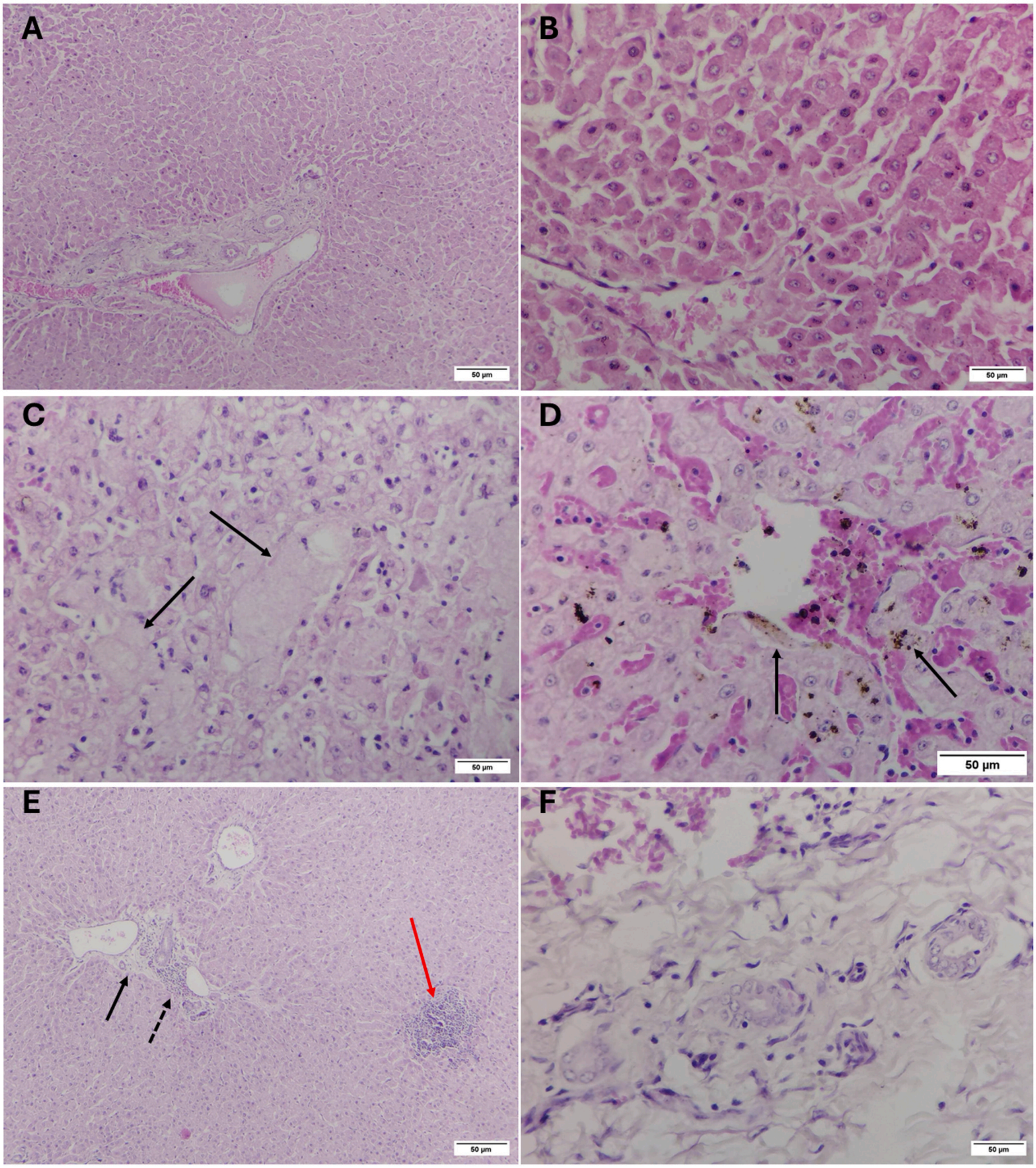

Fig. 1. Liver samples were collected at the time of slaughter, slides stained with hematoxylin and eosin (H&E). Representative image of healthy liver tissue and the main lesions found. Healthy hepatic tissue, original magnification, 20X; (B) Healthy hepatic tissue, original magnification, 40 X; (C) Hepatic tissue with foci of necrosis (arrow), original magnification, 40X; (D) Hepatic tissue with foci of bile stasis (arrow), original magnification, 40X; (E) Hepatic tissue with focus of biliary duct hyperplasia (continuous arrow), portal inflammation (dashed arrow) and hepatitis (red arrow), original magnification, 20X; (F) Biliary duct hyperplasia in the hepatic tissue, original magnification, 40X.

trigger apoptosis (Krizoová et al., 2021; Suthiram et al., 2023; de Sá et al., 2024). Nonetheless, further research is needed to determine their impact on ruminants under practical production conditions.

The CMFA used in this study consists of a combination of mineral adsorbents and plant extracts, with potential to mitigate adverse effects associated with high-concentrate diets. Previous studies have shown that supplementation with additives containing clay minerals can increase intestinal microbiota diversity, reduce serum levels of inflammatory metabolites, and improve liver function in ruminants fed starch-rich diets. These effects are attributed to the adsorptive capacity of clay minerals against deleterious microbial metabolites, as well as the hepatoprotective properties of plant extracts (Pacífico et al., 2021; Humer et al., 2019; Neubauer et al., 2019). In this context, the tendency accompanied by a moderate effect size for higher BW, ADG, and improved G:F ratio observed from day 1 to day 28 of the feedlot period suggests that additive inclusion may have contributed to reducing initial stress and promoting a more efficient dietary transition (Humer et al., 2019), although inflammatory metabolites were not directly measured in this study. Furthermore, the additive promoted greater ADG and final BW during the 96-day confinement period, also resulting in higher HCW, and daily HCW gain, effects that may be related to attenuation of inflammatory processes and improved liver integrity, as indicated by reduced serum AST concentrations and lower incidence of liver lesions in histological analyses. Our results were consistent with the findings of Pacífico et al. (2021), who reported similar effects in dairy cows fed high-concentrate diets supplemented with clay minerals; to our knowledge, the present study is the first demonstration representing beef cattle feedlot systems.

Elevated serum levels of liver enzymes such as AST, GGT, and ALT are widely recognized as indicators of compromised liver function associated with hepatocellular damage of various etiologies (Giannini et al., 2005; Moreira et al., 2012). Mycotoxin ingestion is known to cause such hepatocellular damage and increase serum concentrations of AST, GGT, and urea (Fink-Grammels, 2008; Chaiyotwittayakun, 2010; Ashiq, 2015). However, the mycotoxin contamination levels observed in this study were considerably lower than those reported in the literature as harmful to liver function. Exposure to endotoxins and inflammatory mediators resulting from GIT acidification also causes hepatic inflammation and increased serum liver enzyme concentrations (Zhou et al., 2023). Humer et al. (2019) and Pacífico et al. (2021) demonstrated that CMFA use can mitigate these adverse effects by reducing serum levels of liver enzymes and inflammatory markers, especially under high-energy dietary conditions.

Higher grain consumption is associated with the etiology of liver abscesses, which result from the translocation of pathogenic microorganisms such as *Fusobacterium necrophorum* and *Trueperella pyogenes* from the ruminal environment to the bloodstream through lesions in the ruminal epithelium caused by ruminal acidosis (Nagaraja and Lechtenberg, 2007; Broadway et al., 2024), with well-established effects on animal performance (Batista and Holland, 2022). No occurrence of liver abscesses was observed in any treatment in the present study. However, it is important to highlight that endotoxemia, although less explored, can trigger acute inflammatory responses capable of compromising liver tissue (Soppert et al., 2023). Endotoxins can be released both in the rumen and large intestine under acidosis conditions (Plaizier et al., 2008; Khafipour et al., 2009; Monteiro and Faciola, 2020). However, endotoxin occurrence in the intestinal lumen has gained increasing attention, since intestinal epithelial integrity is more fragile, facilitating the translocation of these toxins into the bloodstream (Abeyta et al., 2023b).

The literature consistently describes the capacity of endotoxins to induce oxidative stress and morphological alterations in liver tissue, including inflammation of hepatocytes and bile ducts, apoptosis, cellular necrosis, and increased serum AST and ALT levels (Lim et al., 2021; Zhou et al., 2023; Babu and Mohanty, 2023). Endotoxemia, mainly triggered by LPS, leads to a cascade of effects that impair cellular antioxidant capacity, increasing concentrations of malondialdehyde, nitric oxide, and pro-inflammatory cytokines, thereby inducing an acute inflammatory response. This response promotes infiltration of neutrophils, macrophages, and lymphocytes into the affected tissue, exacerbating tissue damage (Zhao et al., 2021).

In the present study, feeding CMFA resulted in better histological integrity of liver tissue, combined with lower presence of inflammatory infiltrates, absence of necrosis, and reduced degree of bile duct hyperplasia, corroborating the lower serum AST levels observed. These interrelated indicators suggest a better response to oxidative and inflammatory challenge. These effects may be attributed to the adsorptive capacity of bentonites, but also to the presence of plant extracts from *Silybum marianum* and *Ascophyllum nodosum*, active components of CMFA with antioxidant and anti-inflammatory properties (Rašković et al., 2011), capable of increasing the activity of CYP complex enzymes involved in toxin metabolism and reducing the expression of inflammatory cytokines (Tian et al., 2019), as well as reducing nitric oxide and reactive oxygen species synthesis (Liang et al., 2021), and caspase activation — key enzymes responsible for triggering apoptotic processes (Surai, 2015), thus improving liver health and reducing the incidence of liver lesions (Guo et al., 2016; Al-Kadi et al., 2020; Zhao et al., 2021).

The histological findings of the present study are promising and demonstrate the benefits of CMFA supplementation on liver health in confined cattle. To our knowledge, this is the first report demonstrating that supplementation with CMFA in high-concentrate diets can improve liver health by reducing inflammation and liver lesions in confined cattle. Such evidence may be associated with a reduction in energy expenditure required for inflammatory processes (Sanz-Fernandez et al., 2020; Gouvêa et al., 2022; Horst et al., 2025), as well as an improvement in hepatic detoxification capacity (Humer et al., 2019), which helps explain the better performance observed in supplemented animals. Although, to date, no previous studies have evaluated the effects of clay mineral-based feed additives on feeding behavior responses in feedlot cattle, we observed that animals receiving the additive had higher feed intake — albeit only numerically — as well as greater feed intake per meal, consistent with their improved productive performance.

5. Conclusion

The present study provides evidence that adding a clay mineral-based feed additive, containing specific plant extracts such as Ascophyllum nodosum and Silybum marianum, to high-concentrate diets can be an effective nutritional strategy to enhance performance and support liver health in feedlot cattle.

CRediT authorship contribution statement

D.I.C.G. Gouvêa: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **M.B. Niehues**: Writing – review & editing, Methodology, Formal analysis. **V.V. Carvalho**: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **A. Perdigão**: Writing – review & editing, Supervision, Project administration, Formal analysis. **J. Faas**: Writing – review & editing, Validation. **I. Artavia**: Validation, Conceptualization. **M.C.S. Pereira**: Writing – review & editing. **M.D.B. Arrigoni**:

Writing – review & editing, Validation, Supervision, Methodology. **C.L. Martins**: Writing – review & editing, Validation, Project administration, Methodology.

Ethics approval

All animal procedures in this study adhered to the guidelines set forth by the I&AS Beef Center of DSM Nutritional Products Ethical Committee for Animal Research (protocol number: 003/21).

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

Declaration of Competing Interest

V. V. de Carvalho, A. Perdigão, J. Fass, and I. Artavia are currently employed by DSM-Firmenich. The other authors declare no conflict of interest.

Acknowledgment

This study is part of the dissertation presented to the São Paulo State University "Júlio de Mesquita Filho" (UNESP), Botucatu in compliance with the requirements for the master's degree in animal science by D. I. C. G. Gouvêa (2023). We thank you "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)" and DSM-Firmenich (São Paulo, Brazil) for financially supporting this project.

Financial support statement

"This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001". This study was supported by DSM-Firmenich (São Paulo, Brazil).

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