



OPEN Nutritional protocols for improving meat quality of feedlot Nellore bulls differing in marbling estimated progeny

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This study aimed to assess the effects of nutritional technologies on marbling precursors in feedlot cattle and their impact on meat quality. One hundred and fifty Nellore bulls with an initial body weight of 403.98 ± 23.82 kg were classified according to their estimated progeny difference (EPD) for marbling and blocked into high or low EPD groups. The bulls were randomly allocated into 30 pens and fed for 112 days in a $2 \times 2 + 1$ factorial arrangement of treatments: (1) Finely-ground corn (FGC); (2) High-moisture corn (HMC); (3) Finely-ground corn + calcium salts of fatty acids (FGC + CSFA); (4) High-moisture corn + calcium salts of fatty acids (HMC + CSFA); (5) High-moisture corn + calcium salts of fatty acids + organic zinc and chromium (HMC + CSFA + ZnCr). On day 0, one animal per pen ($n = 30$) was slaughtered to establish baseline meat quality, while the remaining 120 animals were fed until slaughter. Samples of *longissimus muscle*, 6th to 12th ribs, and its subcutaneous and intramuscular fat were collected for proximate composition, pH, shear force, cooking loss, lipid oxidation, color stability, fatty acid profile, and sensory evaluation. Bulls with high marbling EPD fed HMC + CSFA exhibited greater intramuscular fat ($P = 0.03$) and cooking loss ($P < 0.01$), whereas those with low EPD showed higher protein ($P < 0.01$) and collagen content ($P < 0.01$). Meat aged for 14 days demonstrated improved tenderness ($P < 0.05$), although treatments did not influence Warner–Bratzler shear force. Lipid oxidation ($P < 0.01$) and color variation (ΔE , $P < 0.05$) increased during retail display with ZnCr supplementation. Fatty acid profiles (SFA, MUFA, PUFA) were significantly affected by EPD $\times$ treatment interactions ($P < 0.05$). Sensory evaluation indicated improved tenderness and overall satisfaction with CSFA supplementation, regardless of corn processing ($P < 0.05$). In summary, classification by marbling EPD allowed to demonstrate that genetic predisposition interacts with nutritional strategies, highlighting that HMC and CSFA promoted intramuscular fat deposition in high EPD bulls. In contrast, ZnCr supplementation improved oxidative stability and meat color without enhancing marbling.

Keywords Adipose tissue, Chromium, Zinc, Fatty acid profile, Rumen-protect fat

The Nellore breed represents the majority of the Brazilian cattle herd. It is characterized by exceptional muscle deposition but low intramuscular fat content and marbling scores^{1,2}, making it well-suited for feedlot finishing. However, due to high-energy diets being typically offered over relatively short finishing periods, averaging 106 days³, these strategies may not substantially improve meat quality traits.

Improving meat quality requires selecting cattle with favorable genotypes and phenotypes within the breed. Marbling is a complex trait influenced by multiple genes, and gene expression differences may affect adipose tissue deposition in *Bos indicus*^{4–6}. Intramuscular fat deposition is generally low in Nellore cattle⁷ and is associated

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with specific metabolic pathways involved in lipid metabolism⁸. Nevertheless, recent research suggests that the genetic selection strategies applied in Nellore cattle have yielded satisfactory outcomes in muscle growth and backfat deposition⁷.

Several strategies have recently been explored to enhance intramuscular fat deposition in cattle. Among these, one approach is genetic selection, as the expected progeny difference (EPD) for marbling offers a promising tool for improving this trait in *Bos indicus* breeds. Given that marbling is a moderate to highly heritable characteristic⁹, selective breeding may promote greater intramuscular fat deposition in Nellore cattle.

Another complementary strategy is dietary manipulation. Increasing grain inclusion combined with the extensively processed grain content in the diet can enhance glucose availability to adipocytes, thereby favoring intramuscular fat deposition. Since intramuscular fat biosynthesis is closely associated with glucose availability¹⁰, high-concentrate ingredients, such as high-moisture corn (HMC), can enhance starch fermentability in the rumen. This process increases the production of short-chain fatty acids, particularly propionate, a key precursor for gluconeogenesis in ruminants¹¹. In parallel, rumen-protected fat, such as calcium salts of fatty acids (CSFA), has gained attention in meat quality enhancement programs. Beyond increasing the energy density of the diet, CSFA can modulate the fatty acid composition of meat and enhance glucose uptake by intramuscular adipocytes, thereby contributing to marbling development^{12,13}.

Beyond genetic and nutritional strategies, mineral supplementation has also gained attention as a potential tool to enhance intramuscular fat deposition. Zinc, for instance, is essential for DNA and nucleic acid synthesis and has been shown to stimulate the activity of glycerol-3-phosphate dehydrogenase in intramuscular preadipocytes^{14,15}. Chromium, on the other hand, enhances insulin sensitivity in insulin-responsive tissues¹⁶, facilitating glucose uptake^{17,18}, which may also influence immune function¹⁹.

Despite these promising roles, studies evaluating the interaction between EPD for marbling and feedlot protocols, associating different grain processing methods, CSFA, and zinc or chromium supplementation, remain limited for Nellore cattle^{20–22}. This study was designed to test three hypotheses: (1) Feeding HMC increases subcutaneous and intramuscular fat deposition in Nellore cattle meat; (2) Including CSFA in the diet elevates the concentration of unsaturated fatty acids in subcutaneous and intramuscular adipose tissue, thereby enhancing meat marbling; and (3) Supplementation with organic Zn and Cr improves the marbling scores and meat quality of Nellore cattle. Consequently, the objective of this study was to evaluate different nutritional strategies, including HMC, CSFA, organic zinc, and chromium, in the diets of feedlot Nellore cattle, categorized by their EPD for marbling (high vs. low), and assess their impact on meat quality.

Materials and methods

All procedures and protocols involving animals in this study were approved by the Ethical Committee for Animal Research of the São Paulo State University (UNESP), Dracena campus (protocol CEUA 0151/2019). All methods were performed in accordance with the relevant guidelines and regulations.

Animals and treatments

The study was conducted at the São Paulo State University (UNESP) feedlot facility, Dracena Campus, Brazil. A total of 150 Nellore bulls (18 months old, with an initial body weight of 403.98 ± 23.82 kg) were enrolled in the study. At the start of the experiment, 30 bulls were harvested to serve as a baseline reference for subsequent analyses ($n = 1$ bull per pen), while the remaining 120 were housed in 30 pens (4 bulls per pen; 18 m² of pen space per animal and 1.5 m of linear bunk space).

Bulls were from a commercial farm located in Bataguassu, Mato Grosso do Sul State, Brazil, which has been selecting Nellore bulls for three decades. The rancher borrowed the bulls for the university for this study. Before the start of the experiment, marbling data were obtained by ultrasound on day −4, along with EPD values for animals whose parents had been evaluated. These values were used to classify the animals into high and low EPD for marbling. The experimental treatments were planned in a $2 \times 2 + 1$ factorial arrangement as follows: (I) FGC – Finely-ground corn; (II) HMC – High-moisture corn; (III) FGC + CSFA – Finely-ground corn with calcium salts of fatty acids (NutriGordura; Nutricorp Animal Nutrition; Araras, São Paulo, Brazil); (IV) HMC + CSFA – High-moisture corn with the inclusion of calcium salts of fatty acids; (V) HMC + CSFA + ZnCr – High-moisture corn with the inclusion of calcium salts of fatty acids and organic zinc and chromium (Zinpro Animal Nutrition; Piracicaba, São Paulo, Brazil). Sodium monensin (Rumensin 200; Elanco Animal Health, São Paulo, São Paulo, Brazil) was included in all treatments at 25 ppm.

Feeding and management description

At the beginning of the study, all bulls were dewormed and vaccinated against tetanus, bovine viral diarrhea virus, and *Clostridium* spp. (7-way; CattleMaster and Bovishield, Pfizer Animal Health, New York, NY). Cattle were fed ad libitum twice daily at 08h00 (45% of the total ration) and 16h00 (55% of the total ration), with free access to water from a trough (3.00 × 0.80 × 0.20 m).

The study lasted 112 days, including a 14-day adaptation period (Fig. 1). The experimental diets consisted of sugarcane bagasse, *Cynodon dactylon* hay, FGC, HMC, 132 soybean meal, sodium chloride, limestone, mineral supplement, and urea. Experimental diets were formulated according to the Large Ruminant Nutrition System²³ and are shown in Table 1. The fatty acid (FA) profile of the CSFA is presented in Table 2.

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 69%, 74%, and 79% concentrate and were fed for 5, 4, and 5 days, respectively.

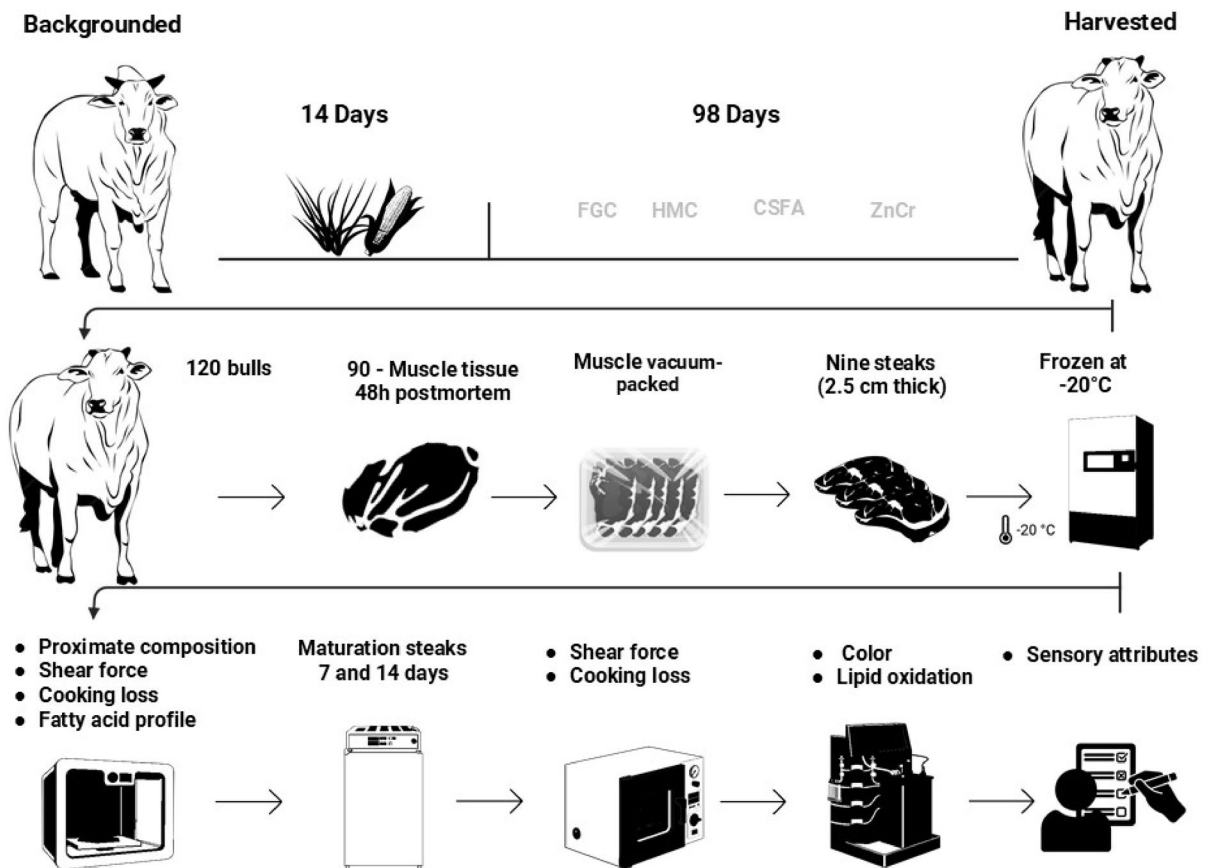


Fig. 1. Schematic diagram of feeding regime for cattle subjected to 5 diets with FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium, and subsequent analyses.

Slaughter and sample collection

On day 0, one animal per pen ($n=30$) was slaughtered to evaluate baseline meat quality. The remaining 120 animals underwent a 112-day feeding period. After 112 days on trial, the cattle were slaughtered at a commercial slaughterhouse (Naturafrig Alimentos, Pirapozinho, SP, Brazil) in accordance with standard industry procedures and good animal welfare practices. Stunning was performed with a penetrating captive bolt device, followed by exsanguination. The carcasses were chilled at 4 °C for 48 h, and samples of the *longissimus muscle* (NAMP #180; Beef Loin, Strip Loin) were randomly collected from 60 animals ($n=2$ per pen; 6 pens and 12 animals per treatment) between the 6th and 12th ribs of the left carcass. In total, 90 animals had meat samples collected for analysis. The samples were vacuum-packed and transported to the Meat Laboratory at the University of Campinas. The *longissimus muscle* was cut into nine steaks (2.5 cm thick). The first three steaks were immediately frozen at -20 °C and analyzed for proximate composition, pH, shear force, and fatty acid profile. The remaining steaks were randomly assigned to aging treatments at 2 °C for 7, and 14 days and analyzed for shear force, cooking loss, color, lipid oxidation, and sensory attributes (Fig. 1).

Centesimal composition and pH

The crude protein, moisture, lipid, sodium, collagen, and ash contents of the *longissimus muscle* were determined using a FoodScan™ device (FOSS North America, Eden Prairie, MN, USA)²⁴. Briefly, 180 g of shredded meat was placed into a 140-mm round plate and scanned, with results expressed as g/100 g. Meat pH was measured using a Sentron SI600 digital pH meter, calibrated with pH 4.0 and 7.0 buffers at room temperature.

Shear force and cooking loss

Shear force was assessed using the Warner-Bratzler Square Shear Force (WBSF) method²⁵. Steaks were thawed for 24 h at 2 °C and cooked in an electric oven (Imequi, Brazil) at 170 °C. The internal temperature was monitored using copper-constantan thermocouples (E5CWL Omron, CSW) inserted into the geometric center of each steak until reaching 71 °C. Cooked steaks were chilled overnight at 4 °C, and cooking loss was determined by weighing the raw and cooked steaks²⁶. Six round cores (1.27 cm diameter) were extracted parallel to the muscle fibers from each steak. Each core was shared at the center using a Bratzler blade attached to a Texture Analyzer (TA-XT Plus, Texture Technologies Corp./Stable Micro Systems, UK) with a 250 mm/min crosshead speed.

Treatments ¹	1	2	3	4	5
Ingredients, % DM					
Hay	3.00	3.00	3.00	3.00	3.00
Sugarcane bagasse	13.00	13.00	13.00	13.00	13.00
Soybean meal	10.71	10.01	10.81	10.41	10.41
Moist corn grain silage	-	51.00	-	48.00	48.00
Finely ground corn	70.30	20.00	67.60	20.00	20.00
Urea	0.70	0.70	0.70	0.70	0.70
Mineral premix	0.98	0.98	0.98	0.98	0.86
Mineral salt	0.30	0.30	0.30	0.30	0.30
Calcium salts of fatty acids	-	-	3.20	3.20	3.20
Dietary calcium	0.60	0.60	-	-	-
Calcium	0.40	0.40	0.40	0.40	0.40
Zn120	-	-	-	-	0.075
Cr1000	-	-	-	-	0.045
Rumensin	0.0125	0.0125	0.0125	0.0125	0.0125
Dry matter	84.00	76.00	85.00	77.00	77.00
Chemical composition, % DM ¹					
Crude protein	14.30	14.20	14.10	14.10	14.10
Neutral Detergent Fiber	24.10	21.10	23.80	20.90	20.90
Non fiber carbohydrate	56.00	59.00	54.00	57.00	57.00
Fat	3.00	3.60	5.50	6.10	6.10
Ca	0.56	0.54	0.56	0.55	0.57
P	0.41	0.41	0.41	0.40	0.40
Cr	-	-	-	-	0.45
Zn	63.99	52.68	63.47	52.94	143.35
peNDF ²	13.00	14.00	13.00	14.00	14.00
NEg, Mcal/kg ³	1.17	1.27	1.26	1.35	1.35

Table 1. Feed ingredients and chemical composition of high-concentrate diets fed to feedlot Nellore bulls during the finishing period. Abbreviations: 1: Finely ground corn grain; 2: High- moisture corn; 3: Finely ground corn grain + Calcium salts of fatty (NutriGordura; Nutricorp Animal Nutrition; Araras, São Paulo, Brazil); 4: High-moisture corn + Calcium salts of fatty; 5: High- moisture corn + Calcium salts of fatty + organic Zn e Cr (Zinpro Animal Nutrition; Piracicaba, São Paulo, Brazil);. ¹Dry matter. ²Physically effective NDF. ³Net energy for gain, Mcal/kg (DM basis).

Lipid oxidation and color

Lipid oxidation was assessed using the thiobarbituric acid-reactive substances (TBARS) method^{27,28}. Results were expressed as mg of malonaldehyde per kg of tissue. Analyses were performed on days 0, 3, and 6 of the retail display using steaks aged 7 days, a period considered an intermediate point where lipid oxidation becomes detectable without compromising sensory quality. The TBARS were not determined after 14 days of ageing because there were insufficient samples.

For color evaluation, steaks were aged under vacuum at 2 °C for 7 or 14 days. After the aging period, samples were thawed for 24 h at 2 °C, sliced in half (exposing an unoxidized surface), and placed in polystyrene trays covered with polyvinyl chloride film to simulate retail display. During retail display, steaks were stored at 4 °C for 6 days, and color measurements were taken daily using a colorimeter (CM2500d - Konica Minolta, São Paulo, Brazil) with illuminant D65, a 30-mm aperture, and a 10° viewing angle. The color parameters L (lightness), a* (redness), and b* (yellowness) were recorded at three different points per steak. Delta E was calculated by comparing initial (day 0) and final (day 6) readings²⁹. In addition, the hue angle (tone) was calculated as $\tan^{-1}(b^*/a^*)$, and chroma (saturation) was calculated as $[(a^*)^2 + (b^*)^2]^{1/2}$, according to Terevinto et al³⁰.

Fatty acid profile

The fatty acid composition was determined in the *longissimus muscle* subcutaneous and intramuscular fat. Lipid extraction³¹, and fatty acid quantification was carried out via gas chromatography^{32,33}. The analysis was conducted using a Chromopack CP-Sil column (0.25 mm × 100 m) with an injection temperature of 270 °C and a detector temperature of 300 °C (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA, USA). Fatty acids were identified based on retention times relative to commercial standards (GLC-68D, GLC-79, GLC-87, GLC-211, and GLC-458; NU-Chek Prep, Inc., Elysian, MN, USA), and their relative percentages were determined from chromatographic peak areas.

Fatty acid, %	Calcium salts fatty acids
SFA ²	53.12
C8:0 (caprylic)	0.033
C10:0 (capric)	0.183
C12:0 (lauric)	2.862
C14:0 (myristic)	1.868
C15:0 (pentadecanoic)	0.080
C16:0 (palmitic)	42.42
C17:0 (margaric)	0.136
C18:0 (stearic)	5.213
C20:0 (arachidic)	0.318
UFA ³	43.69
MUFA ⁴	31.78
C16:1cis9 (palmitoleic)	0.189
C17:1 (heptadecenoic)	0.025
C18:1 cis9 (oleic)	31.51
C20:1 (eicosenoic)	0.057
PUFA ⁵	11.90
C18:2 cis-9, cis-12 (linoleic)	11.72
C20:3 cis-8, cis-11, cis-14 (dihomo- γ -linolenic acid)	0.185
Ratios	
MUFA: SFA	0.59
PUFA: SFA	0.22
UFA: SFA	0.82

Table 2. Fatty acid profile of calcium salts of fatty acids included in high-concentrate diets fed to feedlot Nellore bulls. ¹Calcium salts fatty acids from palm, soybean, and cottonseed oil (NutriGordura; Nutricorp Animal Nutrition; Araras, São Paulo, Brazil). ²SFA= saturated fatty acids. ³UFA= unsaturated fatty acids. ⁴MUFA= monounsaturated fatty acids. ⁵PUFA= polyunsaturated fatty acids.

Sensory evaluation

Sensory analysis was conducted in individual testing booths with 140 consumer panelists (aged 18–65 years; 42% female, 58% male) from Piracicaba, São Paulo, Brazil. Steaks for sensory evaluation were aged for either 7 or 14 days. A semi-structured hedonic scale was used, with panelists rating tenderness, juiciness, flavor/aroma, and overall satisfaction on a 100-mm continuous line scale, ranging from 0 (highly disliked) to 100 (extremely liked)³⁴. Additionally, panelists classified each sample into one of four quality categories: (1) unsatisfactory, (2) suitable for daily consumption, (3) very good, and (4) excellent. Steaks were coded, randomized, and cooked to a medium degree of doneness (63 °C), with internal temperatures monitored using a digital thermometer (ThermoPro TP-16, Duluth, GA, USA). Panelists were provided with water for palate cleansing.

Statistical analysis

The experiment followed a completely randomized block design, with initial BW and EPD used as blocking criteria. Pens were considered the experimental unit ($n=30$), with six replicates per treatment. Treatment and the interaction between EPD $\times$ treatment were considered fixed factors. The block and EPD were considered random effects.

Data was analyzed using the PROC MIXED procedure in SAS (SAS Institute Inc., Cary, NC), with repeated measures when appropriate. Data from the 30 animals slaughtered on day 0 as a baseline were included as covariates in the model. For Thio barbituric acid reactive substances (TBARS) measurements (days 0, 3, and 6) and color stability evaluation (days 0, 1, 2, 3, 4, 5, and 6), a repeated measures mixed model was used to assess treatment effects over time. The best-fitting covariance structure AR (1), ARH (1), ANTE (1), CS, CSH, TOEP, TOEPH, HF, UN, and VC, for each variable was selected based on Akaike's information criterion. The priori significance for the difference between means was $P<0.05$ for all statistical analyses.

Results

Centesimal composition and pH

The results for centesimal composition and pH are presented in Table 2. A significant interaction between EPD $\times$ treatments was observed for fat ($P=0.03$; Fig. 2 - a), and protein ($P<0.01$; Fig. 2 - b). Including CSFA enhanced intramuscular fat deposition, especially in bulls with high marbling EPD fed high-moisture corn, followed by those with low EPD fed finely ground corn. Including CSFA in the HMC diet of low-EPD bulls increased protein content and reduced collagen levels, whereas adding ZnCr to the HMC + CSFA diet resulted in higher collagen content ($P<0.01$). The interaction and treatment effects did not influence moisture, ash, sodium, or pH.

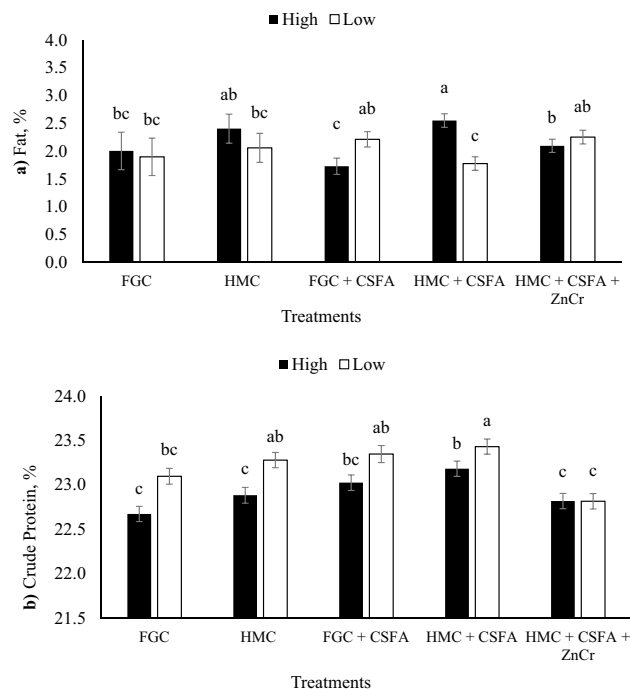


Fig. 2. (a) Influence of EPD for marbling on the centesimal composition of fat in the *longissimus muscle* of Nellore bulls finished in a feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. (b) Influence of EPD for marbling on the centesimal composition of protein in the *longissimus muscle* of Nellore bulls finished in a feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium.

		FGC	HMC		HMC	SEM ¹	P-Value			
Treatments	CSFA -	CSFA +	CSFA -	CSFA +	CSFA + ZnCr		Corn	CSFA	Corn*CSFA	ZnCr
Concentration in the muscle, % of dry matter										
Moisture	72.9	72.7	72.6	72.8	72.8	0.17	0.68	0.92	0.35	0.95
Fat*	2.0	2.0	2.2	2.2	2.2	0.15	0.16	0.88	0.79	0.93
Protein*	22.9	23.2	23.1	23.3	22.8	0.06	0.02	0.01	0.54	<0.01
Collagen	1.0	1.0	0.8	0.8	1.1	0.05	0.01	0.98	0.27	0.01
Ash	0.7	0.7	0.8	0.7	0.7	0.02	0.90	0.73	0.14	1.00
Sodium	0.4	0.4	0.5	0.4	0.4	0.02	0.80	0.06	0.38	0.70
pH	5.6	5.6	5.6	5.7	5.6	0.04	0.85	0.65	0.71	0.14

Table 3. Centesimal composition and meat pH of feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and organic zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing × inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$. *Interaction between treatments × EPD shown on Fig. 1.

Shear force and cooking loss

Results on shear force and cooking loss are shown in Table 3. No interaction or treatment effects were observed for Warner–Bratzler shear force (WBSF; $P > 0.05$). However, an effect of aging time was detected, with meat aged for 14 days showing reduced tenderness compared to samples aged for 4 and 7 days.

A significant interaction between EPD × treatment was observed for cooking loss ($P < 0.01$). Feeding HMC combined with CSFA to high-EPD bulls resulted in greater cooking losses. No significant effects of aging time or interactions between aging × treatment or aging × treatment × EPD were detected for cooking loss.

Treatments		Shear force, kg	Cooking loss, %
FGC	CSFA-	5.6	30.4
	CSFA+	5.5	29.5
HMC	CSFA-	5.5	29.3
	CSFA+	5.3	30.8
HMC	CSFA + ZnCr	5.1	28.0
Maturation time	0	5.7	28.7
	7	5.8	30.2
	14	4.7	29.9
P-Value	SEM ¹	0.14	0.68
	Corn	0.40	0.89
	CSFA	0.38	0.69
	Corn*CSFA	0.95	0.10
	ZnCr	0.23	0.01
	Maturation	<0.01	0.07

Table 4. Shear force and cooking loss of aged meat (0, 7, or 14 days) from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing $\times$ inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$.

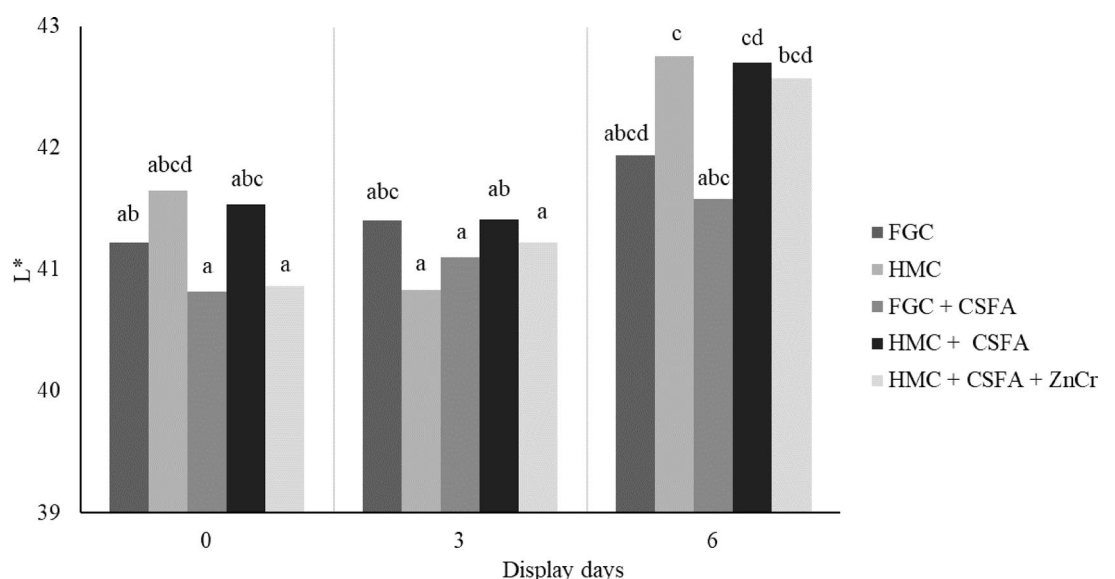


Fig. 3. Influence of retail display days (0, 3, and 6) on L* color values in the *longissimus muscle* aged for 14 days, from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and organic zinc and chromium supplementation. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium.

Lipid oxidation (TBARS) and Color.

The results of instrumental color evaluation and lipid oxidation (TBARS, mg MDA/kg) of the *longissimus muscle* aged for 7- and 14-days during retail display (0, 3, and 6 days) are presents in Table 4; Fig. 3. An effect of retail display time was observed for TBARS values ($P < 0.01$), indicating progressive lipid oxidation during retail display. No significant effects were observed for corn processing, and CSFA ($P > 0.05$), except for ZnCr supplementation on day 6 compared to day 0, suggesting greater lipid oxidation.

All color parameters (L^* , a^* , b^* , chroma, hue angle, and ΔE) were significantly affected by retail display time ($P < 0.01$) at both 7 and 14 days of aging, reflecting the expected deterioration in visual meat quality over time. Significant treatment $\times$ retail display, and treatment $\times$ EPD $\times$ retail display interactions were also observed ($P < 0.01$), indicating that the dietary strategies influenced meat color stability throughout the retail period.

Treatments		7 days of aging							14 days of aging					
		TBARS	L*	a	b	Croma	Hue	ΔE	L*	a	b	Croma	Hue	ΔE
FGC	CSFA-	0.06	41.5	15.2	7.8	17.7	27.1	5.2	40.2	16.1	8.5	18.2	27.8	5.9
	CSFA+	0.06	40.9	15.6	7.9	17.4	26.9	4.4	40.2	15.8	8.0	17.7	27.0	3.2
HMC	CSFA-	0.04	41.9	15.7	8.0	17.6	27.1	5.5	41.5	15.7	8.2	17.7	27.7	5.8
	CSFA+	0.10	42.0	15.6	8.1	17.5	27.5	4.4	40.6	15.8	8.3	17.8	27.8	4.4
HMC	CSFA + ZnCr	0.09	41.7	15.4	8.1	17.4	27.8	5.9	41.1	15.5	8.1	17.5	27.7	6.1
P-Value ¹	SEM ¹	0.02	0.43	0.34	0.29	0.47	0.26	0.60	0.79	0.26	0.25	0.42	0.30	0.40
	Corn	0.58	0.10	0.50	0.35	0.56	0.08	0.83	0.12	0.55	0.95	0.69	0.19	0.24
	CSFA	0.11	0.59	0.71	0.71	0.80	0.75	0.22	0.38	0.84	0.49	0.63	0.22	<0.01
	Corn + CSFA	0.10	0.37	0.45	0.98	0.68	0.09	0.81	0.45	0.45	0.28	0.45	0.10	0.20
	ZnCr	0.80	0.54	0.80	0.94	0.87	0.43	0.03	0.30	0.21	0.33	0.25	0.78	<0.01
	Display	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 5. Instrumental color evaluation and TBARS (mg MDA/kg) of *longissimus dorsi* muscle aged for 7 and 14 days, during 0, 3, and 6 days of retail display, from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing × inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$. *Interaction between treatments × EPD shown on Fig. 2.

At day 7 of aging, lightness (L*) and yellowness (b*) were affected during retail display, while at 14 days, most color parameters were influenced. The HMC diet increased L* at day 6 of retail maturation, whereas CSFA in the HMC diet reduced b* values. The ZnCr supplementation in the HMC + CSFA diet reduced redness (a*) and chroma on day 6 of retail display after 14 days of aging, suggesting that marbling potentially interacts with dietary strategies to affect meat color.

Moreover, ZnCr supplementation significantly increased ΔE values at both ageing periods ($P = 0.03$ at 7 days; $P < 0.01$ at 14 days), indicating reduced color stability during retail display.

Fatty acid profile

The fatty acid profile of the *longissimus muscle* in feedlot Nellore bulls is present in Table 6. The EPD × treatment interaction affected the concentrations of palmitic acid (C16:0; $P = 0.02$; Fig. 4). Bulls with high marbling EPD had greater palmitic acid content when fed FGC + CSFA and, to a lesser extent, FGC alone. No significant differences between EPD classes were observed under HMC-based diets and ZnCr supplementation, suggesting that the genetic potential for marbling may be modulated by diet composition.

The CSFA supplementation increased the proportion of SFA ($P = 0.01$), especially lauric, myristic, and stearic acids (C12:0, C14:0, and C18:0; $P = 0.01$, $P = 0.02$, and $P = 0.01$, respectively), while reducing the concentration of UFA acids ($P = 0.01$), and particularly palmitoleic acid (C16:1 cis-9; $P = 0.01$). Consequently, the MUFA: SFA ratio was also lower in CSFA-supplemented groups ($P < 0.01$). In addition, Δ⁹-desaturase activity indexes for both C16 and C18 substrates were decreased ($P < 0.01$).

An interaction between corn processing and CSFA inclusion affected MUFA ($P < 0.01$), myristoleic, oleic, arachidonic, PUFA: SFA ratio, and omega-3 fatty acids (C14:1; C18:1 cis-9; C20:4 n-6; $P = 0.04$; $P < 0.01$; $P = 0.01$, respectively), suggesting that the impact of CSFA on this fatty acid depended on the corn processing method. The ZnCr supplementation influenced the concentrations of linoleic acid (C18:2; $P = 0.05$), and omega 6 ($P = 0.05$), indicating additional modulation of the muscle lipid profile.

The fatty acid profile of subcutaneous fat is present in Table 7. A significant interaction between dietary treatment × EPD was observed for the percentage of palmitic and oleic acids ($P < 0.01$; Fig. 5 – A and B, respectively). Bulls with high marbling EPD showed greater palmitic acid concentrations compared to their low EPD group, particularly with the FGC and FGC + CSFA treatments. In addition, bulls fed FGC, particularly those with low marbling EPD, showed the highest oleic acid concentrations.

The CSFA supplementation increased the concentration of stearic acid (C18:0), whereas ZnCr supplementation decreased it. The PUFA concentration increased with CSFA supplementation, along with higher levels of omega-3 and omega-6 fatty acids; in contrast, the combination of HMC + CSFA + ZnCr decreased these concentrations. Additionally, CSFA supplementation decreased the Δ⁹-desaturase index for C18.

An interaction between CSFA × Corn processing method (FGC vs. HMC) was observed for several variables, including SFA, UFA, MUFA ($P = 0.03$; $P = 0.01$; $P = 0.01$), UFA: SFA and MUFA: SFA ratio ($P = 0.04$; $P = 0.03$), and the Δ⁹-desaturase activity indexes for C16 ($P = 0.04$). The concentration of SFA increased in bulls fed the HMC + CSFA diet, whereas ZnCr supplementation reduced its levels. In contrast, UFA concentration was higher with the FGC + CSFA diet but decreased when HMC + CSFA was provided; however, ZnCr supplementation promoted an increased UFA level. The MUFA concentration increased only with HMC + CSFA and was further enhanced by ZnCr. Although an interaction was observed for both UFA and the MUFA: SFA ratio, no differences

Total FA, %	FGC		HMC		HMC	SEM ¹	P-Value			
Treatments	CSFA-	CSFA+	CSFA-	CSFA+	CSFA + ZnCr		Corn	CSFA	Corn*CSFA	ZnCr
SFA	41.8	43.8	41.7	43.3	41.8	0.62	0.69	0.01	0.74	0.15
C12:0	0.07	0.09	0.07	0.08	0.08	0.01	0.72	0.01	0.60	0.83
C14:0	2.7	2.5	2.3	3.0	2.8	0.11	0.83	0.02	0.43	0.38
C16:0*	23.4	24.0	23.5	24.3	23.6	0.39	0.64	0.09	0.90	0.23
C18:0	12.0	13.4	12.0	12.5	12.0	0.38	0.14	0.01	0.15	0.38
UFA	57.9	55.7	58.1	56.5	57.9	0.63	0.46	0.01	0.63	0.21
MUFA	50.9	45.6	49.3	49.8	49.3	0.91	0.10	0.01	< 0.01	0.63
C14:1	0.87	0.80	0.75	0.83	0.82	0.03	0.19	0.91	0.04	0.77
C16: cis-9, cis-12	3.4	3.0	3.4	3.2	3.4	0.10	0.34	0.01	0.17	0.21
C18:1 cis-9	41.2	36.0	39.7	38.7	39.0	1.01	0.38	< 0.01	< 0.01	0.79
PUFA	7.1	10.1	8.7	6.7	8.6	1.08	0.30	0.56	0.01	0.12
C18:3 n-3	0.12	0.09	0.11	0.12	0.09	0.01	0.33	0.39	0.05	0.10
C18:2 cis-9, cis-12	4.1	6.0	4.7	3.5	5.5	0.96	0.20	0.65	0.03	0.05
C20:4 n-6	1.4	2.0	1.9	1.4	1.4	0.15	0.77	0.75	0.01	0.89
UFA: SFA	1.4	1.3	1.4	1.3	1.4	0.03	0.69	0.01	0.59	0.20
MUFA: SFA	1.2	1.0	1.2	1.2	1.2	0.04	0.29	< 0.01	0.02	0.49
PUFA: SFA	0.2	0.2	0.2	0.2	0.2	0.03	0.35	0.78	0.01	0.13
Omega 3	0.56	0.68	0.72	0.57	0.54	0.04	0.52	0.69	0.01	0.58
Omega 6	4.1	6.0	4.8	3.5	5.5	0.96	0.20	0.65	0.03	0.05
Omega 6/Omega 3	8.1	8.9	6.6	6.2	10.0	1.36	0.04	0.82	0.51	0.08
Δ -9/Delta-16 des.	12.8	11.0	12.5	11.7	12.6	0.37	0.57	< 0.01	0.21	0.10
Δ -9/Delta-18 des.	77.4	72.8	76.7	75.5	76.3	0.71	0.16	< 0.01	0.03	0.43

Table 6. Fatty acid profile of *longissimus muscle* from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing $\times$ inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$. *Interaction between treatments $\times$ EPD shown on . 3.

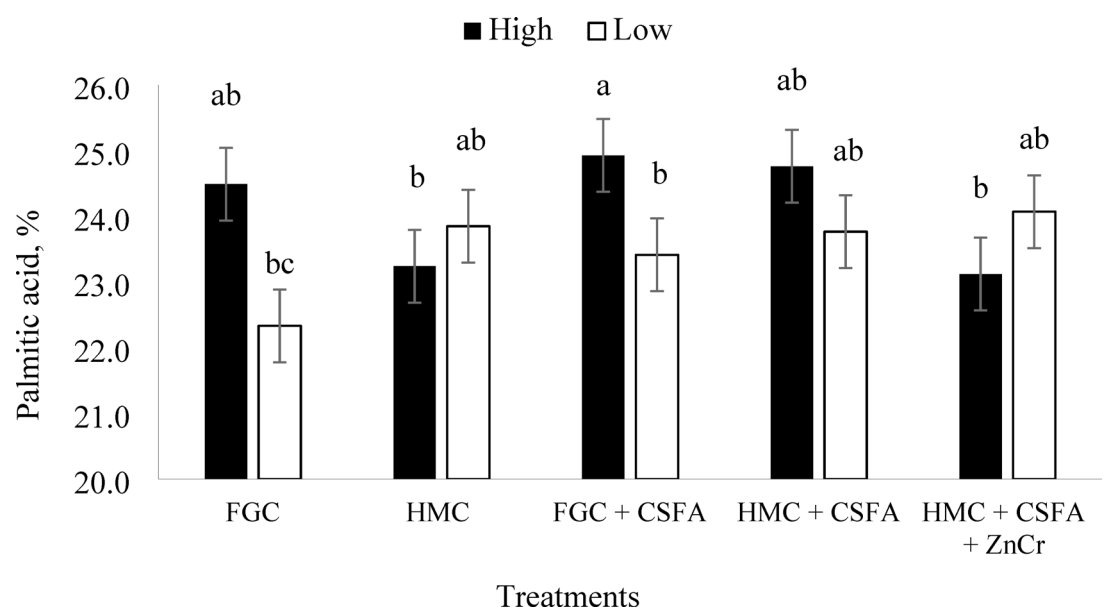


Fig. 4. Influence of EPD on the percentages of palmitic acid in the *Longissimus muscle* of Nellore bulls finished in feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. x.

Treatments	FGC		HMC		HMC	SEM ¹	P-Value			
	CSFA-	CSFA+	CSFA-	CSFA+	CSFA + ZnCr		Corn	CSFA	Corn*CSFA	ZnCr
SFA	43.6	43.3	42.9	45.1	42.8	0.60	0.27	0.08	0.03	0.01
C12:0	0.08	0.09	0.09	0.09	0.09	0.01	0.27	0.01	0.38	0.46
C14:0	3.6	3.5	3.6	3.8	3.3	0.15	0.26	0.78	0.59	0.03
C16:0*	24.1	23.9	23.8	24.7	21.9	0.52	0.40	0.33	0.12	0.14
C18:0	11.6	12.1	10.9	12.5	10.9	0.62	0.68	0.02	0.26	0.02
UFA	56.8	57.3	57.6	55.3	57.6	0.66	0.28	0.13	0.01	0.01
MUFA	53.9	53.7	54.7	52.0	54.7	0.60	0.30	0.01	0.01	<0.01
C14:1	1.3	1.3	1.4	1.2	1.2	0.09	0.75	0.03	0.22	0.41
C16:1	3.7	3.7	4.0	3.5	3.6	0.16	0.70	0.09	0.18	0.76
C18:1*	43.7	42.9	43.3	39.5	39.4	0.98	<0.01	<0.01	0.01	0.96
PUFA	1.9	2.5	1.8	2.5	2.0	0.11	0.91	<0.01	0.62	0.13
C18:3 n-3	0.09	0.08	0.09	0.08	0.10	0.01	0.75	0.03	0.60	0.12
C18:2 cis-9	1.2	1.6	1.1	1.5	1.2	0.07	0.32	<0.01	0.41	0.09
C20:4 n-6	0.01	0.01	0.01	0.10	0.01	0.02	0.30	0.26	0.29	0.29
UFA: SFA	1.3	1.3	1.3	1.3	1.4	0.03	0.39	0.18	0.04	0.01
MUFA: SFA	1.2	1.3	1.3	1.2	1.3	0.03	0.38	0.05	0.03	<0.01
PUFA: SFA	0.04	0.06	0.04	0.06	0.05	0.01	0.97	0.01	0.76	0.25
Omega 3	0.10	0.09	0.09	0.12	0.11	0.01	0.46	0.48	0.24	0.93
Omega 6	1.3	1.6	1.1	1.6	1.2	0.07	0.33	<0.01	0.39	0.08
Omega 6/3	14.1	18.5	12.5	15.4	12.6	0.74	<0.01	<0.01	0.21	0.03
Δ-9/Delta-16 des.	13.3	13.3	14.3	12.5	18.3	1.14	0.84	0.03	0.04	0.17
Δ-9/Delta-18 des.	79.0	77.9	79.9	76.0	74.0	2.03	0.51	0.01	0.09	0.68

Table 7. Fatty acid profile of subcutaneous fat from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing × inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$. *Interaction between treatments × EPD shown on Fig. 4.

were detected among treatments, except for the ZnCr-supplemented group, which showed higher ratio values. The Δ^9 -desaturase index for C16 increased in bulls fed HMC alone and HMC + CSFA + ZnCr.

Sensory evaluation

The sensory evaluation results are presented in Table 8. A significant interaction between treatment × EPD was observed for steak tenderness in samples aged 7 days ($P = 0.04$; Fig. 6) and 14 days ($P = 0.02$; Fig. 6). Low-EPD bulls fed the FGC + CSFA diet showed higher tenderness acceptability at 7 days of aging, whereas the inclusion of CSFA and ZnCr in the HMC diet improved tenderness at 14 days.

In contrast, corn processing method, supplementation with CSFA, and the addition of organic ZnCr did not influence juiciness, and flavor for steaks aged 7 or 14 days ($P > 0.05$). However, for *longissimus muscle* aged 7 days, including CSFA significantly improved overall satisfaction ($P = 0.02$), and ZnCr supplementation affected the ranking score ($P = 0.05$). No treatment effects were observed on the other sensory parameters.

Discussion

Grain processing

Including ensiled HMC, with approximately 30% moisture, is a recognized strategy to improve nutrient solubility and increase proportionate in the rumen^{35,36}. Replacing FGC with HMC was hypothesized to increase starch digestion in the rumen and small intestine, resulting in higher glucose availability and net energy supply³⁷. This enhanced fermentative capacity would lead to greater production of propionate, the primary gluconeogenic precursor in ruminants¹¹. Since glucose is the primary substrate for fatty acid biosynthesis in intramuscular adipose tissue, increased glucose availability is crucial in marbling development. Therefore, this dietary approach might improve centesimal composition and overall meat quality³⁸.

In the present study, we observed overall average values of 72.7% moisture, 23% protein, 2.1% fat, and 0.73% ash, consistent with values reported in the literature⁴⁰. However, the companion study³⁹ found that blood glucose availability was not affected by feeding HMC diets, which may explain why the intramuscular fat content in the *longissimus muscle* remained unchanged, despite the corn processing. In addition, dry matter intake decreased when FGC was replaced by HMC, which may have limited the overall energy intake and contributed to maintaining blood glucose levels unchanged.

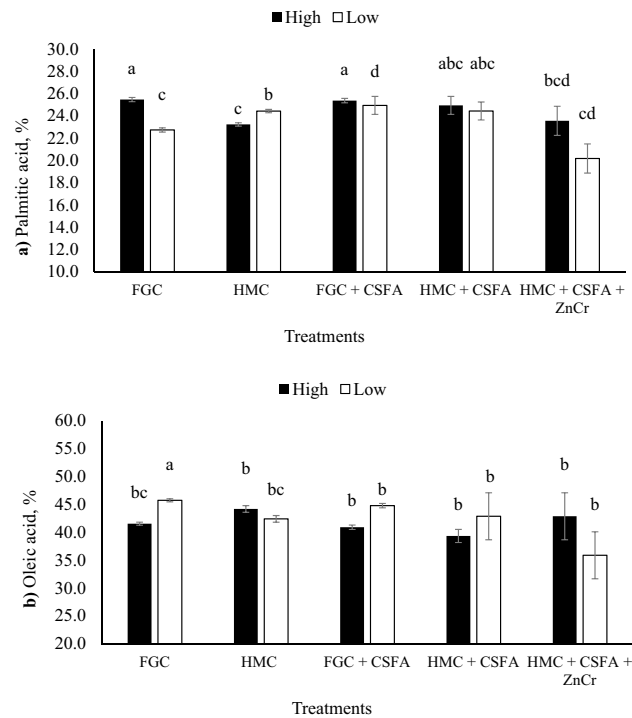


Fig. 5. (a) Influence of EPD on the percentages of palmitic acid in the *Longissimus muscle* of Nellore bulls finished in feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. (b) Influence of EPD on the percentages of oleic acid in the subcutaneous fat of Nellore bulls finished in feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium.

Treatments		7 days of aging					14 days of aging				
		Tenderness*	Juiciness	Flavor	Satisfaction	Ranking	Tenderness*	Juiciness	Flavor	Satisfaction	Ranking
FGC	CSFA-	63.6	69.2	66.0	67.9	2.5	70.5	68.5	68.8	69.1	2.6
	CSFA+	63.6	66.3	67.0	67.5	26	69.1	66.4	68.9	70.4	2.6
HMC	CSFA-	61.2	65.9	66.5	69.5	2.5	69.1	66.7	67.8	69.6	2.6
	CSFA+	64.9	63.9	66.0	68.2	2.5	70.8	67.7	69.8	70.2	2.6
HMC	CSFA + ZnCr	65.2	66.3	68.2	68.0	2.6	72.4	68.5	69.5	72.6	2.7
P -Value	SEM ¹	1.56	1.57	1.39	1.73	0.06	1.90	2.17	2.14	2.19	0.09
	Corn	0.73	0.09	0.87	0.94	0.51	0.95	0.90	0.99	0.96	0.83
	CSFA	0.27	0.21	0.85	0.92	0.74	0.94	0.81	0.62	0.87	0.64
	Corn*CSFA	0.29	0.86	0.58	0.39	0.25	0.50	0.47	0.67	0.67	0.74
	ZnCr	0.86	0.30	0.28	0.94	0.05	0.54	0.79	0.92	0.43	0.82

Table 8. Sensory evaluation of aged *longissimus dorsi* muscle from feedlot Nellore bulls fed high-energy diets with different corn processing methods, calcium salts of fatty acids, and zinc and chromium supplementation. Abbreviations: FGC: Finely-ground corn; HMC: High-moisture corn; CSFA -: Absence of calcium salts of fatty acids; CSFA+: Inclusion of calcium salts of fatty acids; Corn*CSFA: interaction between corn processing × inclusion of calcium salts of fatty acids; ZnCr: Inclusion of organic zinc and chromium. ¹Standard error of the mean. Values within a row with different superscripts differ significantly at $P < 0.05$. *Interaction between treatments × EPD shown on Fig. 5.

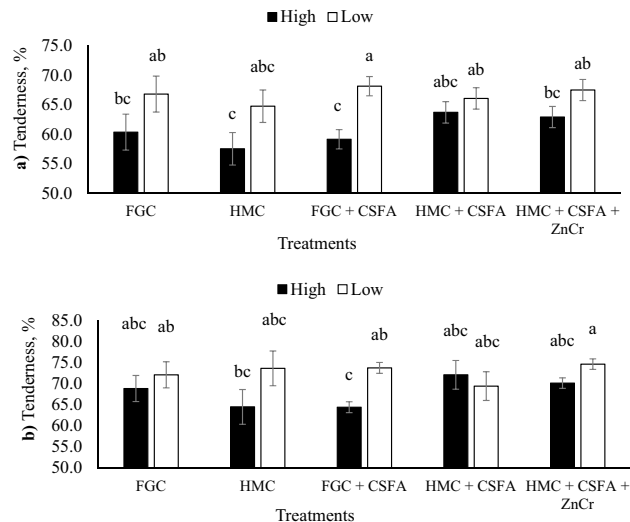


Fig. 6. (a) Influence of EPD on the percentages of palmitic acid in the subcutaneous fat of Nellore bulls finished in feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. (b) Influence of EPD on the percentages of oleic acid in the subcutaneous fat of Nellore bulls finished in feedlot. Black bars represent bulls classified with high EPD, and white bars represent low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. (a) Influence of EPD on the tenderness acceptability scores from a sensory panel of meat aged 7 days from Nellore bulls finished in feedlot. Black bars represent animals classified with high EPD, and white bars represent animals with low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium. (b) Influence of EPD on the tenderness acceptability scores from a sensory panel of meat aged 14 days from Nellore bulls finished in feedlot. Black bars represent animals classified with high EPD, and white bars represent animals with low EPD for marbling. Different letters indicate differences among treatment means, regardless of marbling EPD class ($P < 0.05$). FGC: Finely ground corn; HMC: High-moisture corn; CSFA: calcium salts of fatty acids; ZnCr: organic zinc and chromium.

Meat quality is often associated with intramuscular fat levels ranging from 3% to 7.5%, corresponding to USDA quality grades from “Select” ($\approx 3\%$) to “High Choice” ($\approx 8\%$);⁴⁰. The average intramuscular fat content of 2.1% observed in this study was below this threshold, which may negatively impact sensory attributes such as tenderness, juiciness, and flavor. Previous studies have reported similar findings regarding the effect of grain processing on carcass traits. Silva et al.⁴¹ observed no significant differences in intramuscular fat between dry and high-moisture corn diets supplemented with CSFA in Nellore steers (3.44 vs. 3.51%), corroborating with the lower values obtained in the current study. It is noteworthy to mention that using bulls instead of steers or heifers in this study may have had a negative impact on marbling deposition.

Nevertheless, increased intramuscular fat deposition in high EPD bulls fed the HMC + CSFA diet may be explained by a greater genetic predisposition for marbling and enhanced glucose uptake by the adipocytes, since blood glucose levels remained unaltered by replacing FGC with HMC. Higher glucose uptake by adipocytes could have contributed to increased lipid synthesis in the intramuscular depot. This may be associated with the fermentation profile of HMC, which promotes greater short-chain fatty acid production⁴². However, glucose uptake by adipocytes was not determined in this study.

Understanding intramuscular fat development is crucial for designing nutritional strategies to enhance meat quality. In cattle, adipocyte hyperplasia occurs mainly during late gestation and around 250 days of age, after which adipocyte hypertrophy drives marbling through increased fat deposition until slaughter^{43–45}. This data underscores the importance of feeding strategies that ensure adequate energy availability during the finishing phase. Ramírez-Zamudio et al.⁴⁶ found no significant effects of protein supplementation during gestation on intramuscular fat in Nellore cattle, suggesting that postnatal nutrition and diet composition are likely more decisive factors in determining marbling outcomes.

Although intramuscular fat increased only in the high-EPD group, the combination of HMC and CSFA supplementation enhanced muscle protein content in low-EPD bulls, possibly indicating a shift in nutrient utilization toward muscle tissue deposition. This effect may be attributed to the limited genetic potential for marbling, resulting in an allocation of nutrients toward muscle growth. As suggested by Batistel et al.⁴⁷, the type of energy precursor glucogenic versus lipogenic can influence the magnitude and direction of such carryover effects. The CSFA may have spared glucose from oxidation⁴⁸, while the HMC likely increased propionate

production, supporting gluconeogenesis and protein synthesis⁴⁹. Together, these dietary components may have promoted muscle fiber synthesis over fat deposition, especially in bulls genetically predisposed to lower marbling.

CSFA inclusion

An alternative strategy to enhance marbling is to feed ruminants with rumen-protected fat that are either wholly or partially protected from ruminal modification, such as CSFA. The supplementation would increase energy intake without disrupting ruminal fermentation, as the fatty acids bypass the rumen and escape biohydrogenation⁵⁰. Consistent with expectations, supplementation with CSFA did not affect ruminal fermentation parameters, such as acetate, propionate, butyrate, lactate, or total short-chain fatty acid production, indicating that fiber fermentation was maintained⁵¹.

Feeding CSFA has been reported to improve intramuscular fat scores in cattle due to rich content of oleic and linoleic acids⁵². However, feeding fats of different origins and saturation levels to ruminants has produced variable responses in ruminant performance and carcass traits⁵⁰.

In the present study, bulls fed HMC diets exhibited reduced collagen content compared to those fed FGC or the combination of HMC + CSFA + ZnCr, which may be beneficial for improving meat tenderness by limiting collagen cross-linking. According to Coró et al⁵³, *Bos indicus* cattle typically exhibit more stable and heat-resistant collagen cross-links than *Bos taurus*, contributing to tougher meat. Moreover, collagen cross-linking becomes more resistant to heat degradation as cattle age⁵⁴. Interestingly, changing the corn processing method reduced collagen content. These effects suggest a potential dietary intervention on improving meat tenderness.

Similarly to our findings, studies evaluating the inclusion of CSFA in the diets of Nellore and crossbred cattle ($\frac{1}{4}$ Nellore $\times$ $\frac{1}{4}$ Santa Gertrudis $\times$ $\frac{1}{2}$ Braunvieh) reported no significant effects on shear force or cooking loss^{46,54,55}. None of these studies observed meat samples with shear force values below the 4.4 kg threshold for tenderness. In the present study, however, the inclusion of CSFA, whether in FGC or HMC diets, and the addition of ZnCr, increased the frequency of bulls with shear force values equal to or below 4.4 kg. While these results suggest the potential benefits of both dietary strategies on tenderness, the literature remains scarce regarding the specific effects of ZnCr supplementation on meat quality traits.

Although CSFA is rich in C16:0 and C18:1, its supplementation did not significantly increase C18:1 cis-9 concentrations in the *longissimus muscle* and subcutaneous fat. However, bulls with high EPD for marbling showed elevated C16:0 levels in the *longissimus muscle* when fed the FGC + CSFA diet, suggesting a genotype-dependent response to fat supplementation. Additionally, the bulls fed CSFA diets showed increased percentages of C18:0, but this fatty acid was not converted to C18:1 cis-9 by delta-9 desaturase action, and C18:0 may have been lost during the process. Therefore, CSFA may promote the lengthening of C16:0 to C18:0, but without fatty acid desaturation.

The hypothesis that adding CSFA would increase the deposition of unsaturated fatty acids in the meat was not supported, as there was a decrease in UFA when CSFA was added to the diet. Furthermore, the results for MUFA showed that adding CSFA to FGC decreased MUFA while adding CSFA to HMC increased MUFA in high EPD bulls. Similar results were found by Silva et al⁵⁶, who observed an increase in C18:0 (13.6 vs. 15.1%) and a decrease in C18:1 cis-9 (41.1 vs. 37.1%) in comparison to the control group.

Costa et al⁵⁷ did not find significant differences for C18:1 cis-9, C18:2 cis-9, cis-12, SFA, MUFA, PUFA, and C16:0 in Nellore cattle supplemented with CSFA. However, they observed that the control group presented higher concentrations of C18:0. In contrast, Nascimento et al⁵⁸ found that CSFA promoted higher concentrations of MUFA and a greater MUFA: SFA ratio due to higher concentrations of palmitoleic (C16:1 cis-9) and oleic acids. These studies also showed higher linoleic acid concentrations (C18:2) and a greater trend of PUFA concentration. Adding CSFA to the diet also increased PUFA deposition in subcutaneous fat, although this percentage did not influence greater lipid oxidation.

Zn and cr supplementation

Organic Zn and Cr supplementation has been linked to improved immune function and carcass traits. Zinc stimulates glycerol-3-phosphate dehydrogenase in intramuscular preadipocytes⁵⁷ and regulates PPAR γ expression via ZFP423, promoting adipocyte differentiation⁵⁹. Chromium enhances insulin sensitivity and glucose uptake in target tissues^{16,18,60}, potentially increasing marbling by improving glucose availability for lipogenesis⁶¹.

It was expected that zinc and chromium may support enzymatic processes during aging, contributing to greater tenderness. Nevertheless, we have not observed the effects of ZnCr supplementation on fat intramuscular deposition or sensorial parameters. Vellini et al²² reported increased tenderness (2.86 kg) in meat aged 28 days but did not find differences in the fatty acid profile of meat from *Bos indicus* supplemented with ZnCr in the diet of Nellore cattle.

On the other hand, bulls supplemented with ZnCr showed higher lipid oxidation between days 0 and 6 of exposure compared to other treatments. Despite these effects, the TBARS values stayed below the sensory threshold of 2.28 mg MDA/kg of meat, which may prevent consumers from perceiving the rancid flavor^{62,63}. The TBARS values observed in the present study were lower than those reported in the literature, suggesting that lipid oxidation remained limited throughout the maturation period, and may associate with the lower intramuscular fat content in the meat⁶⁴.

Additionally, regardless of aging time, the ΔE values were higher for ZnCr group supplementation than those that fed only the HMC + CSFA diet. However, limited research has been conducted on the effect of Zn and Cr supplementation on lipid oxidation in cattle. Color variation (ΔE) reflects the difference between the initial and subsequent color measurements during storage and is perceptible to the human eye when values exceed 3.5⁶⁵. In the present study, meat aged 7 and 14 days exhibited average ΔE values around 5, indicating noticeable color

changes to consumers. Similarly, Andrade et al.⁶⁶ observed a linear increase in ΔE values in aged meat from Nellore cattle: 3.37 at 7 days, 11.95 at 14 days, and 12.81 at 21 days.

Controlling oxygen exposure is essential for maintaining the bright red appearance of fresh meat; however, prolonged exposure accelerates oxidation and reduces freshness, thereby negatively affecting sensory quality⁶⁷. In the present study, although no treatment effects were observed, aging time significantly influenced beef color. Extending aging from 7 to 14 days is known to reduce the water-holding capacity of muscle fibers due to proteolysis and weakening of the myofibrillar structure, leading to water exudation and greater light reflectance^{68,69}. This mechanism explains the tendency toward a lighter appearance, higher L^* in meat, subjected to longer aging, as previously reported^{70,71}.

On day 0 of exposure, steaks aged 14 days showed slightly higher a^* values compared with those aged 7 days; however, no corresponding increase in L^* was observed. According to Holman et al.⁷², consumers prefer beef with a^* values above 14.5, yet wet-aged steaks generally fall below this threshold after 5 to 6 days of display. Similarly, in our study, a^* values dropped below 14.5 by the fifth day, regardless of aging period, reinforcing that wet-aged meat should ideally be marketed within the first days of display to maximize consumer appeal.

Furthermore, chroma (C^*) and hue angle (h°) provide complementary information on color saturation and stability. Consistent with recent studies^{69,73}, higher h° values observed over time reflected a progressive shift from red to yellow, while decreases in C^* indicated fading color intensity. These patterns confirm the expected discoloration process during storage and highlight that extended aging primarily affects beef color stability through combined effects on water-holding capacity and myoglobin oxidation^{74,75}.

Marbling EPD groups

In addition to nutrition, genetic improvement plays a crucial role in determining meat quality. Selecting cattle with high EPDs for marbling increases intramuscular fat deposition in *Bos indicus*, as marbling is a trait with moderate to high heritability^{75,76}. A recent review reported heritability values ranging from 0.34 to 0.68, reinforcing that marbling can be effectively enhanced through genetic selection⁷⁷. Furthermore, selection for higher marbling has been shown to reduce fat deposition in other body regions⁷⁸, potentially minimizing carcass-trimming losses caused by excessive subcutaneous or visceral fat⁷⁹.

Bos taurus cattle, such as Angus, generally exhibit higher intramuscular fat contents (6.5–8.3%) compared with *Bos indicus*, like Nellore, which often remain below 5%^{79,80}. The differences between breeds are attributed mainly to variations in energy metabolism and the regulation of genes involved in adipogenesis⁸¹. Nonetheless, considerable within-breed genetic variability exists, and molecular studies have identified candidate genes associated with contrasting marbling potentials, reinforcing the opportunity for genetic selection in *Bos indicus* cattle^{82,83}.

Vieselmeier et al.⁸⁴ reported that *Bos taurus* cattle with positive EPDs for marbling achieved a higher percentage of marbling (74%) of USDA “Choice” carcass grades compared to those with low marbling EPDs (47%). The “Choice” grade corresponds to a marbling score ranging from 2 to 3 on the USDA scale. Similarly, Detweiler et al.⁷⁹ observed that Angus cattle with high marbling EPDs had greater proportions of “Prime” carcasses. Similarly, in the present study, Nellore bulls with higher marbling EPDs exhibited increased fat deposition in the *longissimus muscle*, suggesting that selection for this trait can enhance the efficiency of dietary energy use for intramuscular fat deposition even in *Bos indicus* breeds^{79,84,85}.

Altogether, these findings indicate that the interaction between genetic potential and diet composition is crucial for marbling development. While *Bos indicus* cattle present inherent challenges due to their lower intramuscular fat deposition capacity, the use of marbling EPDs coupled with energy-dense diets represents a promising strategy to improve beef quality⁸¹. According to Corbin et al.⁸⁵, customers select more tender cuts with higher marbling levels than equally tender cuts with lower marbling. Marbling can significantly influence palatability and flavor by enhancing organoleptic attributes⁸⁷. In the present study, the increased fat content in high-EPD bulls may reflect a genetic predisposition for fat deposition influenced by diet. However, the panelists did not sense differences in juiciness and flavor between meat aged for 7 or 14 days.

Differences in tenderness were observed among treatments and EPD groups. Bulls with low EPD for marbling fed the FGC + CSFA diet received higher tenderness acceptability scores than high EPD cattle under the same treatment and aging times. Additionally, low-EPD cattle showed lower cooking loss than high-EPD cattle within the FGC + CSFA treatment.

This finding may be associated with the greater frequency of cattle fed the FGC + CSFA group exhibiting shear force values ≤ 4.4 kg, rising from 0% to 16.7% with CSFA inclusion. The USDA has established shear force standards for tenderness certification, with meats having shear force values of 4.4 kg or less classified as “tender” and 3.9 kg or less as “very tender”⁸⁸. However, this experiment did not observe shear force values that met USDA criteria for tender meat. Nonetheless, the cattle had uniform carcass finish, with an average of 6 mm, which may have helped avoid issues related to cold shortening.

Several factors can influence meat tenderness, such as breed, age, glycogen reserves, rigor mortis, intramuscular fat, pH, and aging period. The higher shear force values found in this study (5.43 kg) may be attributed to factors such as the breed and sexual condition of the bulls. *Bos indicus* tend to have more collagen crosslinks and higher calpastatin enzyme activity, which inhibits calpain proteolysis, leading to tougher meat⁸⁹.

In conclusion, our findings demonstrate that replacing FGC with HMC increased intramuscular fat deposition, particularly in bulls with high marbling EPD, highlighting the interaction between genetic potential and diet composition. Bulls with high marbling EPD exhibited an efficient use of dietary energy for fat deposition when fed energy-dense diets and CSFA. However, CSFA supplementation did not significantly increase the levels of unsaturated fatty acids or improve marbling scores. Supplementation with organic Zn and Cr improved oxidative stability and meat color during retail display but did not enhance marbling or other meat quality traits. Finally, an aging period longer than 14 days is recommended to optimize tenderness in Nellore beef.

These results underscore the importance of considering genetic traits in conjunction with targeted nutritional strategies to enhance meat quality.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Luana D. Felizari: Conduction of the study in the feedlot; Lab analyses; Conceptualization. Mariana M. Squizatti: Conduction of the study in the feedlot; Lab analyses; Formal analysis. Antônio M. Silvestre: Conduction of the study in the feedlot; Lab analyses; Formal analysis. Breno L. Demartini: Conduction of the study in the feedlot; Lab analyses; Formal analysis. Thaiano I. S. Silva: Conduction of the study in the feedlot; Lab analyses; Formal analysis. Jonata H. R. Souza: Lab analyses; Formal analysis. Johnny M. Souza: Writing – review & editing; Investigation; Formal analysis. Sergio B. Pflanzer: Writing – review & editing; Investigation; Formal analysis. Dante P. D. Lanna: Writing – review & editing; Investigation; Formal analysis. Bradley J. Johnson: Writing – review & editing. Ariany F. Toledo: Writing – review & editing. Danilo D. Millen: Writing – review & editing; Supervision; Methodology; Funding acquisition; Data curation; Conceptualization.

Declarations

Competing interests

The authors declare no competing interests.

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.

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Ethics statement

This study is reported in accordance with the ARRIVE guidelines (<https://arriveguidelines.org>). All experimental procedures involving animals were conducted in compliance with institutional and national guidelines for the care and use of laboratory animals and were approved by the Ethical Committee for Animal Research (protocol CEUA 0151/2019) of the São Paulo State University (UNESP), Dracena campus.

Additional information

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