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Effects of monensin only, monensin and virginiamycin combination, or monensin and a blend of organic trace minerals and yeast on meat quality of crossbred bulls finished in feedlot individual pens and fed with high-grain diets

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Abstract

This study assessed carcass characteristics and meat quality of bulls finished in individual pens and fed with different diets. A completely randomized design determined how to feed 24 crossbred bulls (European × Nellore) with four diets over 84 days: CONT) without additives; MONE) inclusion of 30 mg of monensin/kg DM; MO + VI) inclusion of 30 mg of monensin + 30 mg of virginiamycin/kg DM; and MO+AD) inclusion of 30 mg of monensin/kg DM + 1.57 g of a blend of organic trace minerals, live yeast, beta-glucan, and mannans per kg DM (Advantage-Confinamento). MO+VI resulted in lower pH ($P < 0.05$) and lighter meat ($P < 0.05$) compared with other treatments. Cooking loss was less ($P < 0.05$) with MO+AD at 14 days of aging time. At 14 days, Warner-Bratzler shear force was higher for meat from bulls fed with CONT and MONE diets and slower ($P < 0.05$) for meat from bulls fed with MO+VI and MO+AD diets. In conclusion, including monensin combined with virginiamycin and monensin combined with a blend of organic trace minerals and yeast in the diets of bulls finished in individual pens can improve the color, Warner-Bratzler shear force of meat, and lower cooking losses.

Keywords: additives; antibiotics; beef quality; cattle; ionophores; *Saccharomyces cerevisae*.

Practical Application: A combination of organic minerals with conventional additives, such as monensin, is suitable for supplementation of cattle finished in individual pens and fed with high-grain diets aiming at meat quality, lipid oxidation, and antioxidant activity.

1 INTRODUCTION

Beef industries are no longer concerned only with the efficiency of farms. There is growing interest on meat and co-product quality. Brazil, the largest exporter of beef, has significantly increased the efficiency of the production systems in farms over the past 20 years. During the 1990s, Brazil had 170 million cattle and produced approximately 4.5 million tons of meat (FAPRI, 2023). In this decade, the Brazilian cattle herd has approximately 210–220 million cattle and produces ~10 million tons of meat (FAPRI, 2023). Thus, over the past 30 years, there was 25% of herd growth, but a 120% increase in beef production (FAPRI, 2023). This increase in productivity in recent years was due to genetic gains (Prado et al., 2008), improvements in pastures, disease control and eradication, professional management of properties, and implementation of more productive systems such as pasture supplementation (El-Memari Neto et al., 2003) and finishing cattle in individual pens fed with high-grain diet (Ornaghi et al., 2020; Rivaroli et al., 2020; Torrecilhas et al., 2021).

The diets used to feed cattle in individual pens are rich in starch, non-fiber carbohydrates (Ornaghi et al., 2017; Rivaroli et al., 2017). The rapid rate of degradation of these carbohydrates in the rumen can increase the risk of metabolic disorders and ruminal acidosis. To avoid the risks of ruminal disorders, antimicrobial compounds such as ionophores and antibiotics (Duffield et al., 2012) have been used, which impact ruminant metabolism by increasing energy metabolism efficiency, improving

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nitrogen metabolism, and reducing acidosis risk (Schelling, 1984). These compounds can change the ratio of short-chain fatty acids in the rumen, increase the production of propionic acid, and reduce the production of butyric and acetic acids (Owens et al., 1998). Increased production of propionic acid from the rumen increases hepatic gluconeogenic flux (Schönhusen et al., 2013). Changes in the flow of short-chain fatty acids in rumen, and subsequently in the liver, could affect the composition of the meat (Eiras et al., 2014; Françozo et al., 2013; Prado et al., 2016).

It is well known that the ionophore monensin and the antibiotic virginiamycin have minimal effects on meat quality of feedlot cattle when administered alone or in combination (Rigueiro et al., 2020). In the same manner, it has been shown that live yeast generally has a slight and non-significant effect on meat quality in cattle (Cozzi et al., 2009). In a previous study, Gomes et al. (2009) reported that the combination of monensin with live yeast had no important effects on carcass traits and on meat quality of feedlot finished steers.

On the contrary, there is growing concern about residues from antimicrobials in meat, which restricts the commercialization of meat (Cameron & McAllister, 2016; Schäberle & Hack, 2014). Thus, alternatives are needed. Yeasts, such as *Saccharomyces cerevisiae*, have been widely used in the industry because it is generally regarded as a safe product, presenting health benefits to the rumen and ruminants (Elghandour et al., 2020) and improving the digestibility of food while maintaining animal performances of ruminants (Elghandour et al., 2020).

Studies concerning the effect of the combination of antimicrobial compounds and trace minerals and yeast have been focusing on animal performance, feed efficiency, and ruminal modulation (Rigueiro et al., 2020). However, few studies have explored single additives, or their combination, on meat characteristics in beef cattle (Castagnino et al., 2018). Moreover, the results of these studies show that the addition of antimicrobial compounds in the diets of cattle finished in individual pens has less influence on the meat characteristics (Wang et al., 2020). Finally, there is a lack of knowledge regarding the effect of the combinations among antimicrobial compounds with minerals trace and yeast on meat quality, lipid oxidation, and antioxidant activity for beef cattle.

Therefore, we hypothesize that the inclusion of antimicrobial compounds (monensin or virginiamycin) in combination with a blend of organic trace minerals and yeast could alter meat quality.

This study was conducted to evaluate the inclusion of monensin only or monensin and virginiamycin combination or monensin and a blend of organic trace minerals and yeast on animal performance and meat quality of bulls finished in individual pens fed with a high-grain diet and to evaluate if they had the residues from monensin and virginiamycin in the meat.

2 MATERIALS AND METHODS

2.1 Ethics committee, location, animals, and diets

Details of the cattle, diets, and slaughter procedures are given by Duarte et al. (2023). This study was approved by the Department of Animal Production and Research Ethics Committee at the State University of Maringá, Brazil (protocol nº 1103290719).

Briefly, 36 crossbreed (European × Nellore) bulls were used in a completely randomized design in a feeding trial lasting 84 days. At the beginning of the experiment, the average weight and age of bulls were 385.1 ± 3.84 kg and 24 ± 3.2 months, respectively. During the feeding trial, nine bulls were used per treatment. However, for meat analysis, only six bulls were sampled and the criterion used was random. The bulls were weighed at the beginning of the experiment and assigned to individual pens (10 m^2), with concrete floors, and partially covered. The animals had free access to clean water throughout the experiment.

Bulls were randomly distributed into four diets according to initial BW, with an adaptation period of 14 days. The basal diet comprised 850 g/kg of cracked corn and soybean meal and 150 g/kg of corn silage, which was offered *ad libitum* for 84 days+14 days for adaptation. The basal diet was formulated to be isonitrogenous and isoenergetic and was similar for all animals. The four experimental diets were used as follows: CONT) control without additives, MONE) inclusion of 30 mg of monensin/kg of dry matter (DM), MO+VI) inclusion of 30 mg of monensin/kg DM+30 mg of virginiamycin/kg DM, and MO+AD) inclusion of 30 mg of monensin/kg DM+1.57 g of Advantage-Confinamento (kg/DM). The Advantage-Confinamento (Alltech, Inc.) is a blend of organic trace minerals and yeast (*S. cerevisiae*), beta-glucan, and mannans. Monensin, virginiamycin, and the blend of organic trace minerals and yeast, beta-glucan, and mannans were obtained from Rumensin (Elanco Animal Health, Indianapolis, IN, USA), V-max 50 (Phibro Animal Health, Ridgefield Park, NJ, USA), and Advantage-Confinamento (Alltech, Lexington, KY, USA), respectively.

2.2 Meat sampling

On the final day of the trial (day 84), the bulls were weighed after 16 h of fasting and transported to a commercial slaughterhouse (Campo Mourão, Paraná state, Brazil). Bulls were transported for 80 km, and the truck stock density was 0.8 ± 0.2 bulls/m^2. Bulls were stunned with a captive-bolt pistol and bled through exsanguinations by cutting the neck vessels, following the industry guidelines. Then, the carcasses were divided medially from the sternum and spine, and the half-carcasses were washed only with water, identified, and stored in a chilling chamber at 4°C for 24 h.

The *longissimus* muscle (LM) was excised from the left half of the carcass from the sixth to the last lumbar vertebra and transported to the laboratory under ice (+4ºC). The LM was sliced into seven 2.5-cm-thick steaks, weighed, vacuum-packed (99% vacuum, Sulpack SVC 620) in polyamide/polyethylene pouches (120 μm; 1 cm^3/m^2/24 h O_2 permeability and 3 cm^3/m^2/24 h CO_2 permeability, at 5°C and 75% relative humidity; 3 g/m^2/24 h water vapor transmission rate at 38°C and 100% relative humidity; 97°C Vicat softening temperature; 1.3 g dart drop strength), and aged for either 1, 3, 7, or 14 days at +4°C in the dark.

2.3 Monensin and virginiamycin analyses

The samples of meat (*longissimus lumborum*) were sent to the Agro Safety Laboratory (Piracicaba, São Paulo state, Brazil) and analyzed for the presence of monensin and virginiamycin. An amount of 2 g sample was mixed with 5 mL of the extraction solution composed of acetonitrile/ethanol/water (80:10:10; v/v/v) in 1% of formic acid and vortexed for 1 min. Then, 0.2 g of NaCl and 1 g of $MgSO_4$ were added and stirred vigorously for 1 min. Next, this content was centrifuged at 3,100 rpm for 10 min, and a 1 mL aliquot of the supernatant was transferred to a tube of a 15-mL C18 column, which was stirred for 1 min and centrifuged at 3,100 rpm for 10 min. The extract was filtered through a 0.22-µm nylon filter and then analyzed in the LC-MS/MS system set in the full scan mode. An Accela LC system (Thermo Scientific, San Jose, CA, USA) coupled to an Orbitrap model QE Exactify high-resolution mass spectrometer equipped with a Phenomenex Sinergy analytical column (150 × 2.0 mm, 4 µm) was used. The mobile phase was composed of eluent A (0.1% formic acid in distilled water; v/v) and eluent B (0.1% formic acid in acetonitrile; v/v). A gradient mode was applied to start with 5% eluent B increasing to 90% for 2 min and maintained for 16.5 min, after which it was immediately reduced to 5% eluent B, with a rebalancing time of 5 min. The column and the automatic sampler were maintained at a constant temperature of 40°C. A 2-µL aliquot of the sample extract was injected into the chromatographic system for reading, and the data were processed using the Xcalibur 4.3 software (Thermo Scientific, San Jose, CA, USA).

2.4 Meat quality

A pH meter (Hanna instruments model HI99163, Romaria, Brazil) was used to evaluate the pH, which was calibrated using buffer solutions of known pH (4 and 7, respectively) before the measurements. The electrode was inserted into the LT muscle on days 1, 3, 7, and 14 after slaughter.

The parameters L* (lightness), a* (redness), and b* (yellowness) were evaluated using the CIELab system with a Minolta CR-400 Chroma meter (Japan) (with a 10° view angle, D65 illuminant, 8 mm of aperture with a close cone). Each sample was exposed to oxygen for 30 min before reading under room temperature. Six measurements at randomly selected points were recorded per sample.

The water-holding capacity of meat measured as drip loss was assessed using two steaks of each animal collected 24 h *post-mortem*. Each steak was suspended in a net inside a hermetic container and kept at +4°C. After 24 h, the samples were removed from the container and net, dried with absorbent paper, and weighed. Results were expressed as a percentage of the initial weight.

The quantification of weight loss during cooking was performed by recording the sample weights before and after cooking. For this, samples were wrapped in aluminum foil and cooked on a grill (Grill Philco Jumbo Inox, Philco SA, Brazil; previously heated up to 200°C) until an internal temperature of 72°C was achieved. The samples were then removed from the grill and kept at the environmental temperature to cool until reached 25°C, and then the samples were weighed again. Results were expressed as a percentage of the weight before cooking.

The Warner-Bratzler shear force of the previously cooked steaks was measured using a texturometer (TAXT Plus Texture Technologies Corp., Godalming, Surrey, UK) equipped with a Warner-Bratzler blade. The instrument was set to a speed of 2 mm/s, the distance target was 30 mm, and the trigger was 10 g. The samples were cut perpendicular to muscle fibers into rectangular pieces of 1 cm^2, and the measures were performed in six different locations.

The lipid oxidation was measured in triplicate as malonaldehyde (MDA) equivalent to thiobarbituric acid (TBARS). Notably, 5 g of samples and a TCA solution (10 mL; 7.5% TCA, 0.1% gallic acid, and 0.1% EDTA) were mixed, homogenized (Ultra Turrax), and then centrifuged (4,000 rpm; 4°C; 15 min). The supernatant was filtered (Whatman filter paper: 0.16 mm thickness, 20–25 s filtration speed, 4–12 Â µm particle retention) and mixed with TBA reagent (1:1; v/v). The mixture was boiled for 15 min at 100°C and cooled, and the absorbance was measured at 532 nm using a spectrophotometer (Evolution TM 300, Thermo Fisher Scientific, UK). The results were expressed as mg MDA/kg of meat.

In general, according to more recently published studies (Kempinski et al., 2017; Monteschio et al., 2017; Vital et al., 2016), it was observed that the inclusion of different additives in the diet of cattle can increase the antioxidant power in meat, as well as a reduction in lipid oxidation. Thus, the antioxidant activity and lipid oxidation of meat were determined to verify if monensin, virginiamycin, organic minerals, and yeasts have a similar capacity as these additives can alter the animal's immune status. Thus, meat samples were homogenized with methanol (1:1; w/v) using an Ultra-Turrax (IKA® – T10, USA). Then, the samples were centrifuged (4,000 rpm, 15 min) and filtered using filter paper (grammage 80 g/m^2, thickness 205 m, pores 14 m). The antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) assays.

For DPPH, sample extract (150 µL) was mixed with a DPPH methanolic solution (60 µM) (2,850 µL). Absorbance was read at 515 nm after 30 min in dark conditions (Vital et al., 2016). The antioxidant activity was calculated according to the Equation 1:

$$\text{DPPH radical scavenging activity (\%)} = (1 - (A_{\text{sample t}}/A_{\text{sample t}=0})) \times 100 \quad (1)$$

Where:

$A_{\text{sample t}}$: the absorbance of the sample at 30 min;

$A_{\text{sample t}}$: 0 is the absorbance of the sample at time zero.

The ABTS assay was evaluated according to Vital et al. (2016). The $ABTS^+$ was formed by the interaction of 7 mM ABTS

(5 mL) with 140 mM potassium persulfate (88 µL) for 16 h at room temperature in dark conditions. The extract (40 µL) was mixed with $ABTS^{+}$ solution (1,960 µL), and the absorbance was measured after 6 min at 734 nm. The antioxidant activity (%) was calculated according to the Equation 2:

$$\text{ABTS radical scavenging activity (\%)} = (1 - (A_{\text{sample t}}/A_{\text{sample t}} = 0) \times 100 \quad (2)$$

Where:

$A_{\text{sample t}}$: the absorbance of the sample at 6 min;

$A_{\text{sample t}}$: 0 is the absorbance of the sample at time zero.

2.5 Statistical analyses

All parameters were tested for normality (Shapiro–Wilk test) and had a normal distribution. The analysis of variance (ANOVA) was evaluated using the SAS statistical software (Statistical Analysis System, ver. 9.4, Proc GLM).

For drip loss, the statistical model was as follows (Equation 3):

$$Y_{ijk} = \mu + D\text{i} + a_k + e\text{i}_k \quad (3)$$

Where:

Y_{ijk}: the dependent variable;

µ: the overall mean;

D_i: the fixed effect of the diet;

a_k: the random effect of the animal;

ei_k: the random error.

For the other parameters, the statistical model was as follows (Equation 4):

$$Y_{ijk} + \mu + D_i + A_j + a_k + (D_i \cdot XA_j) + e_{ijk} \quad (4)$$

Where:

Y_{ijk}: the dependent variable, µ is the overall mean;

D_i: the fixed effect of the diet;

A_j: the fixed effect of aging;

a_k: the random effect of the bulls;

$(D_iX \cdot A_j)$: the interaction effect of diet and aging;

e_{ijk}: the random error.

In the two models, the mean and standard error of the mean were calculated. Differences between means were evaluated using Tukey's test ($P < 0.05$).

3 RESULTS AND DISCUSSION

3.1 Animal performance and carcass characteristics

Data on animal performance and carcass characteristics were presented in the study by Duarte et al. (2023). The final body weight was greater ($P < 0.05$) for bulls fed with MO+VI (558.7 kg) and MO+AD (554.6 kg) diets, intermediate for bulls fed with MONE (529.3 kg) diet, and lowest for bulls fed with CONT (514.6 kg; data not shown). Similarly, ADG was greater ($P < 0.05$) for bulls fed with MO+VI (2.02 kg) and MO+AD (2.02 kg) diets, intermediate for bulls fed with MONE (1.72 kg) diets, and lowest for bulls fed with CONT (1.57 kg; data not shown). Dry matter intake (DMI, 8.83 kg/day) and the intakes of other nutrients were similar among treatments (data not shown). Feed efficiency was similar for bulls fed with MO+VI and MO+AD diets, with both being more efficient than bulls fed with MONE and CONT diets (data not shown). The worst feed efficiency ($P < 0.05$) was observed for bulls fed with CONT diet. The hot carcass and cold carcass weights were highest for bulls fed with MO+AD and MO+VI diets, intermediate for bulls fed with MONE, and lowest for bulls fed with CONT diet (data not shown). Hot carcass and cold carcass dressing were similar ($P > 0.05$) among the diets with means at 56.8% and 55.2%, respectively.

3.2 Monensin and virginiamycin analyses

It is well accepted that there is a need for studies to confront the growing problem of antibiotics and ionophores in animal feed about possible residues in meat. In this study, no residues of monensin or virginiamycin were observed in the meat of cattle fed with the inclusion of either 30 mg of monensin/kg DM (MONE), 30 mg of monensin/kg DM+30 mg of virginiamycin/kg DM (MO+VI), or 30 mg of monensin/kg DM+3.0 g of Advantage-Confinamento/100 kg BW (MO+AD) in the diet of bulls finished in individual pens for 84 days.

3.3 Meat pH

Meat pH from bulls fed with MONE and MO+AD diets was lower ($P < 0.05$) than meat pH from bulls fed with CONT and MO+VI diets until day 7 of aging (Table 1). The meat pH from bulls fed with MO+AD diet showed a synergistic positive effect as it resulted in lower meat pH (value below 5.8 for 7 days of meat aging), while meat pH from bulls fed with CONT diet was 6.1 before aging. Thus, the addition of MO+AD resulted in a meat pH value that is closer to the ideal pH value of 5.6 (Page et al., 2001). According to some studies, the addition of monensin or virginiamycin separately in the diet of cattle finished in individual pens did not alter the meat pH (Castagnino et al., 2018).

The aging time reduced ($P < 0.05$) the meat pH of all diets from day 1 to day 7, reducing it from 6.0 to 5.7. At 14 days of aging, there was an increase ($P < 0.05$) in the pH of the meat in all treatments (Table 1). The treatments MONE and MO+VI

Table 1. Effect of additives inclusion in the diet and days of aging on pH and meat color on *longissimus* muscle from crossbred bulls ($n = 6$) finished in individual pens.

Parameters	Diets				SEM[E]	*P*-value
	CONT[A]	MONE[B]	MO+VI[C]	MO+AD[D]		
Days			pH_{24h}			
1	6.09aA	5.92A	6.05bA	5.90A	0.033	0.002
3	5.90aB	5.71bB	5.86aB	5.80bB	0.028	0.004
7	5.70aC	5.63bC	5.78aB	5.68bC	0.026	0.017
14	6.24aA	6.02bA	6.02bA	6.33aA	0.060	0.021
SEM[5]	0.052	0.027	0.055	0.032		
P-value	0.009	0.014	0.013	0.006		
			L*			
1	38.14bB	39.86aB	37.52bB	39.73aB	0.206	0.001
3	39.78cA	42.75aA	40.54bA	41.87aA	0.233	0.001
7	38.02bB	38.99aB	38.01bB	40.19aB	0.282	0.017
14	36.01C	35.67C	35.43C	36.09C	0.258	0.790
SEM[5]	0.317	0.269	0.287	0.275		
P-value	0.003	0.001	0.001	0.001		
			a*			
1	12.94B	13.27B	12.82B	11.85B	0.193	0.057
3	12.75B	13.05B	13.71AB	13.75A	0.140	0.055
7	14.00A	13.80B	14.02A	13.57A	0.165	0.757
14	14.31A	15.38A	13.88AB	14.33A	0.193	0.041
SEM[5]	0.217	0.161	0.158	0.184		
P-value	0.023	0.001	0.031	0.001		
			b*			
1	12.04	12.99B	12.14B	12.00C	0.163	0.103
3	13.03b	13.93abA	13.97aA	14.58aA	0.132	< 0.001
7	12.94b	13.42abA	13.34abA	13.84aAB	0.123	0.078
14	12.34	12.82B	12.16B	12.94B	0.172	0.319
SEM[5]	0.181	0.128	0.161	0.148		
P-value	0.159	0.009	0.001	< 0.001		

[A]CONT: no additives added; [B]MONE: inclusion of 30 mg of monensin/kg DM; [C]MO+VI: inclusion of 30 mg of monensin/kg DM + 30 mg of virginiamycin/kg DM; [D]MO+AD: inclusion of 30 mg of monensin/kg DM + 1.57 g of Advantage Confinamento/kg DM; [E]SEM: standard error of means. Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different from Tukey's test ($P < 0.05$).

showed the lowest values at 14 days of aging ($P < 0.05$). Thus, the aging time of up to 7 days reduces the meat pH, but a longer period (14 days) increases it. This increase in late storage may be caused by the growth of spoilage bacteria leading to the accumulation of alkaline components (e.g., ammonia and trimethylamine). Similar results were obtained by Herrera-Mendez et al. (2006).

The observed meat pH overall in this study (5.9), although acceptable, is slightly high. The relatively high pH in this experiment could be explained, in part, by animal genetics. In general, Zebu cattle show a higher pH after slaughter due to reactive behavior during transport and handling before slaughter. Other studies in similar conditions observed a meat pH above 6.2 for crossbred cattle (*Bos taurus taurus* × *Bos taurus indicus*) cattle finished in individual pens (Françozo et al., 2013; Maggioni et al., 2012).

3.4 Meat color

At 1, 3, and 7 days of aging, L* values were greater ($P < 0.05$) for meat from bulls fed with MONE and MO+AD diets (Table 1). Lower L* values were observed in meat from bulls fed with CONT and MO+VI diets (darker meat). This might be explained by the high pH values (Hughes et al., 2017) found in this study. Thus, according to the L* values, the meat that may be the most attractive to the consumer before and at days 3 and 7 of aging was meat from bulls fed with MONE and MO+AD diets, with values close to 40 (Table 1). On the last evaluation day (day 14), the L* values were similar ($P > 0.05$) for meat from bulls fed with all diets (Table 1). Those values were lower compared with before aging (35.8 and 38.8, respectively), characterizing darker meat, therefore, and would most likely be viewed as unattractive to the consumer. The L* ideal value in red meat is considered from 38 to 40 points (Page et al., 2001).

Monensin, virginiamycin, and Advantage-Confinamento inclusion in the diets did not alter ($P > 0.05$) a* values in the meat of bulls (Table 1). The aging time led to an increase ($P < 0.001$) in a* values in the meat from bulls fed with all diets (Table 1). In this context, aging results in meat that is most likely to be more attractive to the consumer, as higher a* values represent a red color (Page et al., 2001). However, under similar conditions of feeding, handling, and genetic groups of bulls, a* values ranged from 11 (Monteschio et al., 2017) to 18 (Rivaroli et al., 2016, 2020) with intermediate values from 14 to 17 (Fugita et al., 2018; Ornaghi et al., 2020). Thus, a* values observed in this experiment are close to the values observed for crossbred bulls (*B. taurus taurus* × *B. taurus indicus*) under similar feeding and handling conditions.

The b* values characterize the yellow color of meat. Higher b* values characterize meat that is more yellowish, less dark, and therefore more attractive to the consumer. Except for meat from bulls fed with CONT diet, aging increased the b* value from day 1 to day 7 (Table 1), thus making the meat more likely to be attractive to consumers. On days 3 and 7 of aging, the b* values were higher ($P < 0.05$) for the meat from bulls fed with the addition of additives to the diets, but without differences among diets with additives (Table 1). However, on day 14, the value of b* declined to a value similar to that observed before aging. The parameter b* shows that aging for up to 7 days would most likely aid visual color appearance and improve perceived freshness to the consumer. However, a longer aging time (14 days) has no added visual benefit for the meat. In general, meat b* value from bulls finished in individual pens and slaughtered close to 24 months and fed with high-grain diets for 90 days varies from 10 to 14 (Fugita et al., 2018; Monteschio et al., 2017; Ornaghi et al., 2020). Thus, the values observed in this experiment for the b* parameter are close to normal values.

3.5 Drip loss and cooking loss

At 24 h after slaughter, monensin, virginiamycin, and Advantage-Confinamento inclusion in the diets did not alter ($P > 0.05$) water-holding capacity assessed as drip loss compared with CONT (Table 2). Drip losses were low and varied from 1.4 to 1.8%. Lactic acid formation post-mortem is responsible

Table 2. Effect of additives inclusion in the diet and days of aging on water-holding capacity and drip and cooking losses *on longissimus* muscle from crossbred bulls (n = 6) finished in individual pens.

Days	Diets				SEM[E]	*P*-value
	CONT[A]	MONE[B]	MO+VI[C]	MO+AD[D]		
Drip losses (% over 24 h)						
1	1.52	1.43	1.80	1.52	0.132	0.827
Cooking losses (%)						
1	29.35A	31.64A	32.51A	31.66A	0.492	0.100
3	18.89bB	23.37aB	23.12aB	23.74aB	0.173	0.001
7	22.92bB	21.52bB	25.08aB	25.93aB	0.142	0.001
14	20.63bB	26.1aB	21.99bB	18.89cC	0.150	0.001
SEM[E]	0.283	0.547	0.336	0.425		
P-value	< 0.001	< 0.001	< 0.001	0.003		

[A]CONT: no additives added; [B]MONE: inclusion of 30 mg of monensin/kg DM; [C]MO+VI: inclusion of 30 mg of monensin/kg DM + 30 mg of virginiamycin/kg DM; [D]MO+AD: inclusion of 30 mg of monensin/kg DM + 1.57 g of Advantage Confinamento/kg DM; [E]SEM: standard error of means. Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different from Tukey's test ($P < 0.05$).

Table 3. Effect of additives inclusion in the diet and days of aging on meat Warner-Bratzler shear force (kgf/cm^2) on *longissimus* muscle from crossbred bulls (n = 6) finished in individual pens.

Days	Diets				SEM[E]	*P*-value
	CONT[A]	MONE[B]	MO+VI[C]	MO+AD[D]		
1	7.16bA	9.84aA	9.12aA	9.42aA	0.423	0.001
3	6.15bB	8.35aB	5.83bB	6.72bB	0.339	0.005
7	5.69bB	7.13aC	5.33bB	5.15bC	0.288	0.082
14	5.36aC	5.53aD	4.85bC	3.86bD	0.245	0.001
SEM[E]	0.367	0.448	0.325	0.270		
P-value	0.012	0.001	0.001	0.001		

[A]CONT: no additives added; [B]MONE: inclusion of 30 mg of monensin/kg DM; [C]MO+VI: inclusion of 30 mg of monensin/kg DM+ 30 mg of virginiamycin/kg DM; [D]MO+AD: inclusion of 30 mg of monensin/kg DM+ 1.57 g of Advantage Confinamento/kg DM; [E]SEM: standard error of means. Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different from Tukey's test ($P < 0.05$).

for the drop in pH and consequently lowers the ability of the meat to retain water (Monteschio et al., 2017). A high meat pH was observed in this study, which can result in the retention of water in the cells due to fewer losses to capillary forces (gravity). Drip loss 24 h after slaughter was similar to those reported by Monteschio et al. (2017) and Ornaghi et al. (2020) for meat from crossbred bulls finished in individual pens and fed with a high-grain diet.

Before aging, meat cooking losses were similar ($P > 0.05$) for meat from bulls fed with all diets (Table 2). At 3 days of aging, cooking losses were lower ($P < 0.05$) for meat from bulls fed with CONT diet. On day 7 of aging, meat cooking losses were lower ($P < 0.05$) from bulls fed with CONT and MONE. On the contrary, in the last aging time (14 days), cooking losses were lower ($P < 0.05$) for meat from bulls fed with MO+AD diets (18.9%). The greatest cooking losses ($P < 0.01$), regardless of diet, were observed before aging (day 1), close to 31.3% in comparison with the other aging days (Table 2). Thus, the greatest loss of water by cooking occurred before meat aging.

During aging, meat water loss is expected as a consequence of changes in muscular fibers caused by rigor mortis and modifications of myofibrillar structure (Huff-Lonergan & Lonergan, 2005). It is known that cooking denatures the muscle proteins, which directly influences the structural characteristics (Pearce et al., 2011). The changes lead to a substantial cooking loss in the range of 20–30% (Ornaghi et al., 2020; Rivaroli et al., 2016, 2020). However, the amount of cooking losses is dependent on the cooking method, cooking time, and end-point temperature (Pearce et al., 2011).

3.6 Warner-Bratzler shear force

The Warner-Bratzler shear force (kgf) measured on the first day of the evaluation was lower ($P < 0.001$) for the meat from bulls fed with CONT diet in comparison with meat shear force from bulls fed with the addition of additives in the diets (Table 3). On days 3 and 7 of evaluations, shear force values from bulls fed with MONE diet were higher ($P < 0.05$) when compared with shear force values from bulls fed with the other three diets. On the last evaluation day (14 days), shear force values from bulls fed with MO+VI or MO+AD diets were lower ($P < 0.05$) when compared with shear force values from the meat of bulls fed with CONT and MONE diets. In the general context of the 4 days of evaluations, the shear force values coincided with a tougher shear force for the meat from bulls fed with MONE diet in comparison with meat from bulls fed with other diets. In this way, MO+VI or MO+AD addition resulted in more tender meat.

Before aging, the mean shear force was close to 8.9 kgf, which is not considered tender meat. The shear force values in this study varied from 8.9 kgf before aging to 4.96 kgf 14 days after aging at 4°C. Meat aging time linearly reduced the meat Warner-Bratzler shear force of bulls fed with either of the four diets. Bulls with Zebu genetics such as Nellore bulls used in this study have less tender meat compared with bulls with European genetics due to the calpain-calpastatin complex (Shackelford et al., 1994), which inhibits calpain activity that is responsible for the degradation of myofibrillar proteins during *rigor mortis*. This proteolysis is an important process in the establishment of tenderness. Several studies carried out with crossbred bulls (*B. taurus taurus* × *B. taurus indicus)*, finished in individual pens with age close to 24 months showed a shear force varying from 9 to 4 kgf during the aging period for 14 days (Eiras et al., 2016; Monteschio et al., 2017; Ornaghi et al., 2020).

3.7 Lipid oxidation

Monensin, virginiamycin, and Advantage-Confinamento inclusion in the diets of bulls finished in individual pens did not alter ($P > 0.05$) lipid oxidation (Table 4). Thus, none of the dietary treatments altered the meat's protection against lipid oxidation. On the contrary, lipid oxidation increased with meat aging (Table 4). The TBARS values increased from 0.37 mg MDA/kg of fresh meat on day 1 to 0.71 mg MDA/kg of fresh meat on day 14 of aging. The aging time is the factor that has the greatest effect on lipid oxidation due to the presence of oxygen that accelerates the oxidation process (Shahidi & Zhong, 2010).

Table 4. Effect of the inclusion of additives in the diet and days of aging on meat lipid oxidation (mg/malonaldehyde/kg^{-1} of meat) and antioxidant activity on *longissimus* muscle from crossbred bulls (n = 6) finished in individual pens.

Days	Diets				SEM[E]	*P*-value
	CONT[A]	MONE[B]	MO+VI[C]	MO+AD[D]		
Thiobarbituric acid (TBARS)						
1	0.35C	0.40B	0.37C	0.36C	0.015	0.729
3	0.47B	0.43B	0.51B	0.49B	0.027	0.805
7	0.49B	0.46B	0.54B	0.50B	0.025	0.775
14	0.70A	0.74A	0.70A	0.68A	0.036	0.891
SEM[E]	0.034	0.040	0.031	0.033		
P-value	0.001	0.003	0.002	0.001		
2,2-Diphenyl-1-picrylhydrazyl (DPPH, %)						
1	22.93	21.36	25.04	21.57	0.966	0.541
3	25.72	19.47	27.25	25.53	1.149	0.080
7	23.93	21.63	27.33	22.95	0.723	0.076
14	22.14	23.78	24.80	22.36	0.824	0.659
SEM[E]	1.310	1.262	0.847	0.736		
P-value	0.320	0.080	0.607	0.060		
2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS,%)						
1	43.40C	45.09C	46.54C	47.40B	1.519	0.823
3	47.25B	50.56CB	51.54BC	44.43B	1.244	0.162
7	55.04ABb	63.74Aba	63.53Aa	61.63Aa	1.038	0.003
14	62.64A	66.50A	63.10AB	63.19A	1.833	0.888
SEM[E]	1.899	2.231	2.064	2.152		
P-value	0.001	0.004	0.009	0.001		

[A]CONT: no additives added; [B]MONE: inclusion of 30 mg of monensin/kg DM; [C]MO+VI: inclusion of 30 mg of monensin/kg DM + 30 mg of virginiamycin/kg DM; [D]MO+AD: inclusion of 30 mg of monensin/kg DM + 1.57 g of Advantage Confinamento/kg DM; [E]SEM: standard error of means. Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different from Tukey's test ($P < 0.05$).

Before aging, the TBARS values were high (0.37 mg MDA/kg fresh meat) when compared with the values observed by other authors (Monteschio et al., 2017; Prado et al., 2015; Rivaroli et al., 2016, 2020). The TBARS values were close to 0.7 mg MDA/kg of fresh meat at the end of the aging period (14 days). MDA/kg values of fresh meat above 2.0 mg make it rancid with rejection to human consumption (Campo et al., 2006). In this experiment, these values were well below, therefore, without harmful effect on meat consumption.

3.8 Antioxidant activity

The meat antioxidant activity was measured using two methodologies: DPPH and ABTS (Table 4). The inclusion of monensin, virginiamycin, and Advantage-Confinamento ($P > 0.05$) did not cause either a positive or negative effect on meat antioxidant activity, unlike the inclusion of vegetable oils that significantly increases the antioxidant activity (Monteschio et al., 2017; Vital et al., 2018). Likewise, the aging time did not influence ($P > 0.05$) the antioxidant activity in meat measured by DPPH methodology (Table 4). Thus, the aging time does not harm meat antioxidant activity from bulls finished in individual pens and fed with antibiotics, ionophores, and trace minerals. However, when the antioxidant activity was measured using the ABTS methodology, the values on day 7 of the evaluation were lower ($P < 0.05$) for the meat from bulls fed with CONT diet in comparison with meat from bulls fed with additives addition in the diets (Table 4). Moreover, an increase in antioxidant activity was observed during the aging time, i.e., the values increased from 43.4% before aging to 66.5% after 14 days of aging.

Although the yeast could have some antioxidant effect, the inclusion in the MO+AD treatment did not show a positive effect during the 84 days, which was not sufficient to influence the antioxidant activity of the meat. Among these compounds are mannans and glucomannans that have antioxidant activity (Singh et al., 2018). Also, organic selenium is present in its composition, which has antioxidant activity related to the antioxidant enzyme glutathione peroxidase acting in delaying *post-mortem* oxidation reactions in muscle tissue.

4 CONCLUSION

The inclusion of monensin and virginiamycin with or without trace minerals + yeast to the diet of cattle did not result in meat containing the respective antibiotic residues. Furthermore, monensin and virginiamycin potentially enhanced some parameters of meat quality, such as the color of meat. In particular, the combination of monensin and a blend of organic trace minerals and yeast (Advantage-Confinamento) resulted in lower cooking losses and Warner-Bratzler shear force, which may beneficially impact meat quality to the consumer.

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