South African Journal of Animal Science 2024, VOL 54

Natural additives as a source of antioxidants improve lipid oxidation, antioxidant activity, and shelf-life of beef

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(Submitted 30 August 2023; Accepted 22 February 2024; Published 26 March 2024)

Abstract

Forty young bulls were finished in feedlot with diets that contained different dosages of a combination of several natural additives (NA), i.e., clove essential oil, cashew and castor oil, and rumen-protected eugenol, vanillin, and thymol. The animals were randomized in five diets containing four different inclusion levels of NA and a control diet (*n* = 8 animals per treatment): basal diet without NA (CON); NA15 = 1500 mg/day; NA30 = 3000 mg/day; NA45 = 4500 mg/day, and NA60 = basal diet with 6000 mg/day of the natural additives blend. Colour, antioxidant activity (DPPH, ABTS and FRAP assays), lipid oxidation, and visual acceptability were evaluated through display (until 14 d in vacuum or film packages). Both factors (diet and display) affected all parameters evaluated. The highest dosage, NA60, was able to improve the antioxidant potential, decreasing beef oxidation to produce a higher visual acceptability. The results of this research provide evidence that NA included in the diet of beef cattle can improve overall meat quality and extend shelf-life, thus, providing higher visual acceptability through the colour perception of consumers.

Keywords: Bioactive compounds; essential oils; eugenol; natural antioxidants; oxidation stability; thymol
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Introduction

Consumer awareness of the impacts of food on human health has increased in recent decades, especially for products of animal origin (Clonan *et al.*, 2015; Ducatti *et al.*, 2009). There is an increasing interest in products that are safe, healthy, ethical, with a low impact on the environment that are affordable (Bosona & Gebresenbet, 2013; Monteschio *et al.*, 2017, Monteschio *et al.*, 2020; Vital *et al.*, 2018). Natural additives (NA), such as plant extracts and essential oils, are a promising option to be used in the diet of ruminants as shown by evidence of improved animal performance and meat quality (Beauchemin *et al*., 2020; Hayajneh, 2019; Ornaghi *et al.*, 2020; Pateiro *et al.*, 2018; Rivaroli *et al.*, 2016, 2020).

The main causes of meat deterioration are microbiological and non-microbiological factors. Fletcher *et al.* (2018) reported a decrease in meat quality due to microbial spoilage (i.e., breakdown the

meat components due to bacterial and fungal growth). The latter is caused by oxidation of lipids and proteins of meat products during storage, thus affecting sensory properties such as colour, odour, flavour, texture, and ultimately consumer preference.

Beef is enriched with saturated (~45%, based on total fatty acids), unsaturated fatty acids (~40 %, based on total fatty acids) and has a minor proportion of polyunsaturated fatty acids (~5%, based on total fatty acids) (Ducatti et al., 2009; Rotta et al., 2009). Fatty acids, especially unsaturated, are prone to oxidation and rancidness, directly affecting meat odour (Xiao *et al.*, 2011). Meat colour is another key factor that will be affected by this reaction influencing consumer preferences. The cherry red colour of meat is correlated to freshness and is highly desired by consumers (Monteschio *et al.*, 2017; Passetti *et al.*, 2017; Torrecilhas *et al.*, 2018). Meat oxymyoglobin exposed to air will rapidly be oxidized to metmyoglobin, resulting in a brown-like colour, which is rejected by consumers (Suman & Joseph, 2013).

The susceptibility of meat components to oxidation can be influenced by several ante-mortem factors such as: animal species, breed, diet, stress, livestock management, muscle fibre type, and anatomical location (Min *et al*., 2008). Cellular systems are responsible for oxidative stress and the production of free radicals, which will induce muscle/meat oxidation. Thus, accumulation of free radicals can cause functional and structural damage to muscle organelles, cells, and tissues, even post-mortem (Sies *et al.*, 2017).

Natural additives can be a simple and manageable option to modulate the antioxidative status of the meat by exploiting plant extracts with free radical scavenging activity (Al-Zubairi *et al.*, 2017; Kleinberg *et al.*, 2019). Eugenol and thymol are some of the bioactive compounds from clove and thyme essential oils, respectively, and have some of the highest antioxidant activities known among natural compounds due to their molecular form (phenols), which can reduce free radical formation and scavenge of ROS (reactive oxygen species). However, improving meat quality and increasing storage time (shelf-life) of red meat using these bioactive compounds in the diet is challenging due to the nature of the rumen, a fermentation chamber that hosts bacteria, fungi, protozoa, and bacteriophages, which degrade and modify dietary components (Richardson *et al.*, 2019). Thus, including these promising compounds in a rumen-protected manner could be effective in increasing the absorption of bioactive compounds.

Exploration of farm-level practices to tackle meat industry challenges, such as oxidative instability of lipids in meat, is needed. The hypothesis of the current study was that NA with bioactive compounds of high antioxidant activity included in the finishing diet of beef cattle able to withstand rumen conditions would reduce beef lipid oxidation, improve colour stability, and increase shelf life.

Materials and Methods

All animal care and experimental procedures were conducted under the surveillance of the Animal Care and Use Committee of the Universidade Estadual de Maringá, Brazil (protocol no. 8583060318). A total of 40 young bulls (½ Angus x ½ Nellore) of 16 ± 2.2 months of age and with a body weight of 385.82 ± 20.67 kg were used.

The basal diet was offered *ad libitum* for 62 days and consisted of 72% concentrate (cracked corn, soybean meal, mineral mixture, limestone, and yeast) and 28% corn silage. The animals were randomized to five diets (n = 8 animals per treatment): basal diet without NA (CON); basal diet with 1500 mg/day of a blend of NA (153.1 mg/animal/kg of dry matter basis; NA15); basal diet with 3000 mg/day of a blend of NA (305.2 mg/animal/kg of dry matter basis; NA30); basal diet with 4500 mg/day of a blend of NA (444.7 mg/animal/kg of dry matter basis; NA45); basal diet with 6000 mg/day of a blend of NA (594.6 mg/animal/kg of dry matter basis; NA60). The blend of NA was composed of essential oil from the leaves of clove (*Syzygium aromaticum*; Ferquima®, São Paulo, Brazil), functional oils from castor (*Ricinus communis*; Safeeds®, Cascavel, Paraná, Brazil) and cashew (*Anacardium occidentale*; Safeeds®, Cascavel, Paraná, Brazil), and a commercial blend composed by a mix of rumen-protected vanillin, eugenol, and thymol (Safeeds®, Cascavel, Paraná, Brazil). Sources and doses were defined based on the previous findings from our research group investigating the synergistic effects between compounds (Monteschio *et al.*, 2017; Ornaghi *et al.,* 2017; Valero *et al*., 2016).

At day 62 in the feedlot, the bulls were weighed after 16 h of fasting (482 ± 31.9 kg) and transported to a commercial slaughterhouse. The young bulls were slaughtered following the usual practices of the Brazilian beef industry, according to the Brazilian Regulation of Industrial and Sanitary Inspection Animal Products (RISPOA). The Longissimus muscle was excised from the left half of the carcass from the seventh to the last lumbar vertebra 24 h after slaughter and stored until further analysis. Steaks of 2.5 cm thickness were prepared for colour, antioxidant activity, and lipid oxidation evaluations. Steaks of 2.0 cm thickness were used for visual analysis. Vacuum storage was used to evaluate oxidation

stability and antioxidant activity (at days 1, 3, 7, and 14). Film packaging was used to evaluate shelf life by displaying the steaks from 1 to 14 days, analysing oxidative stability, antioxidant activity, colour, and visual perception by consumers.

The FRAP method was performed according to the method of Zhu *et al.* (2002). Samples were mixed with methanol and an aliquot (250 µL) was mixed with 50 mM sodium phosphate buffer (pH 7; 1.25 mL) and 1% potassium ferricyanide (1.25 mL), and incubated at 50 °C for 20 min. Then, 1.25 mL of trichloroacetic acid (TCA; 10%) was added and the mixture was centrifuged at 3,000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 500 µL 0.1% ferric chloride and the absorbance was measured at 700 nm. Results were expressed as mg of gallic acid equivalent (GAE) g^{-1} oil, mg of GAE g^{-1} coating, and mg of GAE per 100 g^{-1} of meat. The standard curve of gallic acid was 0−300 mg per l^{-1}.

The ABTS assay was conducted according to the method of Re et al. (1999), with modifications (Vital *et al.*, 2016). ABTS·+ was generated by the interaction of 7 mM ABTS (5 mL) with 140 mM potassium persulfate (88 µL). The mixture was incubated in the dark at 25 °C for 16 h. The ABTS-activated radical was diluted with ethanol to an absorbance of 0.70 ± 0.02. The radical scavenging activity (%) was measured at 734 nm. Samples (40 µL) were mixed with ABTS·+ solution (1960 µL) and absorbance was recorded after 6 min. The radical scavenging activity (%) was calculated as:

$$\text{ABTS activity (\%)} = (1-(\text{Abs } t/\text{Abs } t=0))*100 \quad (1)$$

where: A sample t = 0: absorbance of the sample at time zero; A sample t: absorbance of the sample after 6 min.

DPPH activity was determined according to the method of Li *et al.* (2009), with modifications (Vital *et al.,* 2016). Samples (150 µL) were mixed with a methanolic solution (2850 µL) containing DPPH (60 µM) for 30 min. The absorbance was read at 515 nm. Antioxidant activity was calculated as:

$$\text{DPPH activity (\%)} = (1-(\text{Abs } t/\text{Abs } t=0))*100 \quad (2)$$

where: A sample t = 0: absorbance of the sample at time zero; A sample t: absorbance of the sample after 30 min.

Lipid oxidation assays were performed after 1, 3, 7, and 14 days of display (vacuum or film package storage). The malonaldehyde (MDA) content in meat was quantified using the thiobarbituric acid reactive substances (TBARS) assay, according to the method of Souza *et al.* (2011). The absorbance was measured at 540 nm against the MDA standard. Results were expressed as mg MDA/kg of meat.

The colour was evaluated using the CIELab system with a Minolta CR- 400 Chromameter (Japan) with a 10° view angle, D65 illuminant, and 8 mm of aperture with a closed cone. Six measurements at randomly selected points were recorded per sample: lightness (L*), redness (a*), and yellowness (b*). Vacuum-packed samples were allowed to bloom for 30 min before colour evaluation.

Steaks were photographed every 2 d until 14 d on display using a NIKON D3100 digital camera mounted on a photographic stand and containing two D65 fluorescent light tubes as a standard illuminant. An additional grey-colour cardboard was used to cover the entrance of the cabinet to provide evenly-distributed lighting across the sample and to avoid exposure to external light. The camera was fixed perpendicularly, 45 cm away from the surface of the meat sample. following standardized conditions for photography (Passetti *et al*., 2017, 2019). The following camera parameters were used: manual mode; 1/20 shutter speed; F5.3 aperture size; 1600 ISO; and 40 mm focal distance. Images were exported as JPEG files. A Gretag Macbeth Mini-Colour-Checker (Colour-confidence, Birmingham, UK), which contains 24 coloured patches was photographed with each meat sample to check the colour reproduction capability. Consumer-based visual acceptability was conducted with semi-trained evaluators (*n* = 61 evaluators) to evaluate the colour acceptability of the meat. Photos were presented in random order (Passetti *et al.*, 2017). Consumers evaluated the meat using a nine-point, structured hedonic scale (1 = dislike extremely to 9 = like extremely) to assess the visual acceptability of the meat. The shelf-life was limited by the number of days at which the samples were assigned with scores ≥4.5.

Data were tested for normality (Shapiro–Wilk test) and were analysed using analysis of variance in R statistical software (R Development Core Team, 2014). The dietary effect was evaluated using orthogonal contrasts, which were used to assess the effects of the control diet versus diets with NA, with linear and quadratic responses (P <0.05). The effect of display on meat quality variables (colour, antioxidant activity, and lipid oxidation) and the instrumental meat colour were evaluated. Differences between display time means were assessed using Tukey's Test (P <0.05). Once the fitted regression equations were determined, the response surface plots were drawn using R statistical software (R-Core-Team, 2016).

Data of visual acceptability were imported into an Excel matrix after checking for missing data and outliers. Visual acceptability scores were analysed in the IBM Statistical Package for the Social Sciences (SPSS version 24), using a General Linear Model (GLM) with days of display and experimental diet considered as fixed effects. To analyse the evolution of scores among the display period, a simple regression for the effect of days was performed.

In all statistical analyses, the experimental diet was considered as fixed effects and the animal was considered a random effect. Dietary means were computed using the LSMEANS option.

$$Yij = \beta0 + \beta1Xi + \beta2Xi2 + \varepsilon ij \quad (3)$$

where:
Yij observation of the repetition j on diet i;
β0 general coefficient;
β1 linear regression coefficient of the variable observed depending on the levels;
β2 quadratic regression coefficient of the variable observed depending on the levels;
Xi independent variables (experimental diet);
Eij residual error

Results

Increasing antioxidant activity was observed in the meat with increasing levels of NA (P <0.05; Figure 1; Table 1; Supplementary Tables S5–S10). The antioxidant power delayed the oxidation when meat was vacuum packaged (Figure 2; Table 11S) and consequently increased the shelf life through the maintenance of meat colour (Table 2).

Table 1 Regression coefficients of the proposed model for the variables of response surface: Antioxidant analysis (FRAP, ABTS, and DPPH) and TBARS

Factors	FRAP[1]	FRAP[2]	ABTS[3]	ABTS[4]	DPPH[5]	DPPH[6]	TBARS[7]	TBARS[8]
Constant	80.2965	77.8940	19.7859	27.0112	13.8184	14.8393	0.4949	0.4782
Diet	-18.0010	-4.2361	-0.2762	-1.2984	-0.2509	0.1569	-0.0170	-0.0625
Display (Day)	4.2261	-1.7967	-0.5310	-1.3427	-0.0924	-0.1519	0.0397	-0.0068
Diet × Display	0.0098	0.0262	0.0178	-0.0071	0.0139	0.0276	-0.0005	0.0040
R^2	0.4440	0.9020	0.3498	0.2116	0.0339	0.8680	0.4244	0.3843
Lack of fit	0.0461	>0.0001	>0.0001	>0.0001	0.0276	0.0002	0.9523	0.1027
P-value Diet	0.1023	< 0.001	0.0017	0.0005	0.0976	< 0.001	0.0101	0.0478
P-value Display	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P-value Diet × Display	0.004	0.334	0.0059	0.0467	0.4880	0.1231	0.983	0.877

FRAP[1] = vacuum package; FRAP[2] = film package; ABTS[3] = vacuum package; ABTS[4] = film package; DPPH[5] = vacuum package; DPPH[6] = film package; TBARS[7] = vacuum package; TBARS[8] = film package.

Table 2 Colour variables during display of beef from young bulls finished in feedlot with natural additives

Display (Dp);	Diets (Dt)							P-value	
days	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]	SEM[6]	L[7]	Q[8]	0% vs blend
Lightness (L*)									
1	38.93a	38.39	38.66	40.64	39.77	0.254	0.099	0.262	0.254
7	41.29b	40.11	41.36	42.36	42.21	0.313	0.061	0.136	0.769
14	40.19b	40.10	40.25	41.35	41.60	0.395	0.138	0.297	0.520
SEM	0.361	0.360	0.521	0.360	0.500			P (Dt x Dp)[9]	
P-value	0.017	0.720	0.088	0.140	0.111			0.931	
Redness (a*)									
1	13.30a	13.58a	13.68a	13.74a	13.48a	0.142	0.626	0.588	0.406
7	15.39b	15.57b	15.68b	15.72b	15.70b	0.189	0.552	0.803	0.562
14	14.46b	14.59b	14.36ab	14.56ab	14.49ab	0.197	0.971	0.999	0.933
SEM	0.289	0.302	0.247	0.268	0.300			P (Dt x Dp)[9]	
P-value	0.006	0.015	0.001	0.004	0.004			1.000	
Yellowness (b*)									
1	12.45a	12.81a	12.62a	13.51a	12.99a	0.156	0.032	0.080	0.583
7	14.79b	14.45b	15.16b	15.41b	15.47b	0.164	0.037	0.114	0.400
14	14.14b	13.92b	14.06b	14.56b	14.42b	0.168	0.299	0.549	0.806
SEM	0.261	0.209	0.320	0.220	0.290			P (Dt x Dp)[9]	
P-value	0.001	0.002	0.001	0.001	0.001			0.885	

[1]CON = control (without natural additives); [2]NA15 = addition of 1,500 mg/animal/day of natural additives; [3]NA30 = addition of 3,000 mg/animal/ day of natural additives; [4]NA45 = addition of 4,500 mg/animal/day of natural additives; [5]NA60 = addition of 6,000 mg/animal/day of natural additives; [6]Standard error of means; [7]Linear effect; [8]Quadratic effect. [9]P (Dt × Dp): P value interaction Diet × Display. [a-b:] Values within a column with different superscripts differ significantly at $P < 0.05$

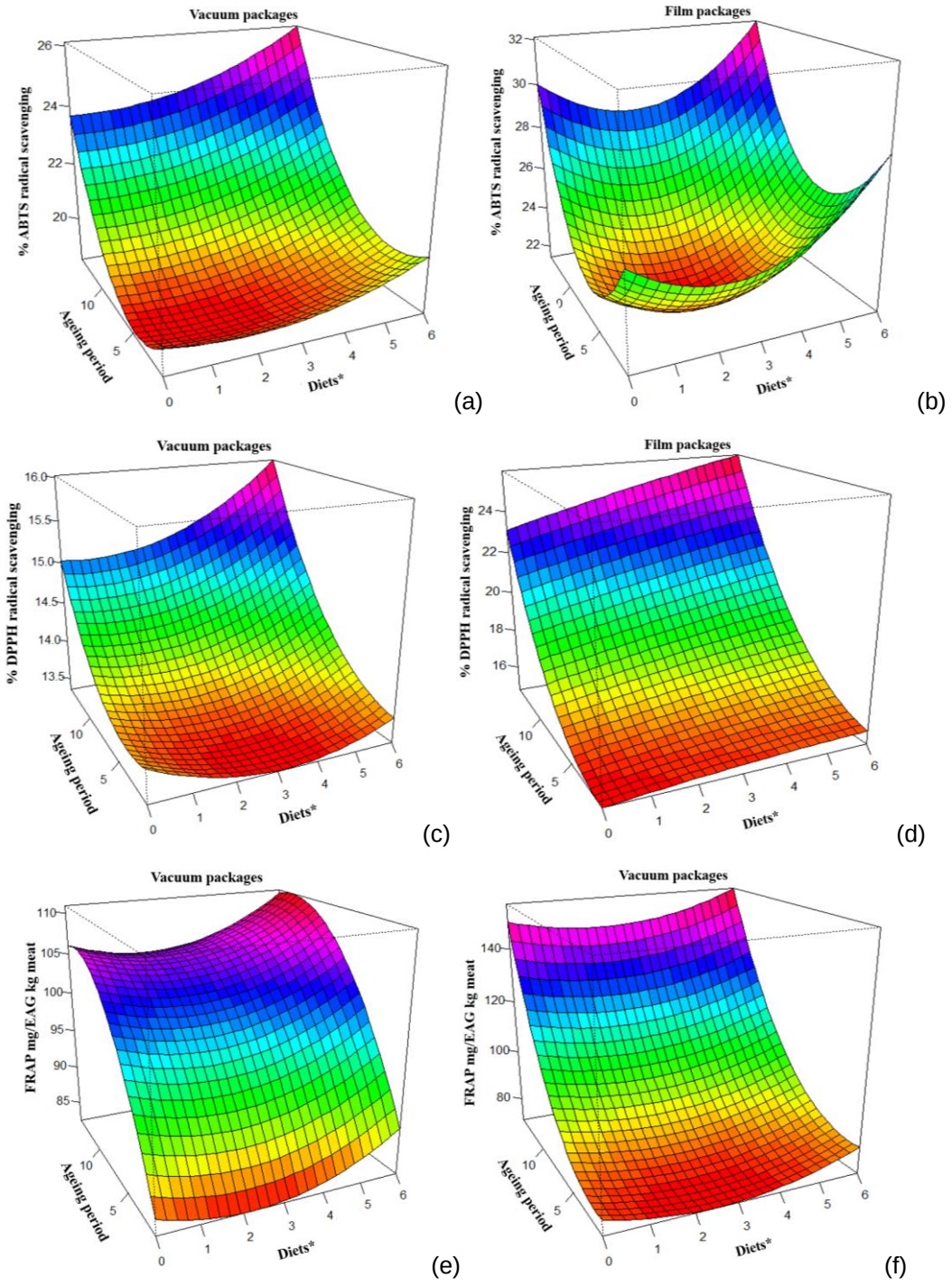

Figure 1 Response surface of the antioxidant activity on meat of young bulls finished in the feedlot with natural additives: (a) ABTS radical scavenging (%) on meat storage in vacuum packages, (b) ABTS radical scavenging (%) on meat storage in film packages, (c) DPPH radical scavenging (%) on meat storage in vacuum packages (d) DPPH radical scavenging (%) on meat storage in film packages, (e) Ferric reducing power (FRAP mg/EAG kg meat) on meat storage in vacuum packages, (f) Ferric reducing power (FRAP mg/EAG kg meat) on meat storage in film packages. *Diets (experimental diets: 0 = without blend; 1–6 = levels of addition of the natural additives blend: 1,500; 3,000; 4,500; 6,000 mg/animal/day, respectively)

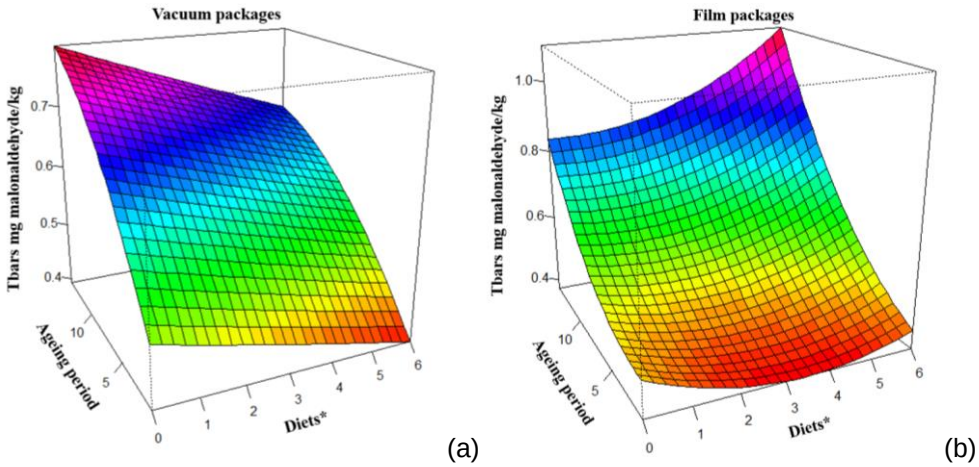

Figure 2 Lipid oxidation on meat of young bulls finished in the feedlot with natural additives: (a) in vacuum packages and (b) film packages; (TBARS) expressed as mg malonaldehyde/kg of meat during storage time. *Diets (experimental diets: 0 = without blend; 1–6 = levels of addition of the natural additive blend: 1,500; 3,000; 4,500; 6,000 mg/animal/day, respectively)

The lipid oxidation of beef, expressed as the MDA production, was affected by the inclusion of NA (P <0.05; Figure 2; Tables S11, S12). Furthermore, an increased concentration of MDA with display time (1, 3, 7, and 14 days) was expected. There was no interaction between diet and display. The L* was similar from 1 to 14 d of storage (P >0.05). However, at day 7 of storage, a tendency (P = 0.06) of an increase in the L* value was observed. The addition of NA had no effect on a* values (P >0.05). The diets influenced (P <0.05; Table 2) the yellowness (b*), where the values increased until day 7, with values ranging from 12.42 to 15.47.

There was an interaction between treatments and display on visual acceptability (P <0.001; Table 3). Visual consumer acceptability scores ranged from 6.23 to 6.78 on the first day. On the third day of evaluation, an increase on the acceptability of meat with NA was observed (P <0.05). There was a linear effect of the addition of NA in the diet of cattle when meat was evaluated at day 14 (P <0.05).

Table 3. Visual acceptability on display[§] of meat of young bulls finished in the feedlot with natural additives as assessed by consumers (n = 61)

Display (Dp);	Diets (Dt)						P-value		
days	CON[1]	NA15[2]	NA30[3]	NA45[4]	NA60[5]	SEM[6]	L[7]	Q[8]	0% vs blend
1	6.58b	6.23bc	6.38bc	6.78b	6.36b	0.031	0.001	0.1543	0.143
3	7.00a	7.40a	7.19a	7.27a	7.15a	0.047	0.381	0.240	0.017
7	5.87c	5.68d	5.86de	6.30c	6.30c	0.032	0.001	0.128	0.382
11	5.59d	5.31e	5.58e	5.67e	5.95c	0.040	0.005	0.395	0.534
13	5.34e	5.29e	5.24f	5.93d	6.10c	0.036	0.001	0.001	0.106
14	4.61f	4.71f	4.58g	4.82f	4.96d	0.050	0.522	0.182	0.481
SEM	0.026	0.035	0.036	0.025	0.025		[9]P (Dt × Dp)		
P-value	0.0001	0.0001	0.0001	0.0001	0.0001		0.0001		

[1]CON = control (without natural additives); [2]NA15 = addition of 1,500 mg/animal/day of natural additives; [3]NA30 = addition of 3,000 mg/animal/ day of natural additives; [4]NA45 = addition of 4,500 mg/animal/day of natural additives; [5]NA60 = addition of 6,000 mg/animal/day of natural additives; [6]Standard error of means; [7]Linear effect; [8]Quadratic effect. [9]P (Dt × Dp): P value interaction Diet × Display. [a-b:] Values within a column with different superscripts differ significantly at P <0.05. [§]Based on an hedonic 9-point scale (1 = dislike extremely; 9 = like extremely)

Discussion

Increased antioxidant capacity was observed in the three methods used to evaluate the antioxidant power, i.e., ABTS, DPPH, and FRAP. This indicates that the addition of NA was likely to be responsible for the enrichment of beef. There was also an effect of the diet and the storage time (Figure 1), which revealed an influence of the NA levels and display days.

There is evidence that NA included in the diet can pass through the rumen and may be deposited in tissues (e.g., meat), resulting in a higher antioxidant power, mostly when the compounds are rumen-protected. According to Falowo *et al.* (2014), some bioactive compounds, such as phenolics (which were part of the NA blend), can attract electrons and delay the oxidation of matrices, and likely protect meat oxidation (Figure 2). Furthermore, such compounds can also activate antioxidant enzymes (e.g., catalase or superoxide dismutase) in the circulatory system (Frankic *et al.*, 2009), which also promotes the antioxidant capacity of meat.

Film packages have higher permeability to oxygen molecules. Thus, meat packed with such material is expected to have higher oxidative responses compared to vacuum-sealed packages. After 14 d of display, the treatments with 3000 or 4500 mg/day inclusion of NA had lower values of MDA compared to CON, NA15, and NA60 (Figure 2; Table S12). Oxidative stress can be delayed by the use of natural antioxidants that improve the balance between production of ROS and the body's defence mechanisms, which prevents future oxidation of tissue after conversion of the muscle to meat (Frankic *et al.*, 2009; Passetti *et al.*, 2019; Rock *et al.*, 1996). Peroxyl radicals can react with unsaturated fatty acids in meat, resulting in a rancid odour and off flavours from the volatile compounds formed in this reaction, thus interfering with meat quality and consumer acceptability. According to Min & Ahn (2005), aldehydes influence the formation of ROS, trigger the deterioration of meat colour and flavour, protein stability, and functionality. The balance between ROS and antioxidant oxidation can be affected by different factors, such as pH, diet, fatty acids, iron content of meat (Gatellier *et al.*, 2007).

In contrast to our results, Rivaroli *et al.* (2016) observed an increase in lipid oxidation of meat in beef cattle following the inclusion of 7000 mg/animal/day of a blend of essential oils. It seems that increased quantities of NA can act as a pro-oxidant (Bakkali *et al.*, 2008). Corroborating our data, Monteschio *et al.* (2017) found a positive effect in a delay in lipid oxidation with the addition of an essential oil blend (rosemary essential oil) and rumen-protected actives (eugenol, thymol, and vanillin blend) in different doses (1330, 2000, or 4000 mg/animal/day) in the diet of the beef heifers. Thus, the dose and the compounds added to the diets of ruminants need to be considered.

The change in colour variables during display is reported in Table 2. This parameter is correlated with the freshness of the meat and, consequently, with higher acceptability of the consumer. The colour is the first attribute that consumers consider in the moment of the purchase (Resconi *et al.*, 2012). The NA addition did not influence in a* values. A superior stability during storage was observed following NA inclusion when analysed each day for the treatments, NA30, NA45, and NA60, compared to control diet or the lowest level of the blend (NA15). This may be associated with the protection caused by the antioxidant incorporation in cell membranes, which delay the myoglobin oxidation. These compounds scavenge the free radicals formed during lipid oxidation, delaying the conversion of the cherry red pigment (oxymyoglobin [oxyMb (Fe^{2+})]) to the brown pigment (metmyoglobin [MetMb (Fe^{3+})]. High levels of ROS in meat can reduce sensory quality, cause a loss of protein functionality, the degradation of polyunsaturated fatty acids of meat, and cause the conversion of oxymyoglobin to metmyoglobin, resulting in the generation of free radicals, which could result in the deterioration of meat protein (Suman & Joseph, 2013).

The consumer acceptability decreased following longer display times, possibly due to oxidation and discoloration of the meat surface. A gradual decline in visual appraisal was expected, due to the natural oxidative process that causes meat deterioration, producing a less attractive appearance of the meat for consumers (Eiras *et al.*, 2017; Ornaghi *et al.*, 2020; Passetti *et al.*, 2019).

The increase in visual acceptability on the third day of evaluation was found. This is likely due to a change from purple-reddish to a cherry red colour of meat. According to Hayes *et al*. (2009), consumers have a preference for cherry-red (oxymyoglobin state), then purple-reddish (deoxymyoglobin state), meat, with the brown colour (metmyoglobin state) being less desirable. The mechanism of myoglobin state changes during display is due to several factors (such as oxidation and spoilage). Thus, the complexity of these compounds and the mixture of NA used in the current study likely had a positive, synergistic effect, reducing meat oxidation and discoloration (Ornaghi *et al.*, 2020).

From the 7^{th} to the 13^{th} day of display, visual acceptability scores remained higher than 5.0. Scores lower than 5.0 reflect rejection by the consumers, which only occurred after the 14th day of display. Past studies observed that red meat could be displayed for up to 6 or 7 d (Eiras *et al.*, 2017; Ornaghi *et al.*, 2020; Passetti *et al.*, 2019). The score at 14 d observed in our study was unexpected. A linear

effect of increasing scores was observed within the treatments on days 7, 11, and 13. The higher scores could be explained by the antioxidant activity present with NA, which reduced the change from the red cherry colour (oxymyoglobin) to the brown colour (metmyoglobin).

The highest dosages, NA45 and NA60, had higher acceptability scores compared to CON, which is likely due to a lower oxidative status of these treatments, as previously commented. A superior stability during storage was observed with the NA30, NA45, and NA60 treatments compared to the control diet or the lower addition of the blend (NA15). The antioxidant power delayed oxidation and consequently, increased the shelf life through the maintenance of the meat colour (Tables 3 and 4).

To determine the display shelf-life of the meat, a regression analyses was performed (Table 4). The CON group presented meat with lower shelf-life of 8.53 d. The inclusion of NA improved the visual shelf-life and the NA60 treatment had the highest shelf-life of 9.58 d. This reflects the beneficial effect of the natural compounds in the blend on the oxidation of myoglobin.

Table 4 Regression analysis of visual acceptability of meat from bulls finished in feedlots fed with or without natural additives

Diets	Days[6]	Equation	R^2	F	*P*-value
CON[1]	8.53	$Y = 12.937 – 0.477x – 0.053x^2$	0.161	275.05	<0.001
NA15[2]	9.36	$Y = 13.618 – 1.014x – 0.010x^2$	0.128	211.28	<0.001
NA30[3]	8.72	$Y = 14.884 – 1.264x + 0.015x^2$	0.174	303.85	<0.001
NA45[4]	8.64	$Y = 11.816 + 0.042x – 0.096x^2$	0.157	268.87	<0.001
NA60[5]	9.58	$Y = 11.916 – 0.357x – 0.038x^2$	0.081	126.92	<0.001

[1]CON = control (without natural additives); [2]NA15 = addition of 1,500 mg/animal/day of natural additives; [3]NA30 = addition of 3,000 mg/animal/ day of natural additives; [4]NA45 = addition of 4,500 mg/animal/day of natural additives; [5]NA60 = addition of 6,000 mg/animal/day of natural additives; [6]Days: Number of days which consumers evaluated meat with scores equal or higher than 5.0

The regression analyses in the study presented low R^2 values; this can be explained by the scores that remained higher than 5.0 after 13 d of evaluation. According to Passetti *et al*. (2019), the number of days of display to be evaluated in visual analyses could be reduced, but it will depend on the inflection point (the day when scores are below 5.0). However, our results show high acceptability results until the last day of evaluation, which suggests that meat in this study would still be accepted even after 14 d of display. Shelf life, as defined by regression equations that compiled the number of days which consumers evaluated meat with scores ≥5.0, was higher with blend addition than in the CON (Table 4). The addition of the lowest and highest dosage of blend of NA added an extra day of shelf-life of the product.

Conclusions

Natural additives with bioactive compounds that are blended to achieve synergistic effects and are also prone to absorption in the rumen and post-rumen portion can be added to the diet of young bulls to improve meat quality. In this study, especially at the highest dose (6000 mg/animal/day), NA reduced lipid oxidation and colour losses when compared to the control diet, due to the improvement in the antioxidant potential in the meat, leading to a higher visual acceptability by consumers. Thus, blending these compounds can be a tool in animal feed to increase meat quality during display. However, the type of essential oil, its constituent compounds, and their concentration must be considered to optimise pro-oxidant responses.

Acknowledgments

The authors thank the Safeeds Nutrição Animal (Cascavel City, Paraná State, Brazil). The trade names or commercial products in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement.

Funding

The current project was funded by Coordination for the Improvement of Higher Education Personnel (CAPES) and the Brazilian Council for Research and Technological Development (CNPq). Research publication was supported by the Universidad CEU–UCH Research Programme.

Authors' Contributions

MGO, RACP, RMP, and INP participated in designing the study, laboratory analysis, and manuscript writing; MGO, RACP, and DFR: involvement in drafting and revising the manuscript for important intellectual content; AG, ACPV, RMP, INP: data analysis and interpretation, involvement in the preparation and revision of the manuscript; AG, ACVP, MAPC: contributions to the acquisition of funding, analysis, and interpretation of data.

Conflicts of Interest

The authors declare no conflict of interest.

References

Al-Zubairi, A. S., Al-Mamary, M. A., & Al-Ghasani, E., 2017. The antibacterial, antifungal, and antioxidant activities of essential oil from different aromatic plants. Glob. Adv. Res. J.Med. Med. Sci, 6, 224–233.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M., 2008. Biological effects of essential oils - A review. Food Chem Toxicol, 46(2), 446-475.

Beauchemin, K. A., Ungerfeld, E. M., Eckard, R. J., & Wang, M., 2020. Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. Animal, 14(S1), s2–s16.

Bosona, T., & Gebresenbet, G., 2013. Food traceability as an integral part of logistics management in food and agricultural supply chain. Food Control, 33(1), 32–48.

Clonan, A., Wilson, P., Swift, J. A., Leibovici, D. G., & Holdsworth, M., 2015. Red and processed meat consumption and purchasing behaviours and attitudes: impacts for human health, animal welfare, and environmental sustainability. Public Health Nutr., 18(13), 2446–2456.

Ducatti, T., Prado, I. N., Rotta, P. P., Prado, R. M., Perotto, D., Maggioni, D., & Visentainer, J. V., 2009. Chemical composition and fatty acid profile in crossbred (*Bos Taurus* vs. *Bos indicus*) young bulls finished in a feedlot. Asian-Australas. J. Anim. Sci., 22(3), 433–439.

Eiras, C. E., Guerrero, A., Valero, M. V, Pardo, J. A., Ornaghi, M. G., Rivaroli, D. C., Sañudo, C., & Prado, I. N., 2017. Effects of cottonseed hulls levels in the diet and aging time on visual and sensory meat acceptability from young bulls finished in feedlot. Animal, 11(3), 529–537.

Falowo, A. B., Fayemi, P. O., & Muchenje, V., 2014. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. Food Res. Int., 64, 171–181.

Fletcher, B., Mullane, K., Platts, P., Todd, E., Power, A., Roberts, J., Chapman, J., Cozzolino, D., & Chandra, S., 2018. Advances in meat spoilage detection: A short focus on rapid methods and technologies. CyTA-Journal of Food, 16(1), 1037–1044.

Frankic, T., Voljč, M., Salobir, J., Rezar, V., Franiič, T., Voljč, M., Salobir, J., & Rezar, V., 2009. Use of herbs and spices and their extracts in animal nutrition. Acta Agric. Slov., 94(2), 95–102.

Gatellier, P., Gomez, S., Gigaud, V., Berri, C., Le Bihan-Duval, E., & Santé-Lhoutellier, V., 2007. Use of a fluorescence front face technique for measurement of lipid oxidation during refrigerated storage of chicken meat. Meat Sci., 76(3), 543–547.

Hayajneh, F. M. F., 2019. Natural feed additives for broiler chickens. S. Afr. J. Anim. Sci., 49(5), 869–875.

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., O'Brien, N. M., & Kerry, J. P., 2009. The effect of lutein, sesamol, ellagic acid, and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems. Meat Sci., 83(2), 201–208.

Kleinberg, M. N., Rios, M. A. S., Buarque, H. L. B., Parente, M. M. V, Cavalcante, C. L., & Luna, F. M. T., 2019. Influence of synthetic and natural antioxidants on the oxidation stability of beef tallow before biodiesel production. Waste and Biomass Valorization, 10(4), 797–803.

Li, W., Hydamaka, A. W., Lowry, L., & Beta, T., 2009. Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. Cent. Eur. J. Biol., 4(4), 499-506.

Min, B., & Ahn, D. U., 2005. Mechanism of lipid peroxidation in meat and meat products: A review. Food Sci. Biotechnol, 14(1), 152–163.

Min, B., Nam, K. C., Cordray, J., & Ahn, D. U., 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. J. Food Sci., 73(6), C439–C446.

Monteschio, J. O., Souza, K. A., Prado, R. M., Vital, A. C. P., Guerrero, A., Pinto, L. M., Valero, M. V., Osório, J. C. S., Castilho, R. A., Sañudo, C., & Prado, I. N., 2020. Acceptability by sensory and visual analyses of meat from Nellore heifers fed with natural additives and finished in feedlot. J. Sci. Food Agric., 100(13), 4782–4790.

Monteschio, J. O., Souza, K. A., Vital, A. A. C. P., Guerrero, A., Valero, M. V, Kempinski, E. M. B. C., Barcelos, V. C., Nascimento, K. F., & Prado, I. N., 2017. Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol, and vanillin blend) on meat quality of feedlot-finished heifers. Meat Sci., 130, 50–57.

Ornaghi, M. G., Guerrero, A., Vital, A. C. P., Souza, K. A., Passetti, R. A. C., Mottin, C., Castilho, R. C., Sañudo, C., & Prado, I. N., 2020. Improvements in the quality of meat from beef cattle fed natural additives. Meat Sci., 163(108059), 1–9.

Ornaghi, M. G., Passetti, R. A. C., Torrecilhas, J. A., Mottin, C., Vital, A. P., Guerrero, A., Sañudo, C., Campo, M. M., & Prado, I. N., 2017. Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. Anim. Feed Sci. Technol., 234, 274–283.

Passetti, R. A. C., Resconi, V. C., Çakmakçı, C., Campo, M. M., Kirinus, J. K., Passetti, L. C. G., Guerrero, A., Prado, I. N., & Sañudo, C., 2019. Number of consumers and days of display necessary for the assessment of meat colour acceptability. Food Res. Int., 121, 387–393.

Passetti, R. A. C., Torrecilhas, J. A., Ornaghi, M. G., Mottin, C., Oliveira, C. A. L., Guerrero, A., del Mar Campo, M., Sañudo, C., & Prado, I. N., 2017. Validation of photographs usage to evaluate meat visual acceptability of young bulls finished in feedlot fed with or without essential oils. Meat Sci., 123, 105–111.

Pateiro, M., Barba, F. J., Domínguez, R., Sant'Ana, A. S., Mousavi Khaneghah, A., Gavahian, M., Gómez, B., Lorenzo, J. M., Khaneghah, A. M., Gavahian, M., Gómez, B., & Lorenzo, J. M., 2018. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. Food Res. Int., 113, 156–166.

R-Core-Team, 2016. R: A language and environment for statistical computing. R. Foundation for Statistical Computing.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26(9–10), 1231–1237.

Resconi, V. C., Escudero, A., Beltrán, J. A., Olleta, J. L., Sañudo, C., & Campo, M. M., 2012. Color, lipid oxidation, sensory quality, and aroma compounds of beef steaks displayed under different levels of oxygen in a modified atmosphere package. J. Food Sci, 71(1), S10–S18.

Richardson, I., Duthie, C. A., Hyslop, J., Rooke, J., & Roehe, R., 2019. Nutritional strategies to reduce methane emissions from cattle: Effects on meat eating quality and retail shelf life of loin steaks. Meat Sci., 153, 51–57.

Rivaroli, D. C., Campo, M. M., Sañudo, C., Guerrero, A., Jorge, A. M., Vital, A. C. P., Valero, M. V, Prado, R. M., & Prado, I. N., 2020. Effect of an essential oils blend on meat characteristics of crossbred heifers finished on a high-grain diet in a feedlot. Anim. Prod. Sci., 60(4), 595–602.

Rivaroli, D. C., Guerrero, A., Valero, M. V., Zawadzki, F., Eiras, C. E., Campo, M. M., Sañudo, C., Jorge, A. M., & Prado, I. N., 2016. Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. Meat Sci., 121, 278–284.

Rock, C. L., Jacob, R. A., & Bowen, P. E., 1996. Update on the biological characteristics of the antioxidant micronutrients: Vitamin C, vitamin E, and the carotenoids. J. Am. Diet. Assoc., 96(7), 693–702.

Rotta, P. P. P., Prado, R. M., Prado, I. N., Valero, M. V., Visentainer, J. V. V, & Silva, R. R., 2009. The effects of genetic groups, nutrition, finishing systems and sex of Brazilian cattle on carcass characteristics and beef composition and appearance: A review. Asian-Australas. J. Anim. Sci., 22(12), 1718–1734,

Sies, H., Berndt, C., & Jones, D. P., 2017. Oxidative stress. Annu. Rev. Biochem., 86, 715–748.

Souza, F. N., Monteiro, A. M., Santos, P. R., Sanchez, E. M. R., Blagitz, M. G., Latorre, A. O., Neto, A. M. F., Gidlund, M., & Della Libera, A. M. M. P., 2011. Antioxidant status and biomarkers of oxidative stress in bovine leukemia virus-infected dairy cows. Vet. Immunol. Immunopathol., 143(1), 162–166.

Suman, S. P., & Joseph, P., 2013. Myoglobin chemistry and meat color. Annu. Rev. Food Sci. Technol., 4, 79–99.

Torrecilhas, J. A., Mottin, C., Ornaghi, M. G., Rodrigo, P. A. C., Valero, M. V, Souza, K. A., Zawadzki, F., Bridi, A. M., & Prado, I. N., 2018. Color and lipid oxidation of meat from young bulls finished in feedlot supplemented with clove or cinnamon essential oils. J. Anim. Sci., 95(Suppl-4), 179.

Valero, M. V, Farias, M. S., Zawadzki, F., Prado, R. M., Fugita, C. A., Rivaroli, D. C., Ornaghi, M. G., & Prado, I. N., 2016. Feeding propolis or essential oils (cashew and castor) to bulls: performance, digestibility, and blood cell counts. Rev. Colomb. de Cienc. Pecu., 29(1), 33–42.

Vital, A. C. P., Guerrero, A., Kempinski, E. M. B. C., Monteschio, J. O., Sary, C., Ramos, T. R., Campo, M. M., & Prado, I. N., 2018. Consumer profile and acceptability of cooked beef steaks with edible and active coating containing oregano and rosemary essential oils. Meat Sci., 143, 153–158.

Vital, A. C. P., Guerrero, A., Monteschio, J. O., Valero, M. V., Carvalho, C. B., Abreu Filho, B. A., Madrona, G. S., & Prado, I. N., 2016. Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. PLoS ONE, 11(8), 1–15.

Xiao, S., Zhang, W. G., Lee, E. J., Ma, C. W., & Ahn, D. U., 2011. Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage. Poult. Sci., 90(6), 1348–1357.

Zhu, Q. Y., Hackman, R. M., Ensunsa, J. L., Holt, R. R., & Keen, C. L., 2002. Antioxidative Activities of Oolong Tea. J. Agric. Food Chem., 50(23), 6929–6934.