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Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers

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ABSTRACT

Forty Nellore heifers were fed (73 days) with different diets: with or without essential oils (clove and/or rosemary essential oil) and/or active principle blend (eugenol, thymol and vanillin). The pH, fat thickness, marbling, muscle area and water losses (thawing and drip) were evaluated 24h *post mortem* on the *Longissimus thoracis,* and the effects of aging (14 days) was evaluated on the meat cooking losses, color, texture and lipid oxidation. Antioxidant activity was also evaluated. Treatments had no effect ($P > 0.05$) on pH, fat thickness, marbling, muscle area, thawing and drip losses. However, treatments affected ($P < 0.05$) cooking losses, color, texture and lipid oxidation. The diets with essential oil and the active principle blend reduced the lipid oxidation and reduced the color losses in relation to control diet. Aging affected ($P < 0.05$) texture and lipid oxidation. The essential oil and active principles or its blend have potential use in animal feed aiming to maintain/improve meat quality during shelf-life.

1. Introduction

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The addition of antibiotics in livestock production systems is a common practice to prevent diseases and metabolic disorders and to improve feed efficiency, particularly when animals are intensively reared (Goodrich et al., 1984; Valero et al., 2014; Zawadzki et al., 2011). However, due to antibiotic resistance and possible risks to human health (residues in the final products) (Russell & Houlihan, 2003), their use has been prohibited in some regions of the world. Consequently, researches are increasingly focused on natural alternatives, which are well accepted by consumers (Chaves et al., 2011; Chaves, Stanford, Dugan, et al., 2008). Thus, alternative solutions to improve cattle performance and improve the food shelf life are required to replace the antibiotics (Moyo, Masika, & Muchenje, 2014; Prado et al., 2016; Valero et al., 2014a; Valero et al., 2014b). Each plant has specific active components that determine its extract characteristics. Essential oils are aromatic extracts from plant material, such as flowers, buds, seeds, leaves, twigs, barks, wood, fruit and roots (Burt, 2004). They may be obtained by fermentation, extraction or most commonly by steam distillation (Burt, 2004; Gershenzon & Croteau, 1991). Chemically, essential oils are variable mixtures of terpenoids that primarily include monoterpenes (C10) and sesquiterpenes (C15), although diterpenes (C20) may also be present. They also include a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and N- and S-containing compounds, such as coumarins, and homologs of phenylpropanoids may also be present (Dorman & Deans, 2000). These products may act as antimicrobials (oils of clove, rosemary, thyme and vanillin are some of the most effective due to the presence of phenolic compounds) and antioxidants, benefiting the immune and digestive system of animals, which is reflected in their performance indices (Benchaar et al., 2008; Jayasena & Jo, 2013). Moreover, when a blend is used, essential

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oils may have a synergistic effect, influencing their mode of action in animal metabolism and affecting beef quality (Prado et al., 2016; Valero et al., 2014b).

Plant species containing thymol and carvacrol show high antioxidant potential due to the presence of phenolic terpenes (Bakkali, Averbeck, Averbeck & Idaomar, 2008). Vanillin, which has a similar structure to eugenol exhibits both antimicrobial and antioxidant properties in soft drink and fruit juice, and table grapes, respectively (Fitzgerald, Stratford, Gasson & Narbad, 2004; Konuk & Korel, 2016)

Rosemary (*Rosmarinus officinalis*) has a higher antioxidant activity than the other essential oils, and several phenolic compounds have been isolated from this oil, such as carnosol, rosmanol, rosmaridiphenol and rosmarquinone (Assis et al., 2009). Moreover, rosemary is reported to have a high antioxidant activity in meat (Djenane, Sánchez-Escalante, Beltrán & Roncalés, 2003).

Clove (*Syzygium aromaticum*) essential oil has received a lot of attention due to its high and diverse content of phenolic compounds, its antimicrobial and antioxidant properties and potential use in meat and derived products (Barbosa et al., 2009; Bensid, Ucar, Bendeddouche & Özogul, 2014; Jayasena & Jo, 2013).

Although some studies have demonstrated that essential oils can influence meat quality (Rivaroli et al., 2016) and prolong the shelf-life of meat (Jayasena & Jo, 2013; Lucera, Costa, Conte & Del Nobile, 2012), as well as studies with other natural compounds (Moyo, et al., 2014; Muthukumar, Naveena, Vaithiyanathan, Sen, & Sureshkumar, 2014). Researches concerning the effects of essential oils on meat quality (used in animal feed) are still limited. Thus, this study aimed to investigate the effects of essential oils (clove and rosemary), their encapsulated active principles (eugenol, thymol and vanillin mixture) and blends thereof on the meat quality of feedlot-finished heifers fed on high-grain diets.

2. Materials and Methods

2.1. Locality, animal and diets

This experiment was approved (no. 3624120116) by the ethical committee of Maringá State University. The study was conducted at Sector Rosa and Pedro, at the Experimental Station, Iguatemi farm, Iguatemi City, Paraná, Brazil. This region (south) has a subtropical climate, with an annual average around 22ºC; and semi-humid (approximately 1590 mm of annual rain). The lowest temperatures are between the months of May to July, while the highest temperatures are from November to March.

In relation to humidity, the city is drier between July to September, while rains are mainly between January, February and March.

Forty purebred Nellore heifers with an initial average weight of 297.6 ± 31.2 kg were randomly assigned to one of five finishing diets (n = 8 per treatment). The heifers were allocated to individual pens, with an adaptation period of 7 days. All animals received the same basal diet (Table 1), which was formulated according to the NRC (2000) and provided *ad libitum*. The heifers were used since in Brazil, part of the beef production (approximately 40%) comes from the slaughter of heifers and cows cull (Annualpec 2015). Moreover, heifers not used for breeding in the herd are destined for fattening and slaughtered at a young age (<24 months) because they produce meat with good quality (Rotta et al., 2009 and Reddy et al., 2015; Rivaroli et al., 2016).

The five experimental diets (based on previous studies) were: CON – without essential oil and active principles (eugenol, thymol and vanillin); ROS – rosemary essential oil (4 g/animal/day); BLE – eugenol, thymol and vanillin active principle

blend (4 g/animal/day); BCL – eugenol, thymol and vanillin active principle blend (2 g/animal/day) + clove essential oil (2 g/animal/day); and BRC – eugenol, thymol and vanillin active principle blend (1.33 g/animal/day) + rosemary essential oil (1.33 g/animal/day) + clove essential oil (1.33 g/animal/day). These concentrations were chosen according to the results of Rivaroli et al. (2016) which showed that a concentration above 3.5 g/animal/day has no effect on animal performance and meat quality. Furthermore, previous studies (Busquet et al., 2006; Benchaar et al., 2008) showed that the most adequate concentrations of the essential oils in the animal diets is between 3 and 5 g/animal /day.

Rosemary and clove essential oils were obtained from Ferquima® (Vargem Grande Paulista, São Paulo, Brazil) and its major compounds were 1,8 cineole and eugenol, respectively (Biondo et al., 2016). The encapsulated blend (eugenol, thymol and vanillin active principles) was obtained from Safeeds® (Cascavel, Paraná, Brazil).

The diets were prepared with a pre-mix containing ground corn. The essential oils were then added to the feed mixer with the other ingredients. As reported by Zulueta, Esteve & Frígola (2009), the oxygen radical absorbance capacity of the essential oils in the diet is retained for up to 30 days of exposure.

The heifers were finished on their respective diets for 73 days until they reached a medium weight of 356.6 ± 32.6 kg. They were then slaughtered at a commercial abattoir, after a solid fasting period of 12 h, in compliance with the slaughter standards of the State Inspection Service legislation in Brazil (Brazil, 2000). The carcasses were then divided medially through the sternum and vertebral column, identified and chilled below 4 ºC for 24 h. The *Longissimus thoracis* (LT) was excised from the left side of the carcass between the 7th and the 13th ribs for subsequent analysis.

2.2. Sampling and meat quality

The LT was sliced into steaks (2.5 cm thick), 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 24 h, or 7 or 14 days, before being frozen and stored (-20 °C) for 1 month for subsequent analyses.

2.3. pH measurements

At 24 h *post-mortem*, the LT pH was measured using a digital pH meter (Hanna – HI99163, Romania - Europe) with a penetration electrode placed at the point of the 3rd lumbar vertebra. The phMeter was calibrated at 20ºC using standard pH 4.0 and 7.0 buffers (Valero et al., 2014b).

2.4. Fat thickness

The thickness of subcutaneous fat from the 12th rib in the LT muscle was measured by a digital caliper at 24 h *post-mortem* and averaged over three points (Eiras et al., 2014).

2.5. Marbling (MAR)

The MAR was measured in the LT muscle area (LMA) (12th rib) at 24 h *post-mortem* using the Brazilian scoring system (18–16, abundant; 15–13, moderate; 12–10, mean; 9–7, small, 6–4, light: and 3–1, traces), according to Muller (1987).

2.6. *Longissimus thoracis* muscle Area (LMA)

The LMA (cm^2) was measured at 24 h *post-mortem* in the 12th rib by a compensating planimeter. The LT muscle area/100 kg carcass (LMC) is defined as the LMA:HCW (hot carcass weight) ratio, multiplied by 100 (Eiras et al., 2014).

2.7. Thawing, drip and cooking losses

The steaks were thawed at 4 °C for 24 h. They were then weighed and the thawing losses were calculated as the percentage difference between the fresh and thawed weights (Rivaroli et al., 2016).

Drip loss was measured using the method described by Honikel (1998). A steak of each animal was taken 24 h *post mortem*, placed in a plastic bag, and kept at 4 °C. After 24 h, the sample was removed from the bag, dried on absorbent paper, and reweighed. Amount of drip at 48 h *post mortem* was expressed as a percentage.

$$\% \text{ drip loss} = \left(\frac{initial\ weight - final\ weight}{initial\ weight} \right) * 100$$

For cooking losses, the steaks were weighed and wrapped in aluminum foil. Each sample was cooked in a pre-heated grill (Grill Philco Jumbo Inox, Philco SA, Brazil) at 200 °C until an internal temperature of 72 °C was reached, which was monitored using an internal thermocouple (Incoterm, 145 mm, Incoterm LTDA, Brazil). The sample was then removed from the heat and left at ambient temperature to cool. Once the steaks

reached 25 °C, each steak was weighed and the cooking losses calculated as the percentage difference in weight before and after cooking.

2.8. Instrumental meat color

The color was evaluated after 30 min of exposure to oxygen at 1, 7 and 14 days of display, by 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). Six measurements at randomly selected points were recorded per sample, obtaining lightness (L*), redness (a*) and yellowness (b*). Chroma and hue values were calculated as follows:

Chroma= $(\sqrt{a*^2 + b*^2})$ and hue angle (h°) = $(\arctan(b*/a*))$.

2.9. Texture measurement

The texture of the previously cooked steaks was analyzed using a Stable Micro Systems TA.XTplus (Texture Technologies Corp., Serial Number 41288, Godalming, Surrey, UK) texture analyzer with a Warner-Bratzler blade, according to Honikel (1998). The meat was cut into rectangular pieces of 1 cm^2 cross-section (eight pieces per animal), which were cut perpendicular to the direction of the muscle fibers.

2.10. Antioxidant capacity

2.10.1 Meat bioactive compounds extract

Meat extracts (1:1 w/v with methanol), were obtained according to Vital et al., 2016 using an Ultra-Turrax homogenizer (IKA® - T10, USA), followed by centrifugation (4.000 rpm, 15 min) and filtration (filter paper (grammage – 80 g/m², thickness - 205 μm, pores – 14 μm). Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays, using meat extracts.

2.10.2. Radical scavenging assay (DPPH)

The DPPH radical scavenging activity was measured according to Li, Hydamaka, Lowry, & Beta (2009), with modifications. Meat extract (150 μL) were mixed with 2850 μL of a methanolic solution containing DPPH (60 μM) and reacted for 30 min. The absorbance at 515 nm was measured against pure methanol. Antioxidant activity was calculated as DPPH radical scavenging activity (%) = $(1 - (A_{sample\ t=0}/A_{sample\ t})*100$, where: $A_{sample\ t\ =\ 0}$ is the absorbance of the sample at time zero, and $A_{sample\ t}$ is the absorbance of the sample at 30 min.

2.10.3. Radical scavenging assay (ABTS)

The ABTS assay was conducted according to Re et al., 1999, with modifications. $ABTS^+$ 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 at 734 nm. The radical scavenging activity (%) was also measured at 734 nm. Meat extract (40 μL) were mixed with $ABTS^+$ solution (1960 μL) and the absorbance was recorded

at 6 min. The ABTS radical scavenging activity (%) was calculated as $1-(A_{sample\ t=0}/A_{sample\ t})*100$, where: $A_{sample\ t=0}$ is the absorbance of the sample at time zero, and $A_{sample\ t}$ is the absorbance of the sample at 6 min.

2.10.4. The FRAP assay

The FRAP method was performed according to Zhu, Hackman, Ensunsa, Holt, & Keen, 2002. Meat extract (250 μL) was then 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, 10% trichloroacetic acid (1.25 mL) was added and the mixture was centrifuged at 4,000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 0.1% ferric chloride (500 μL) and the absorbance was measured at 700 nm. Results were expressed as mg gallic acid equivalents (GAE) g^{-1} oil, mg GAE g^{-1} coating and mg GAE 100 g^{-1} meat. Gallic acid (0−300 mg L^{-1}) was used to establish the standard curve.

2.11. Lipid oxidation

The malondialdehyde (MDA) content in meat was quantified using the thiobarbituric acid reactive substances (TBARS) assay as was adapted by Vital et al. (2016).

The sample (5 g) was mixed with trichloroacetic acid (10 mL), homogenized using an Ultra-Turrax, and then centrifuged (4,000 rpm) at 4 ºC for 15 min. The supernatant was filtered and mixed with TBARS reagent (1:1 v/v). The mixture was boiled (100 ºC) for 15 min, cooled, then the absorbance measured at 532 nm against an MDA standard. Results were expressed as mg MDA kg^{-1} of meat. The assay was performed at 1, 7 and 14 days of display.

2.12. Statistical analysis

The experimental design was completely randomized with five treatments (finishing diets) and eight replications per treatment. Meat attributes were assessed by analysis of variance using the general linear model (GLM) with SPSS (v.19.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows. Means and standard error of mean were calculated for each variable.

On the statistical design the finishing diet was considered as fixed effect, on color, shear force and lipid oxidation (TBARS), the effect of aging (1, 7 and 14 days) was also considered as fixed effect and studied the interaction between diet and ageing days. However, there was no interaction effect among diets and ageing days. Thus, data were presented and discussed as the main effect. Differences between group means were assessed by using the Tukey Test ($P < 0.05$).

3. Results and Discussion

3.1. Meat characteristics

3.1.1. The pH, fat thickness, MAR and LMA

At 24 h after slaughter, the pH of the meat samples obtained from the heifers fed the various diets was similar ($P > 0.05$) (Table 2). The mean pH was 5.8, which, although acceptable, was considered high. Under normal conditions, pH 5.5−5.6 is anticipated (Page, Wulf, & Schwotzer, 2001). The relatively high pH obtained can be explained by the animal breed used in this experiment. In general, Zebu animals present a high pH

after slaughter due to its aggressive behavior during handling (Zawadzki et al., 2011). Rivaroli et al. (2016) evaluated the effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots and found that the addition of essential oils to the diets did not affect ($P > 0.05$) the pH(24h), which was below 5.8.

The fat thickness was not influenced ($P > 0.05$) by the essential oils and active principles added to the diets (Table 2). In general, the fat thickness of feedlot-finished heifers is above 5 mm (Andreotti et al., 2015; Marques et al., 2010). According to the cattle marketing practices in Brazil, the fat thickness must be between 3−6 mm. Thus, the fat thickness of 2.5 mm found in this study is considered low. Consequently, this carcass could be penalized by the Brazilian market. Valero et al. (2014b) evaluated the effect of propolis and essential oils additives in the diets of bulls finished in feedlot and found values for fat thickness of 4.35 for control diet, 5.81 for diets with propolis diet and 4.78 for diets with essential oils, and no significant differences was observed. These differences in fat thickness occur due to the termination system, breed, sex, age, among others. In our studies, some of these factors may be determinants for low fat thickness, such as the short time confined, the Nellore breed and because they are young (heifers).

According to Luchiari Filho (2000), intramuscular fat, or MAR, is the last to be deposited in the carcass and the animal may have considerable amounts of internal and subcutaneous fat without a reasonable amount of MAR. The MAR values were low and were not affected ($P > 0.05$) by the diets evaluated (Table 2). Beef with a MAR score above 5 is deemed to be well accepted by consumers (Marques et al., 2006; Rotta et al., 2009). Low MAR is directly related to fat deposition. As observed from the fat thickness results, the fat deposition was low, which was attributed to the breed used in this experiment (Nellore heifers). Zebu animals have low meat MAR as they are less genetically predisposed to fat deposition than other breeds (Campion, Crouse &

Dikeman, 1975; Rotta et al., 2009). Although low MAR values were found in this experiment, similar values have been reported by other authors for Nellore heifers (Silva et al., 2014; Sousa et al., 2015). Valero et al. (2014a) found values between 4.60 and 5.90 and no differences were observed.

The LMA, which was measured between the 12th and 13th ribs, was also not affected ($P > 0.05$) by the diet (Table 2).

Although pH, fat thickness, MAR and LMA differences were not observed, the results revealed a positive use for the essential oils and their active components. When a new compound/product is added to animal feed, the effects of the addition must be carefully studied, due to the complex digestion system of cattle. The added ingredient could cause some harm to the animal rather than the intended benefit, thereby influencing the quality of the final product. This study showed that the inclusion of clove and rosemary essential oils, and the encapsulated active principles, did not cause any damage to the meat characteristics (pH, MAR and LMA).

3.1.2. Thawing, drip and cooking losses

The water losses by thawing and dripping were not affected ($P > 0.05$) by the diets (Table 3). Freezing and subsequent thawing have a strong impact on the meat water loss due to the formation of ice crystals, which damage the structural integrity of the cell membrane, allowing water to flow out from the intracellular to extracellular region, generating considerable exudates losses (Lagerstedt, Enfält, Johansson & Lundström, 2008; Leygonie, Britz & Hoffman, 2012). However, in this study, the thawing (around 3%) and drip losses (1.62%) were low, which may be associated with the high pH value. Lactic acid formation and the consequent drop in pH *post-mortem* are responsible

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for the decreased ability of the meat to retain water. These reactions cause denaturation and loss of solubility of the muscle proteins as the pH approximates their isoelectric point (IP = 5.2–5.3). If the pH is higher than the pI, the proteins have an overall negative charge, which causes repulsion of the filaments, leaving more space for the water molecules (Roça, 2009) and consequently, increasing the water retention capacity.

At 1 and 7 days of aging, the diets had no effect ($P > 0.05$) on the cooking loss (Table 3). However, at 14 days of aging, the beef from heifers supplemented with BRC had a lower cooking loss than the other treatments (Table 3). Although this difference was not significant, the heifers fed a BRC diet had numerically more MAR that could be associated with its relatively lower cooking loss. Intramuscular fat acts as a barrier against muscular juice losses during cooking, increasing water retention by the meat and, consequently, juiciness (Roça, 2000). Another factor linked to low water loss is meat maturation (aging), which slightly increases the water retention capacity due to a small pH increase and a replacement of divalent for monovalent ions in meat Roça (2009). The cooking losses in this study were approximately 25% but within the normal limits. Generally, beef cooking losses range from 20–28% (Prado et al., 2014; Rivaroli et al., 2016). The meat aging (1, 7 or 14 days) had no effect ($P > 0.05$) on the cooking losses (Table 3).

3.1.3. Instrumental color

The lightness (L* value) increased with aging time ($P > 0.05$), in agreement with previous studies (Prado et al., 2015). Although chemical changes in myoglobin do not influence lightness (McKenna et al., 2005), it is affected by changes in protein structure that occur during aging (proteolysis) (Renerre, 2004). The mean L* value observed was

around 36.6. Thus, the meat was slightly darker than that considered to be attractive (L^* ≈ 38) (Page et al., 2001).

Meat color can be influenced by several factors, including the breed and age of the animals. For instance, Zebu and older animals tend to have darker meat due to the changes in myoglobin composition (Mancini & Hunt, 2005). Thus, the L^* values found in this experiment could be associated with the breed (Nellore) and slaughter age (30 months).

The L^* presented significant difference at day 7 and CON showed the highest value, probably caused by changes in the meat structure related to the highly oxidizing conditions, such as protein conformational changes, which may increase light dispersion (MacDougall, 1982).

The addition of essential oils and active principles in the diets had no effect ($P>0.05$) on the a^* values (redness) at day 1 and 7 of aging. However, at 14 days of aging, the CON a^* value was lower ($P < 0.05$) than the other diets, whose values were not affected by the aging time ($P > 0.05$) and, thus, demonstrated greater protection and maintenance of red color. Furthermore, the mean a^* value of 11 for CON at 14 days of aging was lower than the normal value (Page et al., 2001). This may be explained by the breed (Nellore), age (30 months old) and the high pH value (5.8) observed in this experiment.

Fresh meat generally lightens and becomes less red after a few days. However, the diets with ROS and BCL maintained the lightness of the meat during 7 days of aging (L^* value), while BLE and BRC maintained the original lightness even at 14 days of aging. Moreover, the diets with essential oil and active principle inclusion preserved the red color during display. A diet that can influence meat quality, in particular, maintain or intensify the redness, could lead to an extension of color display life (Cardoso et al.,

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2016). The conversion of oxymyoglobin to metmyoglobin results in meat discoloration and interactions between lipid oxidation and discoloration has been demonstrated (Faustman, Sun, Mancini, & Suman, 2010). Meat that showed less oxidation (those coming from heifers fed with essential oil, active principle and their blends) also has higher color preservation.

The diets and aging period did not change ($P > 0.05$) the meat b* values (yellowness). The b* values ranged from 9.7–11.4 and were close to the values considered normal for beef (Page et al., 2001).

Meat with low chroma values are considered pale (Cardoso et al., 2016) which may adversely affect consumer choice at purchase time. In this study, CON showed a lower chroma value than BRC at day 14 of aging. In contrast, the h^o of CON displayed the highest increase during storage, while BLE and BCL indicated minimal color deterioration ($P < 0.05$).

3.1.4. Shear force

The diet had no effect ($P > 0.05$) on meat tenderness during 7 days of aging (Table 5). At day one, the mean shear force was around 76.5 N, which is not considered a tender meat (shear force > 49 N is considered firm) (Shackelford, Koohmaraie, Miller, Crouse & Reagan, 1991).

The content of connective tissue, sarcoma length and myofibrilar breakdown are sources of meat tenderness variation (Strydom, Lühl, Kahl, & Hoffman, 2016). Meat tenderness also might be influenced by genetics, slaughter age and stress before slaughter. Animals with Zebu blood (e.g. Nellore animals, as used in this experiment) have less tender meat compared to animals with European blood (Shackelford et al., 1991) due to the calpain-

calpastatin complex (the calcium-dependent proteolytic system involved in the *post-mortem* aging process) (Allais et al., 2011; Shackelford et al., 1994). Meat from Zebu animals tends to have higher shear force due to the higher calpastatin concentrations in their muscles, which inhibits calpain activity that is responsible for the degradation of myofibrillar proteins during *rigor mortis,* this proteolysis is the most important process in the establishment of tenderness (Koohmaraie, 1994; Whipple et al., 1990). After 14 days of aging, beef from animals fed the BCR diet showed the lowest shear force ($P < 0.05$). Such tenderness may be associated with the higher amounts of fat present in animals fed this diet and the greater water-holding capacity of the meat during cooking (Tables 2 and 3). Moreover, aging time (Table 5) led to more tender meat ($P < 0.05$).

3.1.5. Antioxidant activity in meat

Three methods (ABTS, DPPH and FRAP) were used to verify if the diet with essential oil and the active principles would have any effect on meat antioxidant activity. All three assays revealed similar results (Table 6).

Compounds with antioxidant activity can be incorporated into the diet, and could be transferred to the muscle, not only to prevent or reduce oxidation in muscle food but also improve meat quality (Falowo, Fayemi, & Muchenje, 2014). Antioxidants are typically added to the feed at moderate levels because high levels of inclusion may lead to adverse effects, like pro-oxidative action (Martin & Appel, 2009).

Generally, among the treatments evaluated, the BLE and BCL diets showed higher antioxidant activity. In contrast, CON showed the lowest antioxidant activity. The higher antioxidant activity may help maintain the meat quality during its shelf-life.

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3.1.6. Lipid oxidation (TBARS)

Lipid and protein oxidation is considered one of the main non-microbial factors that affect meat quality deterioration (Falowo et al., 2014). However, the oxidation susceptibility can be influenced by animal species and breed, the muscle type evaluated, as well as the diet provided to the animals (Falowo et al., 2014; Min, Nam, Cordray & Ahn, 2008).

The lipid oxidation of beef, measured by MDA production, was affected by the various diets ($P < 0.05$) and increased with aging time (1, 7 and 14 days) (Table 6). The initial MDA values were comparable among the treatments evaluated ($P > 0.05$), at around 0.25 mg/kg meat on day one. At day 7 of aging, BLE, BCL and BRC presented lower MDA values among the treatments studied. At 14 days of aging, BLE and BCL had the lowest MDA contents, while CON had the highest. This difference may be associated with the antioxidant potential of the meat from animals fed the BLE and BCL diets (Table 6) that delayed its lipid oxidation, in concurrence with similar studies (Botsoglou, Govaris, Ambrosiadis & Fletouris, 2012; Dal Bosco et al., 2014).

An overall, low lipid oxidation was detected in the meats and can be explained by the history of the animals. The feedlot heifers entered confinement at around 30 months of age. Before termination in confinement, the heifers were kept in pastures under tropical conditions. During this time, there is a large accumulation of β-carotene that can protect the meat against lipid oxidation after a short period of confinement (Realini, Duckett, Brito, Dalla Rizza & De Mattos, 2004), such as that implemented in this study (73 days).

4. Conclusion

The inclusion of essential oil, active principles and their blends had no effect on pH, fat thickness, MAR, LMA and in thawing and drip losses but cooking losses were affected at 14 days of aging by the diet containing clove and rosemary essential oil and the active principles (eugenol, thymol and vanillin). In general, the dietary inclusion of these compounds lessened color degradation, increased antioxidant activity and decreased lipid oxidation in the meat. Thus, these compounds have potential use in animal feed to maintain/improve meat quality during its shelf-life.

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References

Allais, S., Journaux, L., Levéziel, H., Payet-Duprat, N., Raynaud, P., Hocquette, J. F., Renand, G. (2011). Effects of polymorphisms in the calpastatin and μ-calpain

genes on meat tenderness in 3 French beef breeds1. *Journal of Animal Science, 89*, 1-11. doi: 10.2527/jas.2010-3063

Andreotti, C. C., Gusman, J. A. P., Ramos, T. R., Barcellos, V. C., Guerrero, A., & Prado, I. N. d. (2015). Slaughter weight did not alter carcass characteristics and meat quality of crossbred heifers supplemented and finished in a pasture system. *Acta Scientiarum. Animal Sciences, 37*, 173-179.

Assis, M. F., Franco, M. L. R. S., Stéfani, M. V., Franco, N. P., Godoy, L. C., Oliveira, A. C., Hoch, A. L. V. (2009). Efeito do alecrim na defumação da carne de rã (Rana catesbeiana): características sensoriais, composição e rendimento. *Food Science and Technology, 29*, 553-556.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology, 46*(2), 446-475. doi: http://dx.doi.org/10.1016/j.fct.2007.09.106

Barbosa, L. N., Rall, V. L. M., Fernandes, A., Ushimaru, P. I., da Silva Probst, I., & Fernandes, A. (2009). Essential Oils Against Foodborne Pathogens and Spoilage Bacteria in Minced Meat. *Foodborne Pathogens and Disease, 6*(6), 725-728.

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology, 145*(1–4), 209-228. doi: http://dx.doi.org/10.1016/j.anifeedsci.2007.04.014

Bensid, A., Ucar, Y., Bendeddouche, B., & Özogul, F. (2014). Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (Engraulis encrasicholus) during chilled storage. *Food Chemistry, 145*, 681-686. doi: http://dx.doi.org/10.1016/j.foodchem.2013.08.106

Biondo, P. B. F., Carbonera, F., Zawadzki, F., Chiavelli, L., Pilau, E. J., Prado, I. N., & Visentainer, J. (2016). Antioxidant Capacity and Identification of Bioactive Compounds by GC-MS of Essential Oils from Spices, Herbs and Citrus. Current Bioactive Compounds, 12, 1-7. doi: http://dx.doi.org/10.2174/1573407212666160614080846.

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D. E., .Touraille, C. (1981). Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. *Livestock Production Science,* *8*(5), 385-397. doi: http://dx.doi.org/10.1016/0301-6226(81)90061-0

Botsoglou, E., Govaris, A., Ambrosiadis, I., & Fletouris, D. (2012). Lipid and protein oxidation of α-linolenic acid-enriched pork during refrigerated storage as influenced by diet supplementation with olive leaves (Olea europea L.) or α-tocopheryl acetate. *Meat Science,* *92*(4), 525-532. doi: 10.1016/j.meatsci.2012.05.022

Brazil. (2000). Instrução Normativa n.03, de 17 de janeiro de 2000. Aprova o regulamento técnico de métodos de insensibilização para o abate humanitário de animais de açougue. *Diário Oficial [da] República Federativa do Brasil, Brasilia, DF*.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223-253. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Busquet, M., Calsamiglia, S., Ferret, A., Kamel, C. (2006). Plant extracts affect in vitro rumen microbial fermentation. Journal of Dairy Science 89:761-771.

Campion, D. R., Crouse, J. D., & Dikeman, M. E. (1975). Predictive value of usda beef quality grade factors for cooked meat palatability. *Journal of Food Science, 40*(6), 1225-1228. doi: 10.1111/j.1365-2621.1975.tb01057.x

Cardoso, G. P., Dutra, M. P., Fontes, P. R., Ramos, A. d. L. S., Gomide, L. A. d. M., & Ramos, E. M. (2016). Selection of a chitosan gelatin-based edible coating for color preservation of beef in retail display. *Meat Science, 114*, 85-94. doi: http://dx.doi.org/10.1016/j.meatsci.2015.12.012

Chaves, A. V., Dugan, M. E. R., Stanford, K., Gibson, L. L., Bystrom, J. M., McAllister, T. A., Benchaar, C. (2011). A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science, 141*(2–3), 213-220. doi: http://dx.doi.org/10.1016/j.livsci.2011.06.006

Chaves, A. V., Stanford, K., Gibson, L. L., McAllister, T. A., & Benchaar, C. (2008). Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. *Animal Feed Science and Technology, 145*(1), 396-408. doi: 10.1016/j.anifeedsci.2007.04.016

Dal Bosco, A., Gerencsér, Z., Szendrő, Z., Mugnai, C., Cullere, M., Kovàcs, M., Dalle Zotte, A. (2014). Effect of dietary supplementation of Spirulina (Arthrospira platensis) and Thyme (Thymus vulgaris) on rabbit meat appearance, oxidative stability and fatty acid profile during retail display. *Meat Science, 96*(1), 114-119. doi: http://dx.doi.org/10.1016/j.meatsci.2013.06.021

Djenane, D., Sánchez-Escalante, A., Beltrán, J. A., & Roncalés, P. (2003). Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. *Meat Science, 64*(4), 417-426. doi: http://dx.doi.org/10.1016/S0309-1740(02)00210-3

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*(2), 308-316. doi: 10.1046/j.1365-2672.2000.00969.x

Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Research International, 64*, 171-181. doi: http://dx.doi.org/10.1016/j.foodres.2014.06.022

Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science, 86*(1), 86-94. doi: http://dx.doi.org/10.1016/j.meatsci.2010.04.025

Fitzgerald, D. J., Stratford, M., Gasson, M. J., & Narbad, A. (2004). The Potential Application of Vanillin in Preventing Yeast Spoilage of Soft Drinks and Fruit Juices. *Journal of Food Protection, 67*(2), 391-395.

Gershenzon, J., & Croteau, R. (1991). Terpenoids in herbivores: Their interactions with secondary plant metabolites. *In G. A. Rosenthal & M. R. Berenbaum (Eds.), Terpenoids 1*, 165-219.

Goodrich, R. D., Garrett, J. E., Gast, D. R., Kirick, M. A., Larson, D. A., & Meiske, J. C. (1984). Influence of Monensin on the Performance of Cattle1. *Journal of Animal Science, 58*, 1484-1498. doi: 10.2527/jas1984.5861484x

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science, 49*(4), 447-457. doi: http://dx.doi.org/10.1016/S0309-1740(98)00034-5

Jayasena, D. D., & Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science & Technology, 34*(2), 96-108. doi: http://dx.doi.org/10.1016/j.tifs.2013.09.002

Khare, M., Mohanty, C., Das, B. K., Jyoti, A., Mukhopadhyay, B., & Mishra, S. P. (2014). Free Radicals and Antioxidant Status in Protein Energy Malnutrition. *International Journal of Pediatrics, 2014*, 6. doi: 10.1155/2014/254396

Konuk Takma, D., & Korel, F. (2016). Impact of preharvest and postharvest alginate treatments enriched with vanillin on postharvest decay, biochemical properties, quality and sensory attributes of table grapes. *Food Chemistry*. doi: http://dx.doi.org/10.1016/j.foodchem.2016.09.195

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science, 36*(1), 93-104. doi: http://dx.doi.org/10.1016/0309-1740(94)90036-1

Kuss, F., Restle, J., Brondani, I. L., Pascoal, L. L., Menezes, L. F. G. d., Pazdiora, R. D., & Freitas, L. d. S. (2005). Características da carcaça de vacas de descarte de diferentes grupos genéticos terminadas em confinamento com distintos pesos. *Revista Brasileira de Zootecnia, 34*, 915-925.

Lagerstedt, Å., Enfält, L., Johansson, L., & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef M. longissimus dorsi. *Meat Science, 80*(2), 457-461. doi: http://dx.doi.org/10.1016/j.meatsci.2008.01.009

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*(2), 93-98. doi: http://dx.doi.org/10.1016/j.meatsci.2012.01.013

Li, W., Hydamaka, A. W., Lowry, L., & Beta, T. (2009). Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. [journal article]. *Central European Journal of Biology, 4*(4), 499-506. doi: 10.2478/s11535-009-0041-1

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Lucera, A., Costa, C., Conte, A., & Del Nobile, M. A. (2012). Food applications of natural antimicrobial compounds. *Frontiers in Microbiology,* 3, 1-13. doi: 10.3389/fmicb.2012.00287

Luchiari Filho, A. (2000). Livestock raising. *1.ed. Revista Vieira Gráfica e Editora, São Paulo*, São Paulo.

MacDougall, D. B. (1982). Changes in the colour and opacity of meat. *Food Chemistry*, 9, 75-88.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*(1), 100-121. doi: http://dx.doi.org/10.1016/j.meatsci.2005.03.003

Marques, J. A., Prado, I. N., Maggioni, D., Rigolon, L. P., Caldas Neto, S. F., & Zawadzki, F. (2010). Desempenho de novilhas mestiças em diferentes estádios reprodutivos. *Semina: Ciências Agrárias, 31*(2), 507-514.

Marques, J. d. A., Prado, I. N. d., Moletta, J. L., Prado, I. M. d., Prado, J. M. d., Macedo, L. M. A., Matsushita, M. (2006). Características físico-químicas da carcaça e da carne de novilhas submetidas ao anestro cirúrgico ou mecânico terminadas em confinamento. *Revista Brasileira de Zootecnia, 35*, 1514-1522.

Martin, K. R., & Appel, C. L. (2009). Polyphenols as dietary supplements: A double-edged sword. *Nutrition and Dietary Supplements, 2010*(2), 1-12.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70*(4), 665-682. doi: http://dx.doi.org/10.1016/j.meatsci.2005.02.016

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. *Journal of Food Science, 73*(6), C439-C446. doi: 10.1111/j.1750-3841.2008.00805.x

Moyo, B., Masika, P. J., & Muchenje, V. (2014). Effect of feeding Moringa (Moringa oleifera) leaf meal on the physico-chemical characteristics and sensory properties of goat meat. *South African Journal of Animal Science*, 44, 64-70.

Muller, L. (1987). Standards for carcass evaluation and tender of steers. *2.ed. Santa Maria: Universidade Federal de Santa Maria*, *31*.

Muthukumar, M., Naveena, B. M., Vaithiyanathan, S., Sen, A. R., & Sureshkumar, K. (2014). Effect of incorporation of Moringa oleifera leaves extract on quality of ground pork patties. *Journal of Food Science and Technology*, 51, 3172-3180.

NRC. (2000). *Nutrient requirements of beef cattle* (7th rev. ed.). Natl. Acad. Press, Washington, DC, USA.

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science, 79*, 678-687. doi: 10.2527/2001.793678x

Prado, I. N., Campo, M. M., Muela, E., Valero, M. V., Catalan, O., Olleta, J. L., & Sañudo, C. (2014). Effects of castration age, dietary protein level and lysine/methionine ratio on animal performance, carcass and meat quality of Friesian steers intensively reared. *Animal, 8*(9), 1561-1568. doi: 10.1017/S1751731114001591

Prado, I. N., Campo, M. M., Muela, E., Valero, M. V., Catalan, O., Olleta, J. L., & Sañudo, C. (2015). Effects of castration age, protein level and lysine/methionine ratio in the diet on colour, lipid oxidation and meat acceptability of intensively reared Friesian steers. *Animal, 9*(8), 1423-1430. doi: 10.1017/S175173115000580

27

Prado, I. N., Cruz, O. T. B., Valero, M. V., Zawadzki, F., Eiras, C. E., Rivaroli, D. C., & Visentainer, J. V. (2016). Effects of glycerin and essential oils (*Anacardium occidentale* and *Ricinus communis*) on the meat quality of crossbred bulls finished in a feedlot. *Animal Science Production, 56*, 2105-2114. doi: http://dx.doi.org/10.1071/AN14661

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 Radical Biology and Medicine, 26*(9–10), 1231-1237. doi: http://dx.doi.org/10.1016/S0891-5849(98)00315-3

Realini, C. E., Duckett, S. K., Brito, G. W., Dalla Rizza, M., & De Mattos, D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Science, 66*(3), 567-577. doi: http://dx.doi.org/10.1016/S0309-1740(03)00160-8

Reddy, B.V., Sivakumar, A. S., Jeong, D. W., Woo, Y., Park, S., Lee, S.Y., Byun, J.Y., Kim, C. H., Cho, S. H., Hwang, I. (2015). Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. Animal Science Journal, 86(1), 1-16. doi: 10.1111/asj.12266

Renerre, M. (2004). La couleur de la viande et sa mesure. *INRA, 1*, 63-68.

Rivaroli, D. C., Guerrero, A., Velandia Valero, M., Zawadzki, F., Eiras, C. E., Campo, M. d. M., Prado, I. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Science, 121*, 278-284. doi: http://dx.doi.org/10.1016/j.meatsci.2016.06.017.

Roça, R. O. (2000). Meat and derived products technology: Botucatu: Faculdade de Ciências Agronômicas, UNESP, 202.

Roça, R. O. (2009). Meat properties. *http://dgta. fca. unesp. br/docentes/roca/carnes/Roca105. pdf> Acesso em, 10*(11), 2009.

Rotta, P. P., Prado, R. M. d., Prado, I. N. d., Valero, M. V., Visentaine, J. V., & Silva, R. R. (2009). The Effects of Genetic Groups, Nutrition, Finishing Systems and Gender of Brazilian Cattle on Carcass Characteristics and Beef Composition and Appearance: A Review. *Asian Australas. Journal of Animal Science, 22*(12), 1718-1734. doi: 10.5713/ajas.2009.90071

Russell, J. B., & Houlihan, A. J. (2003). Ionophore resistance of ruminal bacteria and its potential impact on human health. *FEMS Microbiology Reviews, 27*(1), 65-74. doi: 10.1016/s0168-6445(03)00019-6

Shackelford, S. D., Koohmaraie, M., Cundiff, L. V., Gregory, K. E., Rohrer, G. A., & Savell, J. W. (1994). Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. *Journal of Animal Science, 72*, 857-863. doi: 10.2527/1994.724857x

Shackelford, S. D., Koohmaraie, M., Miller, M. F., Crouse, J. D., & Reagan, J. O. (1991). An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. *Journal of Animal Science, 69*, 171-177. doi: 10.2527/1991.691171x

Silva, R. M. d., Restle, J., Bilego, U. O., Missio, R. L., Pacheco, P. S., & Prado, C. S. (2014). Características físico-químicas da carne de tourinhos zebuínos e europeus alimentados com níveis de grão de milheto na dieta. *Ciência Animal Brasileira, 15*, 20-31.

Sousa, S. L. G., Neto, O. C., Medeiros, L. F. D., Quirino, C. R., Fontes, C. A. A., Jorge Carlos Dias de Sousa, J. C. D., & Rodrigues, V. C. (2015). Características

qualitativas da carcaça e da carne de animais nelore e f1 sindi – nelore abatidos aos 36 e 48 meses de idade. *Acta. Tecnológica, 10*(1), 32-38.

Strydom, P., Lühl, J., Kahl, C., & Hoffman, L. C. (2016). Comparison of shear force tenderness, drip and cooking loss, and ultimate muscle pH of the loin muscle among grass-fed steers of four major beef crosses slaughtered in Namibia. *South African Journal of Animal Science*, 46, 348-359.

Valero, M. V., Prado, R. M. d., Zawadzki, F., Eiras, C. E., Madrona, G. S., & Prado, I. N. d. (2014a). Propolis and essential oils additives in the diets improved animal performance and feed efficiency of bulls finished in feedlot. *Acta Scientiarum. Animal Sciences, 36*, 419-426.

Valero, M. V., Torrecilhas, J. A., Zawadzki, F., Bonafe, E. G., Madrona, G. S., Prado, R. M. d., Passetti, R. A. C., Rivarroli, D. C., Visentainer, J. V., Prado, I. N. do. (2014b). Propolis or cashew and castor oils effects on composition of Longissimus muscle of crossbred bulls finished in feedlot. *Chilean journal of Agricultural Research, 74*, 445-451.

Vital, A. C. P., Guerrero, A., Monteschio, J. O., Valero, M. V., Carvalho, C. B., de Abreu Filho, B. A., Prado, I. N. do. (2016). Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PLoS One, 11*(8), e0160535. doi:10.1371/journal.pone.0160535.

Whipple, G., Koohmaraie, M., Dikeman, M. E., Crouse, J. D., Hunt, M. C., & Klemm, R. D. (1990). Evaluation of attributes that affect longissimus muscle tenderness in Bos taurus and Bos indicus cattle. *Journal of Animal Science, 68*, 2716-2728. doi: 10.2527/1990.6892716x

Zawadzki, F., Prado, I. N., Marques, J. A., Zeoula, L. M., Rotta, P. P., Sestari, B. B., Valero M. V. & Rivaroli, D. C. (2011). Sodium monensin or propolis extract in

the diets of feedlot-finished bulls: effects on animal performance and carcass characteristics. *Journal of Animal and Feed Sciences, 20*(1), 16-25. doi: 10.22358/jafs/66153/2011

Zhu, Q. Y., Hackman, R. M., Ensunsa, J. L., Holt, R. R., & Keen, C. L. (2002). Antioxidative Activities of Oolong Tea. *Journal of Agricultural and Food Chemistry, 50*(23), 6929-6934. doi: 10.1021/jf0206163

Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry, 114*(1), 310-316. doi: http://dx.doi.org/10.1016/j.foodchem.2008.09.033

Table 1

Composition of ingredients and the diets fed to Nellore heifers (g kg $^{-1}$ of DM)

Ingredients	DM[1]	OM[2]	Ashes	CP[3]	EE[4]	NDF[5]	ADF[6]	TC[7]	NFC[8]	TDN[9]
Corn silage	306	969	30.9	71.1	27.1	424	224	870	446	656
Corn grain	853	984	16.4	96.1	47.1	175	45.8	840	665	858
Soybean meal	850	933	67.0	489	19.0	159	87.8	425	266	810
Yeast	920	954	46.1	331	21.0	26.0	9.22	572	546	
Phosphorus	995	38.0	962							
Mineral salt	986	55.0	945							
CON[10]	710	971	27.3	125	39.1	237	95.4	805	569	797
ROS[11]	701	970	28.1	127	38.6	241	98.8	804	562	793
BLE[12]	725	973	26.2	122	39.9	231	90.4	810	579	804
BCL[13]	720	972	26.7	124	39.6	234	92.7	808	574	801
BRC[14]	724	973	26.2	122	39.9	231	90.6	809	578	803

[1]Dry matter; [2]Organic matter; [3]Crude protein; [4]Ether extract; [5]Neutral detergent fiber; [6]Acid detergent fiber; [7]Total carbohydrates; [8]Non-fiber carbohydrates; [9]Total digestible nutrients; [10]CON – Without essential oil active principles ; [11]ROS – Rosemary essential oil (4 g/animal/day); [12]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [13]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [14]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day).

Table 2

Effect of essential oils and active principles on meat characteristics of Nellore heifers finished in feedlot.

Item	Diets					SEM[6]	P <
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]		
pH	5.79	5.82	5.79	5.84	5.84	0.0	0.895
Fat thickness	2.15	2.20	2.90	2.49	2.87	0.1	0.284
Marbling, points	1.75	2.50	2.50	2.25	3.25	0.2	0.205
LMA[7], cm^2	48.25	47.62	49.57	54.00	54.37	1.2	0.223

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). [6]SEM: Standard error of means. [7]LMA- *Longissimus thoracis* muscle area. Number of animals: n=8 per treatment.

Table 3

Effect of essential oils and active principles on meat losses of Nellore heifers finished in feedlot.

| Item | Diets | | | | | SEM[6] | *P* < |
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]		
Thawing losses, %	3.22	3.31	2.98	3.29	3.27	0.1	0.824
Drip loss, %	1.62	1.58	1.49	1.58	1.86	0.0	0.192
Cooking losses, %, days							
1	27.27	25.77	22.98	25.88	23.79	0.7	0.247
7	25.22	25.27	23.80	23.17	23.29	0.8	0.795
14	25.40[a]	25.22[a]	22.32[ab]	23.05[ab]	19.28[c]	0.7	0.024
SEM[7]	1.0	0.9	0.7	0.9	0.9		
P <	0.665	0.970	0.921	0.342	0.059		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC –eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Means with different lowercase letters in the same line are significantly different (p< 0.05). [6]SEM: Standard error of means from the time effect. [7]SEM: Standard error of means from the treatment effect. Number of animals: n=8 per treatment.

Table 4

L*a*b*, chroma and hue values of meat from Nellore heifers finished in feedlot.

Variables	Diets					SEM[6]	*P* <
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]		
L*, Days							
1	36.25[B]	35.18[B]	35.38	34.07[B]	36.35	0.3	0.184
7	38.46[Aa]	35.44[ABb]	36.68[ab]	35.44[ABb]	36.90[ab]	0.3	0.002
14	38.84[A]	38.41[A]	37.27	36.85[A]	37.82	0.3	0.359
SEM[7]	0.5	0.6	0.4	0.4	0.3		
P <	0.044	0.026	0.170	0.006	0.141		
a*, Days							
1	11.41[A]	11.35	11.60	11.74	11.29	0.2	0.933
7	10.12[A]B	10.61	11.57	10.96	11.00	0.3	0.480
14	9.79[B]b	10.40[ab]	10.47[ab]	10.98[a]b	11.65[a]	0.2	0.020
SEM[7]	0.3	0.2	0.3	0.3	0.2		
P <	0.025	0.270	0.266	0.515	0.445		
b*, Days							
1	10.34	10.18	10.26	9.71	10.34	0.1	0.661
7	10.37	9.97	10.42	10.07	10.51	0.2	0.815
14	10.27	10.41	10.33	10.59	11.36	0.2	0.236
SEM[7]	0.1	0.2	0.2	0.3	0.2		
P <	0.969	0.663	0.946	0.441	0.089		
Chroma, Days							
1	15.55	14.75	15.50	15.25	15.34	0.2	0.843
7	14.67	14.50	15.88	14.93	15.23	0.3	0.616
14	13.90[b]	14.78[ab]	14.73[ab]	15.27[ab]	16.32[a]	0.2	0.020
SEM[7]	0.3	0.3	0.4	0.4	0.3		
P <	0.076	0.927	0.456	0.921	0.185		
Hue, Days							
1	42.70[B]	42.00	41.48[B]	39.65[B]	42.55	0.4	0.138
7	46.36[A]	42.94	43.32[AB]	42.74[AB]	43.70	0.5	0.099
14	47.14[A]	45.23	44.64[A]	43.83[A]	44.45	0.4	0.182
SEM[7]	0.7	0.7	0.5	0.6	0.5		
P <	0.007	0.194	0.037	0.013	0.286		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE –eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL –eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means from the time effect. [7]SEM: Standard error of means from the treatment effect. Number of animals: n=8 per treatment.

Table 5

Effect of essential oils and active principles on meat shear force of Nellore heifers finished in feedlot.

Days	CON[1]	ROS[2]	BLE[2]	BCL[4]	BRC[5]	SEM[6]	$P <$
1	77.35^{A}	80.64^{A}	72.58^{A}	74.58^{A}	77.61^{A}	2.9	0.934
7	57.43^{B}	68.19^{AB}	55.94^{AB}	55.13^{AB}	63.50^{A}	2.1	0.227
14	49.89^{Bab}	56.94^{Ba}	48.85^{Bab}	45.90^{Bab}	41.19^{Bb}	1.7	0.041
SEM[7]	3.3	3.0	4.1	4.3	3.8		
$P <$	0.000	0,002	0,051	0.014	0.000		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means from the time effect. [7]SEM: Standard error of means from the treatment effect. Number of animals: n=8 per treatment.

Table 6

Radical scavenging activity (ABTS and DPPH radical scavenging) and ferric reducing power (FRAP) of meat of Nellore heifers finished in feedlot.

| Method | Diets | | | | | | |
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]	SEM[6]	P <
ABTS (%)	37.81[b]	38.49[b]	44.51[a]	44.27[a]	37.77[b]	0.6	0.001
DPPH (%)	24.91[b]	26.98[ab]	28.64[a]	27.95[a]	27.79[ab]	0.3	0.013
FRAP (mg EAG/g meat)	0.10[b]	0.11B[ab]	0.12[a]	0.12[a]	0.12[a]	0.0	0.001

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. [6]SEM: Standard error of means. Number of animals: n=8 per treatment.

Table 7

Effect of essential oils and active principles on lipid oxidation (TBARS) expressed as mg malonaldehyde kg-1 of meat of Nellore heifers finished in feedlot.

| Days | Diets | | | | | SEM[6] | P < |
	CON[1]	ROS[2]	BLE[3]	BCL[4]	BRC[5]		
1	0.27^C	0.26^C	0.24^C	0.24^C	0.25^C	0.0	0.146
7	0.52^{Ba}	0.47^{Bab}	0.39^{Bd}	0.41^{Bcd}	0.45^{Bbc}	0.0	0.001
14	0.70^{Aa}	0.60^{Ab}	0.51^{Ac}	0.51^{Ac}	0.59^{Ab}	0.0	0.001
SEM[6]	0.0	0.0	0.0	0.0	0.0		
P <	0.001	0.001	0.001	0.001	0.001		

[1]CON – Without essential oil; [2]ROS – Rosemary essential oil (4 g/animal/day); [3]BLE – eugenol + thymol + vanillin blend (4 g/animal/day); [4]BCL – eugenol + thymol + vanillin blend (2 g/animal/day) + clove essential oil (2 g/animal/day) and [5]BRC – eugenol + thymol + vanillin blend (1.33 g/animal/day), rosemary essential oil (1.33 g/animal/day), clove essential oil (1.33 g/animal/day). Different lowercase letters in the same line are significantly different. Different uppercase letters in the same column are significantly different. [6]SEM: Standard error of means. Number of animals: n=8 per treatment.

Highlights

- Essential oil and encapsulated active principles blend (BLE) were used in animal feed;
- The diets reduced lipid oxidation in relation to control after 7 days of storage;
- The diets inhibited the a* value degradation during storage compared to control;
- Diets (BLE and BLE + clove) improved meat antioxidant activity;