JENIFER MAIRA LIMA RAMOS

EFFECT OF VITAMIN OR MINERAL SUPPLEMENTATION ON OXIDATION AND QUALITY TRAITS OF FRESH AND AGED BEEF

Thesis submited to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment for the degree of *Doctor Scientiae*.

Adviser: Mario Luiz Chizzotti

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Author

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Mario Luiz Chizzotti
Adviser

To my father Armindo Santiago Ramos (*In Memorian*), who while he was by my side was the person who most supported and encouraged me to continue my studies.

I DEDICATE

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"A inquietude é o estímulo essencial à pesquisa científica"
Anderson Vailati Ritzmann

ABSTRACT

RAMOS, Jenifer Maira Lima, D.Sc., Universidade Federal de Viçosa, December, 2022. **Effect of vitamin or mineral supplementation on oxidation and quality traits of fresh and aged beef.** Adviser: Mario Luiz Chizzotti.

This study was divided into two experiments. The first experiment with the use of fat-soluble vitamins (ADE) and/or water-soluble vitamins (biotin, niacin and thiamine) via diet in Nellore cattle. The second experiment with the use of vitamins AE and microminerals (Se, Mn, Zn and Cu) injected in crossbred cattle (¾ Angus, ¼ Nellore). Each of the two experiments originated a chapter of this thesis, totaling two chapters. In the first chapter, the effect of supplementation with a mix of B vitamins (biotin, niacin and thiamine), mix of vitamins (ADE) or a combination of these mixes on oxidation parameters, antioxidant status and quality of beef was evaluated. Nellore. Forty Nellore bulls, with a mean age of 8 ± 1 month and mean body weight of 261 ± 27.3 kg, were randomly assigned to four treatments: (a) without vitamin supplementation (Control); (b) vitamin B (B) mix supplementation; (c) mix supplementation of fat-soluble vitamins (ADE); and (d) combination of mixes (ADE + B). At the end of the 140-day period, the *longissimus lumborum* muscle was collected for analysis of meat quality, antioxidant enzyme activities and lipid oxidation at 1h, 24h, 196h and 360h *postmortem*. Supplementation with ADE and ADE+B increased intramuscular fat content, oxymyoglobin (OMb) and decreased metmyoglobin (MMb) at 196h and 360h *postmortem*, and decreased deoxymyoglobin (DMb) 196h *postmortem*, increased the Myofibrillar Fragmentation Index (MFI) 24h, 196h and 360h *postmortem*. In addition, ADE supplementation increased superoxide dismutase (SOD) and Ferric Antioxidant Reducing Power (FRAP), decreased glutathione peroxidase (GPx) and nitric oxide 24h *postmortem*, increased catalase and FRAP 196h *postmortem*, and increased FRAP and decreased malondialdehyde (MDA) 360 hours *postmortem*. In the second chapter, the effects of injectable supplementation of vitamins A and E and micro-minerals (Se, Mn, Zn and Cu) applied at weaning (D), castration (C) and pre-slaughter (PA) or their combination on meat quality, antioxidant status and lipid oxidation of crossbred cattle (¾ Angus ¼ Nellore). One hundred and twenty crossbred cattle, with a mean age of 7 ± 1 month and mean body weight of 304.58 ± 33 kg, were randomly assigned to six treatments: (N=20): (i) 000-Control (saline solution (SS) on D , C and PA; (ii) DCA - Vitamin-mineral complex (MVC) in D, C and PA; (iii) DC0 - MVC in D and C + SS in PA; (iv) D0A - MVC in D + SS in C + CVM in the PA; (v) 00A - SS in the D and C + CVM in the PA; (vi)

0CA– SS in the D + CVM in the C and PA. At the end of the period of 250 days, the collection was performed for the analyzes of meat quality, antioxidant enzyme activities and lipid oxidation in *longissimus thoracis* and *lumborum* muscle 1h, 24h, 196h and 360h *postmortem*. 192h and 360h *postmortem*. The shear force variable showed a difference 24h and 192h *postmortem*. Antioxidant activity (CAT and FRAP) and MDA showed a difference 1h *postmortem*. SOD and FRAP 24h *postmortem*, FRAP and MDA 192h *postmortem* and CAT and FRAP 360h *postmortem*. In the *longissimus lumborum* muscle, the Hue, MMb and OMb variables were different 24 hours *postmortem*. The variables a*, chroma and OMb showed a difference 192 hours *postmortem* and the variables a*, b* and chroma showed a difference 360h *postmortem*. FRAP showed difference 24h and 360h *postmortem*, GPx and SOD 360h *postmortem*. The use of CVM at least in PA reduces the oxidation of OMb to MMb and provides greater antioxidant status and decreased lipid oxidation.

Keywords: Antioxidants. Colour. Myoglobin. Oxidation.

RESUMO

RAMOS, Jenifer Maira Lima, D.Sc., Universidade Federal de Viçosa, dezembro de 2022. **Efeito da suplementação vitamínica ou mineral na oxidação e características de qualidade da carne fresca e maturada.** Orientador: Mario Luiz Chizzotti.

Este estudo foi dividido em dois experimentos. O primeiro experimento com a utilização de vitaminas lipossolúveis (ADE) e/ou hidrossolúveis (biotina, niacina e tiamina) via dieta em bovinos Nelore. O segundo experimento com a utilização de vitaminas AE e microminerais (Se, Mn, Zn e Cu) injetável em bovinos cruzados (¾ Angus, ¼ Nelore). Cada um dos dois experimentos originou um capítulo desta tese, totalizando dois capítulos. No primeiro capítulo avaliou-se o efeito da suplementação de um mix de vitaminas do complexo B (biotina, niacina e tiamina), mix de vitaminas (ADE) ou a combinação desses mix sobre parâmetros de oxidação, *status* antioxidante e qualidade da carne de bovinos Nelore. Quarenta tourinhos Nelore com idade média de 8 ± 1 meses e peso corporal médio de 261 ± 27,3 kg, foram distribuídos aleatoriamente em quatro tratamentos: (a) sem suplementação vitamínica (Controle); (b) suplementação mix de vitaminas B (B); (c) suplementação mix de vitaminas lipossolúveis (ADE); e (d) combinação dos mix (ADE + B). Ao final do período de 140 dias foi realizada a coleta do musculo *longissimus lumborum* para as análises de qualidade da carne, atividades de enzimas antioxidantes e oxidação lipídica 1h, 24h, 196h e 360h *postmortem*. A suplementação com ADE e ADE+B aumentou o teor de gordura intramuscular, a oximioglobina (OMb) e diminuiu a metamioglobina (MMb) aos 196h e 360h *postmortem*, e também diminuiu a desoximioglobina (DMb) 196h *postmortem*, aumentou o índice de fragmentação miofibrilar (IFM) 24h, 196h e 360h *postmortem*. Além disso, a suplementação com ADE aumentou a superóxido dismutase (SOD) e o Poder Antioxidante Redutor Férrico (FRAP), diminuiu a glutationa peroxidase (GPx) e o óxido nítrico 24h *postmortem*, aumentou a catalase e FRAP 196h *postmortem*, e aumentou FRAP e diminuiu malondialdeído (MDA) 360h *postmortem*. No segundo capítulo, avaliou-se os efeitos da suplementação injetável de vitaminas A e E e micro-minerais (Se, Mn, Zn e Cu), aplicados no desmame (D), castração (C) e pré-abate (PA) ou sua combinação, sobre a qualidade da carne, *status* antioxidante e oxidação lipídica de bovinos cruzados (¾ Angus ¼ Nelore). Cento e vinte bovinos cruzados com idade média de 7 ± 1 meses e peso corporal médio de 304.58 ± 33 kg, foram distribuídos aleatoriamente em seis tratamentos: (N=20): (i) 000-Controle (solução salina (SS) no D, C e PA; (ii) DCA - Complexo vitamínico-mineral (CVM)

no D, C e PA; (iii) DC0 - CVM no D e C + SS no PA; (iv) D0A - CVM no D + SS na C + CVM no PA; (v) 00A - SS no D e C + CVM no PA; (vi) 0CA– SS no D + CVM na C e PA. Ao final do período de 250 dias foi realizada a coleta para as análises de qualidade da carne, atividades de enzimas antioxidantes e oxidação lipídica no músculo *longissimus thoracis e lumborum* 1h, 24h, 196h e 360h *postmortem*. No músculo *longissimus thoracis*, as variáveis a* e Hue apresentaram diferença 192h *postmortem* e a MMb em 192h e 360h *postmortem*. A variável força de cisalhamento apresentou diferença 24h e 192h *postmortem*. A atividade antioxidante (CAT e FRAP) e MDA apresentaram diferença 1h *postmortem*. SOD e FRAP 24h *postmortem*, FRAP e MDA 192h *postmortem* e CAT e FRAP 360h *postmortem*. No músculo *longissimus lumborum*, as variáveis Hue, MMb e OMb foram diferentes 24h *postmortem*. As variáveis a*, chroma e OMb apresentaram diferença 192h *postmortem* e as variáveis a*, b* e chroma apresentaram diferença 360h *postmortem*. FRAP apresentou diferença 24h e 360h *postmortem*, GPx e SOD 360h *postmortem*. A utilização de CVM ao menos no PA reduz a oxidação da OMb a MMb e proporciona maior status antioxidante e diminuição da oxidação lipídica.

Palavras-chave: Antioxidantes. Coloração. Mioglobina. Oxidação.

LIST OF TABLES

CHAPTER I

CHAPTER II

SUMMARY

1. INTRODUCTION

Color is a characteristic used by consumers as an indicator of meat ‘freshness’ on which they base their purchasing decisions, becoming a basic criterion in the choice (Cheng et al., 2015; Tomasevic et al., 2021). The attractive red color of meat forms when its surface is exposed to oxygen, allowing oxygenation of myoglobin into the red pigment oxymyoglobin (Seyfert et al., 2006). Over time, oxidative *postmortem* metabolism depletes oxygen concentrations and generates oxidative free radical byproducts in meat, causing myoglobin oxidation to the brown pigment metmyoglobin (Calnan et al., 2019).

The rate of oxidation varies depending on various meat internal conditions such as fat content, fatty acid composition, antioxidants, heme pigments and iron content, as well as the external meat conditions arising from various processing steps. such as cutting, milling, in addition to storage temperature, packaging (Das et al., 2020). Oxidation in meat produces a strange taste, deteriorates meat color and causes loss of functional and nutritional properties, and forms possible toxic compounds (Cheng et al., 2015).

In addition, meat is considered a food rich in protein and content of pro-oxidants such as lipids and myoglobin, which are susceptible to oxidation and oxidative conditions that readily occur in *postmortem* muscle (Bao & Ertbjerg, 2019) and continue progressively until the final product is consumed (Domínguez et al., 2019), causing 'oxidative stress' in the organism (Ponnampalam et al., 2022).

A multi dynamic antioxidant defense system is present in living organisms to protect cells and tissues from damage caused by oxidative stress. This system encompasses non-enzymatic antioxidants (eg vitamins, carotenoids) and enzymatic antioxidants (eg glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase) (Ponnampalam et al., 2022). During the *postmortem* period, cellular antioxidant defenses may not be more strongly activated, under some conditions, the balance between the production of reactive oxygen species (ROS) and the antioxidant defense system is weakened or lost.

Therefore, an antioxidant state must be achieved before slaughter to maximize the protection of muscle lipids against lipid oxidation (Descalzo et al., 2005), in addition to aerobic cells against biological damage from ROS created during metabolic activities (Caglayan et al., 2019). Therefore, *ante-mortem* antioxidant solutions include animal feed management to minimize the onset of oxidative stress in the live animal and therefore contribute to increasing the oxidative stability of muscle tissue after slaughter (Possamai et al., 2018).

Dietary vitamin E supplementation is a proven means of increasing meat color stability (Calnan et al., 2019; Descalzo et al., 2005; Gallardo et al., 2015; Juárez et al., 2012; Karami et al., 2015; ., 2011; Kasapidou et al., 2012; Possamai et al., 2018; Ripoll et al., 2011), improving the antioxidant capacity of the muscle and, therefore, the quality of the meat, avoiding/delaying lipid peroxidation, oxidation of protein and the discoloration of muscle meat, which helps to extend shelf life (Domínguez et al., 2019).

Vitamin A also has considerable antioxidant potential, the most prominent dietary antioxidants being carotenoids (eg, β-carotene-a precursor of vitamin A) (Domínguez et al., 2019). Both antioxidants, vitamin A and E can exert a cooperative antioxidant activity at different positions within the membrane (Descalzo et al., 2005), vitamin A breaks the chain located in the hydrophobic region of biological membranes and vitamin E breaks the chain and protects membranes from oxidation (Gülçin, 2012).

In addition to these vitamins previously known as antioxidants, vitamin D supplementation has been reported to improve meat color stability (Hansen et al., 2012; Lahucky et al., 2007; Strydom et al., 2011). Lahucky et al., (2007) used vitamin D and related color improvement with increased antioxidant capacity and suggested that a higher level of Ca^{2+} (bivalent ion) in the muscles caused a positive influence on lipid oxidation.

In addition, other important vitamins are the B complex vitamins that act as cofactors in various reactions: metabolic (Thiamine), redox (Niacin), and also as a coenzyme in the metabolism of fatty acids, amino acids and gluconeogenesis (Biotin) (Duplessis et al., 2022), in addition to providing an increase in muscle fat content (Biotin) (Poolthajit et al., 2022), however, B complex vitamins are poorly studied in beef cattle, as cattle are capable of to synthesize them in the rumen, but despite this, they are stored in small amounts, in addition to being easily excreted in the urine (Berchielli, 2006). Knowledge about the effect of individual or combined use of B vitamins on meat quality parameters remains limited, most research aimed at the application of these alternatives remains focused only on dairy cattle.

In addition to antioxidant vitamins, some microminerals such as manganese (Mn), Copper (Cu), zinc (Zn) and Selenium (Se) are essential for the formation or normal functions of the body and exist in the structure of SOD, CAT and GPx enzymes (Descalzo & Sancho, 2008). The action between selenium and vitamin E can increase GPx production (Idamokoro et al., 2020). SOD is Mn dependent (Arthington & Ranches, 2021).

Normally, the supply of microminerals is dietary, however, there is a great viability in the consumption among animals of the same cattle with such a strategy (Manzano et al., 2012).

Another alternative is the use of injectable mineral supplementation, which rapidly increases the micromineral status of animals compared to dietary supplementation (Genther & Hansen, 2014; Hartman et al., 2018), is not interfered with by dietary antagonists (Arthington et al. , 2014; Hartman et al., 2018) and increase the control of oxidative stress (Soldá et al., 2017; Teixeira et al., 2014; Vedovatto et al., 2019). (Pogge et al., 2012) reported that plasma Cu, Mn, Se and Zn were elevated 8 to 10 hours after injection. Therefore, pre-slaughter injectable supplementation may be a good alternative to improve antioxidant status.

Meat quality is negatively affected by oxidative stress and therefore the inclusion of different antioxidants with synergistic effects can have positive effects. Furthermore, to our knowledge, no study has evaluated the effects of the combined supra nutritional inclusion of vitamin A, D, E and B vitamins (thiamine, niacin and biotin) on meat quality traits of beef cattle or the combination of vitamins A, E and microminerals (copper, selenium, zinc, manganese) injectable.

Given the above, the presented thesis was divided into two chapters. In the first chapter, it was hypothesized that the supplementation of vitamins ADE and complex B (biotin, niacin and thiamine) together will increase the antioxidant status and improve the meat quality of precocious Nellore cattle. The aim was to evaluate the effect of supplementation with vitamins ADE or vitamins of the B complex (biotin, niacin and thiamine), or their combination, on oxidation parameters, antioxidant status and meat quality of precocious Nellore cattle.

In the second chapter, it was hypothesized that injectable supplementation of vitamins A and E and micro-minerals (Copper, Zinc, Manganese, Selenium) for finishing crossbred cattle improves meat quality, antioxidant status and decreases oxidation. The aim was to evaluate the effects of injectable supplementation of vitamins A and E and micro-minerals (Copper, Zinc, Manganese, Selenium) applied at weaning or castration or pre-slaughter or their combination, on meat quality, antioxidant status and oxidation. lipid profile of bovines in the finishing phase.

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2. CHAPTER I

Effect of vitamin supplementation on oxidative stability and beef quality traits of Nellore bulls[1]

Jenifer Maira Lima Ramos[2]*, Mario Luiz Chizzotti[2]

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[2] Department of Animal Science at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-000.

*Corresponding author: Ramos, Jenifer – E-mail: jenifer.ramos@ufv.br

Abstract

Lipid oxidation can negatively impact the flavor, texture, and appearance of meat, and also decrease meat shelf life. An alternative to reducing oxidative action is the use of dietary vitamins that can promote an increase in the antioxidant capacity of muscle tissue. Therefore, the objective of this study was to evaluate the effect of supplementation with a mix of B vitamins (biotin, niacin, and thiamine), a mix of fat-soluble vitamins (ADE), or the combination of these two mixtures on oxidation, antioxidant status, and beef quality traits. Forty young Nellore bulls with a mean age of 8 ± 1 month and a mean body weight of 261 ± 27.3 kg were used. The experiment was conducted in a completely randomized design. The animals were randomly distributed into four treatments, as follows: (a) no vitamin supplementation (Control); (b) supplementation of B vitamins mix (B); (c) supplementation with fat-soluble vitamins mix (ADE); and (d) combination of the two mixtures (ADE + B). Ao final do período de 140 dias foi realizada a coleta do musculo *longissimus lumborum* para as análises de composição (teor de gordura intramuscular) de qualidade da carne (cor, perdas por exsudatos, força de cisalhamento e índice de fragmentação miofibrilar (IFM), atividades de enzimas antioxidantes, oxidação lipídica 1h, 24h, 196h e 360h *postmortem*. A suplementação com ADE e ADE+B aumentou o teor de gordura intramuscular, além de ter aumentado a oximioglobina (OMb) e diminuiu a metamioglobina (MMb) aos 196h e 360h *postmortem*, e também diminuiu a desoximioglobina (DMb) 196h *postmortem*, aumentou o IFM 24h, 196h e 360h *postmortem*. Além disso, a suplementação com ADE aumentou a superóxido dismutase (SOD) em comparação ao controle, o Poder Antioxidante Redutor Férrico (FRAP) também foi maior para ADE e ADE+B em comparação ao controle, ADE e ADE+B diminuíram a glutationa peroxidase (GPx) e o óxido nítrico 24h *postmortem*, aumentou a catalase e FRAP 196h *postmortem*, e aumentou FRAP e diminuiu malondialdeído (MDA) 360h *postmortem*. O uso de suplementação vitamínica com ADE e ADE+B proporciona maior atividade antioxidante em carnes 196h e 360h *postmortem.* No entanto, apesar de ADE e ADE+B apresentarem resultados semelhantes, a utilização apenas das vitaminas do complexo B, não acompanharam tais resultados,

Keywords: ADE vitamin. Aging**.** Antioxidant. Colour. Myoglobin. Oxidation. Shelf life.

2.1. Introduction

Oxidation is the main non-microbiological cause that affects the quality and shelf life of meat and its products (Domínguez et al., 2019). Meat is susceptible to oxidative deterioration due to high concentrations of unsaturated lipids, heme pigments, metallic catalysts, and a range of oxidizing agents in muscle and adipose tissue (Domínguez et al., 2019; Falowo et al., 2014).

Oxidation is one of the biggest economic problems in the meat industry (Sampels, 2013). It leads to the formation of several undesirable compounds (hydrocarbons, aldehydes, alcohols, and volatile ketones), which are responsible for attributing unpleasant flavors and odors to the products (Ahn & Lee, 2002; Chaijan, 2008; Lorenzo et al., 2013; Mapiye et al., 2012).

Muscle and adipose tissue are susceptible to oxidation and oxidative conditions that can fast occur in *postmortem* muscle (Bao & Ertbjerg, 2019) and progressively continue until the final product is consumed (Domínguez et al., 2014, 2019).

Extending meat shelf life by delaying oxidative deterioration is important to the meat industry. However, the acceptability of synthetic additives by consumers is low (Gupta & Abu-Ghannam, 2011), since some toxicity and carcinogenicity were identified products (Faine et al., 2006). Because of that, the meat industry's interest in using natural antioxidants has increased considerably (Kumar et al., 2015), guided by the demands of consumers.

The presence of exogenous antioxidants in the animal diet can increase the lipid stability of meat (Falowo et al., 2014; Jiang & Xiong, 2016; Possamai et al., 2018; Suman et al., 2014), with great potential to incorporate into the final product produced, and consequently, benefit the entire production chain.

Dietary supplementation of vitamin E (α-tocopherol) increases meat color stability (Calnan et al., 2019; Descalzo et al., 2005; Gallardo et al., 2015; Juárez et al., 2012; Karami et al., 2011; Kasapidou et al., 2012; Possamai et al., 2018; Ripoll et al., 2011) due to the ability of vitamin E to neutralize free radicals that trigger myoglobin oxidation. In addition, vitamin A (β-carotene) and vitamin D also play an important role in the oxidative stability of meat (Domínguez et al., 2019; Lobo-Jr. et al., 2012). Vitamin D can increase *postmortem* proteolysis, improving meat tenderness (Duffy et al., 2017; Karges et al., 2001; Montgomery et al., 2000).

Normally, beef cattle do not receive vitamin B complex supplementation due to their ability to synthesize them in the rumen. However, B vitamins are stored in small amounts in the body and are easily excreted in the urine, thus continuous supplementation in the diet is

needed (Berchielli, 2006). In addition, B-complex vitamins act as cofactors in metabolic reactions (Thiamine). In redox reactions (Niacin) they act as coenzymes in the metabolism of fatty acids, amino acids, and gluconeogenesis (Biotin) (Duplessis et al., 2022). Also, they provide increased fat content in the muscle (Biotin) (Poolthajit et al., 2022). However, the knowledge about the effect of individual or combined use of B vitamins on meat quality parameters remains limited. Most research applying these alternatives remains have focused on dairy cattle.

Thus, we hypothesized that the supplementation of fat-soluble vitamins (ADE) and complex B (biotin, niacin, and thiamine) will increase the antioxidant status and improve beef quality of young Nellore bulls. The aim of the present study was to evaluate the effect of a mixture supplementation of B vitamins (biotin, niacin, and thiamine), mixture supplementation of fat-soluble vitamins (ADE), and the combination of these two mixtures on oxidation parameters, antioxidant status and beef quality of young Nellore bulls.

2.2. Material and methods

2.2.1. Facilities, animals, diets, and experimental design

The experiment was carried out at the experimental feedlot facilities of the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais. Forty non-castrated Nellore males with an average age of 8 ± 1 month and body weight of 261 ± 27.3 kg from the Beef Cattle Sector of the Animal Science Department of the Universidade Federal de Viçosa were used.

The experiment was conducted in a completely randomized design, in which the animals were randomly assigned to four treatments. Each treatment group (n=10) received the experimental diet as follows: (a) no vitamin supplementation (Control); (b) mix supplementation of B vitamins (B); (c) mix supplementation of fat-soluble vitamins (ADE); (d) combination of the two mixtures (B + ADE).

The experiment trial lasted 170 days, of which the first 30 days were the adaptation period to the experimental conditions and 140 days for evaluation. The animals were identified, weighed, and treated against ecto and endoparasites. During the 30-day adaptation period, all animals received the same diet *ad libitum*, in which the roughage:concentrate ratio gradually

decreased (at 10% intervals) every 5 days from a 70:30 ratio until reaching the ratio 30:70 (DM basis).

The diets were isoproteic, with 120g of crude protein/kg (DM base), formulated for an average daily gain of 1.2 kg/day according to the recommendations of BR-CORTE 3.0 (Valadares Filho et al., 2016). The animals were kept in collective pens with a concrete floor and a total area of approximately 25 m², equipped with electronic feeders and drinkers (model AF-1000 Master; Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015).

The vitamins were added to the diet according to the vitamin supplementation guidelines (Casals and Calsamiglia, 2012) proposed by DSM Nutritional Products Ltd (Basel, Switzerland). The supplemented amounts of each blend of vitamins were added in the premix for each treatment, following the recommendations of Optimum Vitamin Nutrition - OVN® (OVN, 2016), which is recommended by the company DSM®. The levels of each vitamin per kg of Dietary MS were: 3.3 mg of biotin (D-biotin), 111.1 mg of niacin (niacin), 28.9 mg of thiamine (thiamine tydrochloride), 6666.7 IU of vitamin A (retinol acetate), 5111.1 IU of vitamin D (13% D3 -cholecalciferol and 87% 25-Hydroxyvitamin D 3 - Hy-D®), and 70 IU of vitamin E (DL-alpha-tocopherol acetate). The experimental mineral supplements were produced in a commercial feed mill following all manufacturing standards (DSM Produtos Nutricionais Brasil SA, Mairinque, SP, Brazil). All vitamins used in the study were obtained from DSM Produtos Nutricionais Brasil SA. The chemical composition and proportion of ingredients in the diets are presented in Table 1.

2.2.2. Harvesting, pH and temperature measurements

At the end of the 140 day experimental period, the animals were kept deprived of solids for 16 hours, stunned by a pneumatic gun, and bled following the Normative Instruction N° 9.013/MAPA 2017. After slaughter, the pH and temperature were measured in the *longissimus thoracis* muscle with a portable meat pH meter coupled with a thermometer (Model HI 99163 - Hanna Instruments). Each carcass was divided into two half carcasses, which were weighed, then chilled at 4°C for 24 hours. After the chilling period, the pH and temperature were measured again in the same spot. The pH meter probe calibration was performed in buffers at pH 4 and 7 at 4 °C before use.

2.2.3. Collection and analysis of muscle and meat

Following the chilling period, samples of the *longissimus lumborum* muscle were collected in each right half carcass, then sectioned into three portions, adequately identified, and vacuum-packed. One portion of each sample was frozen immediately, and the two remaining portions were aged at 4ºC for 196h and 360h before freezing.

2.2.4. Intramuscular fat

The edges of unripe steaks were cut, avoiding subcutaneous and intermuscular fat, they were chopped and lyophilized approximately 90g. Later, dry samples were ground using a stainless-steel ball mill. The fat content was analyzed in duplicate by extraction with petroleum ether using the Ankom XT4 filter bag and the Ankom XT15 fat extractor (ANKOM Technology, Macedon, NY, USA), following the manufacturer's recommendations. Intramuscular fat is expressed in $g.kg^{-1}$ of fresh meat.

2.2.5. Meat quality analyses

Three steaks of one inch (2.54 cm) thick were obtained from the frozen portions of the *longissimus lumborum*. One steak was reserved for objective color analysis, another for estimating thawing and cooking losses, and then to evaluate shear force, and the third steak was used for the analysis of the myofibrillar fragmentation index. The analyses were carried out at the Meat Science Laboratory of the Animal Science Department at the Universidade Federal de Viçosa.

Steak samples were thawed for 16 hours at 4ºC for color evaluation. After thawing, steaks were removed from the package and exposed to air for reoxygenation for 30 minutes at 4ºC. CIELab readings L*, a*, and b* were taken using a HunterLab MiniScan EZ 45/0 LAV spectrophotometer, adjusted to the illuminant source D65 and for an observer 10º angle. Five readings were performed for each sample at different points, and then the average value was used.

The wavelength reflected from the instrument were recorded in the range of 400–700 nm at 10 nm intervals, and the reflectance ratio at 630 nm and 580 nm (R630/580) was directly used to assess color stability during display. The reflectance (R) at 473, 525, 572, and 700 nm was converted to reflex attenuation (A) using the equation: $A = \log (1/R)$, and the relative

percentage of three redox forms of myoglobin was calculated following the AMSA (2012) equations:

$$\%MMb = \left(1.395 - \frac{A572-A700}{A525-A700}\right)$$

$$\%DMb = \left[2.35 \times \left(1 - \frac{A473-A700}{A525-A700}\right)\right] \times 100$$

$$\%OMb = 100 - (\%MMb + \%DMb)$$

The exudation losses were performed according to the methodology described by Bruce et al. (2004), with modifications described by Silva et al., (2019). Steaks were weighed before and after thawing, which lasted 16 hours at a temperature of 5°C until they reached an internal temperature of 2 to 5°C. Losses were expressed as a percentage of the weight of the steak before and after the process: Loss (%) = [(weight before – weight after)/weight before] x 100.

The shear force was measured in the same steak used to estimate exudation losses. After cooking, the steaks were refrigerated for 16 hours at 4ºC. Then, five cylindrical samples, 1.27 cm in diameter, were removed from each steak, parallel to the orientation of the muscle fibers, using a stainless-steel sharpened sampler. The cylindrical samples were sheared perpendicularly to the orientation of the muscle fibers, using a V-cut blade, with an angle of 60º and a thickness of 1.016 mm, and a fixed speed of 20 cm/min, coupled to the texture analyzer (G-R Electrical Manufacturing Company, Manhattan, KS, USA). The maximum forces used to cut the cylindrical samples were taken in the five cylindrical samples. The final shear force value (N) resulted from the average of the five cylindrical samples of each steak.

The sarcomere length was estimated according to the laser diffraction technique described by Cross & West, (1981). The average value of sarcomere length was obtained from six evaluations of each steak, following the equation below.

$$\text{Sarcomere length } (\mu m) = \frac{0.6328 \times D \times \sqrt{\left(T/D\right)^2 + 1}}{T}$$

In which: D = the distance in mm between the blade fixing support and the location of collection of the diffuse laser bands (120 mm was used in the present study) and T = distance in mm between the extreme bands divided by 2.

The Myofibrillar Fragmentation Index (MFI) was performed according to the methodology described by Culler et al. (1978), with modifications described by Hopkins et al. (2004). The protein concentration of the final suspension was determined by the biuret method (Gornall et al., 1949). In duplicate, aliquots of the myofibrillar suspension were diluted in a

buffer so that the protein concentration reached 0.5 mg/ml. The absorbance of the diluted myofibrillar suspension was immediately measured at 540 nm. The average of the duplicated absorbance was multiplied by 150 to give the MFI index value.

2.2.6. Evaluation of antioxidant activity and lipid peroxidation

A tissue homogenate (3g/10mL) was prepared and used for the assay of the antioxidant activities of the superoxide dismutase (SOD), glutathione peroxidase (GPx), and Catalase (CAT) enzymes. The same homogenate was used to determine the concentrations of malondialdehyde (MDA), nitric oxide (NO), and antioxidant activity (FRAP - Ferric Reducing Antioxidant Power).

The preparation of the homogenate was carried out according to the methodology described by Walsh et al. (1993). The antioxidant activity of SOD and GPx enzymes was determined using test kits (Ransod and Ransel, respectively) supplied by Randox Laboratories. The SOD concentration was determined following the methodology described by Walsh et al. (1993). Protein concentrations were estimated by the method described by Bradford (1976) and enzyme activities were expressed in IU per g of soluble protein.

The catalase activity (CAT) was determined according to the methodology described by (Hadwan & Abed, 2016), with minor modifications. Hydrogen peroxide (H_2O_2) was used as a substrate. A 5 µL volume of sample was incubated with 100 µL of the reaction mixture containing H_2O_2 and phosphate buffer at pH 7.4. After 3 min of incubation, 150 µL of 32.4 mM ammonium molybdate ($(NH_4)6Mo7O_24.4H_2O$) was added. For each incubated sample, a blank solution was done using 5 µL of meat sample, 100 µL of phosphate buffer solution, and 150 µL of ammonium molybdate. The consumption of hydrogen peroxide was determined using a spectrophotometer at 374 nm (Thermo Scientific® model Multiskan GO). The values are expressed in U/mL CAT/min per g of soluble protein.

Tissue lipid peroxidation was evaluated by measuring MDA concentrations using the test for thiobarbituric acid reactive substances (TBARS) according to the methodology described by Buege & Aust, (1978). The results are expressed in nmol equivalents of MDA per g of soluble protein.

The nitric oxide (NO) content was quantified indirectly by the standard Griess reaction (Tsikas, 2007). The determination of antioxidant activity was measured by the FRAP (Ferric

Reducing Antioxidant Power) methodology (Benzie & Strain, 1996). NO and FRAP results were expressed in µmol equivalents per g of soluble protein.

2.2.7. Statistical analysis

For statistical analyses, the Tukey mean comparison test was used. A 5% tolerance for type I error was adopted. The statistical model used was:

$Yij= \mu + Di + eij$

In which: µ = general constant; Di = effect of treatment i (fixed); eij = residual random effect. Data were submitted to analysis of variance, considering significant differences when P-value was less than 0.05 using SAS 9.4 (Statistical Analysis System).

2.3. Results and Discussion

The variables pH_{24h} and sarcomere length are presented in Table 2. The vitamin supplementation used was not able to affect these variables, which did not differ between treatment groups ($P > 0.05$, Table 2).

A higher percentage of intramuscular fat in the *longissimus lumborum* muscle ($P<0.05$, Table 2) was obtained by the ADE and ADE+B treatments, which differed statistically from the control and B treatment, which can be explained by the vitamin E supplementation, some research has evaluated the effect of vitamin E or α-tocopherol in the diet on the expression of transcription factors and found that vitamin E may be acting as a regulatory factor for the transcriptional control of genes related to lipid metabolism (SREBF1 and receptor gamma activated by peroxisome proliferator (PPARG)) in the *longissimus* muscle of lambs (González-Calvo et al., 2015). Therefore, vitamin E supplementation could influence the expression of genes involved in lipid metabolism due to the regulation of expression and activity of transcription factors (Ladeira et al., 2018). Furthermore, according to Calnan et al. (2019), a higher concentration of intramuscular fat may have a negative impact on lipid oxidation and meat color stability, however, in the present study, increased intramuscular fat was not able to influence these characteristics.

No effects were observed on meat and fat color (L*, a*, b*) ($P>0.05$, Table 3) of the *longissimus lumborum* muscle at 24h, 196h and 360h *postmortem*. Likewise, the parameters of the redox form of myoglobin (OMb, DMb, MMb) showed no difference between the treatments

used in the *longissimus lumborum* muscle 24h *postmortem*.

However, the ADE and ADE+B treatments improved the color stability of the *longissimus lumborum* muscle 196h and 360h *postmortem* on the parameters of the redox form of myoglobin (OMb, DMb, MMb). The DMb variable showed higher values ($P<0.05$) for the treatment that did not receive any type of vitamin supplementation (control) 196h *postmortem*. In addition, as expected, a significant increase in MMb% and a decrease in OMb% ($P<0.05$, Table 3) was observed with the increase in storage time 196h and 360h *postmortem*, for the Control and B treatments.

After 196h *postmortem*, the DMb variable showed higher values ($P<0.05$) for the treatment). The MMb variable showed higher values ($P<0.05$) for animals that were supplemented with ADE and ADE+B 196h and 360h *postmortem*. Some authors relate color deterioration due to increased metmyoglobin levels to protein and lipid oxidation (Kim et al., 2010; Lagerstedt et al., 2011; Zakrys et al., 2008).

In this study, the variations in the parameters of the redox form of myoglobin (OMb and MMb) in the *longissimus lumborum* muscle 360h *postmortem*, both influenced by the redox state of the heme pigment, may be related to oxidation, since it accompanied the increase in the content of MDA ($P<0.05$, Table 4). This could be attributed to the gradual oxidation of myoglobin and the simultaneous accumulation of metmyoglobin over time (Mancini & Hunt, 2005). Wang et al. (2021) reported similar observations, who demonstrated that lipid oxidation inversely impacted redness.

These OMb results demonstrate that supplementation with vitamins ADE probably modified the number of antioxidants present in the meat, increasing the affinity of myoglobin for oxygen, improving oxymyoglobin stability (Hansen et al., 2012) which gives the meat a cherry-red color, increasing the attractiveness of the product. Since supplementation only with complex B was not able to express the same results. This can be explained because vitamins A and E are considered potential antioxidants, in addition to exerting a cooperative antioxidant activity in different positions within the membrane (Descalzo et al., 2005), vitamin A breaks the chain located in the hydrophobic region of biological membranes and vitamin E breaks the chain and protects membranes from oxidation (Gülçin, 2012).

In addition, the use of vitamin D may also have helped in this result. Lahucky et al., (2007) used vitamin D and related color improvement with increased antioxidant capacity and suggested that a higher level of Ca^{2+} (bivalent ion) in the muscles caused a positive influence

on lipid oxidation. Additionally, vitamin D supplementation has been reported to improve meat color stability (Hansen et al., 2012; Lahucky et al., 2007; Strydom et al., 2011).

The results of the present study did not show treatment effects (P>0.05, Table 4) on the variables of exudate loss and shear force in the *longissimus lumborum* muscle at 24h, 192h and 360h *postmortem*.

However, the Myofibrillar Fragmentation Index (MFI) (P < 0.05) was higher for animals that received ADE and ADE+B in the meat at 24h, 192h and 360h. This may have occurred due to a possible beneficial effect of vitamin D in modulating intracellular Ca (increasing proteolysis by calpains) may have been offset by the reduction in oxidative stress by vitamin E (decreasing degradation of calpastatin by caspases), oxidative conditions in *postmortem* muscle can inactivate or modify the activity of the calpain system (Harris et al., 2001), it has been suggested that increasing the level of antioxidants in meat can improve tenderness (Lonergan et al., 2010).

Therefore, the immobilization of calcium in animals supplemented with vitamin D may have favored greater myofibrillar fragmentation (Lobo-Jr. et al., 2012). Such results corroborate those reported, in which vitamin D-enriched diets beneficially increase *postmortem* proteolysis and improve myofibrillar fragmentation, thus improving beef tenderness (Duffy et al., 2017; Karges et al., 2001; Lobo- Jr. et al., 2012; Montgomery et al., 2000, 2002, 2004; Rezende et al., 2021) by increasing the concentration of free calcium in the muscle as well as the antioxidant capacity (Lobo-Jr. et al., 2012).

For 24h *postmortem* meat, there was a difference in the SOD variable, which obtained higher means for the treatment supplemented with ADE and was different from the control, showing greater antioxidant activity for animals that were fed with ADE vitamins. It is reported that deficiency of antioxidant nutrients such as vitamin E can result in a low level of SOD (He et al., 2018). SOD functions as one of the main enzymatic antioxidant defenses against highly reactive superoxide radicals, and its antioxidant activity consists of removing the O2- ion and producing oxygen and H_2O_2 (Domínguez et al., 2019).

The results showed that GPx activity 24h *postmortem* was higher (P>0.05, Table 5) in the control and B-complex treatment, similar to that observed by Hamid et al., (2011), in which vitamin E reduced GPx activity in mice, likely due to its synergistic anti-oxidative defense. In addition, vitamin E has a glutathione (GSH) sparing effect, so GPx uses GSH and when there is GSH sparing, GPx action is not affected (Hamid et al., 2011), the reason for this is that

vitamin E may have taken over oxidative defense and thus reduced GPx induction (Singh et al., 1994)

GPx belongs to the class of peroxidase enzymes found in mammalian erythrocytes that prevent the organism from oxidative damage, its biochemical function is to reduce lipid hydroperoxides generated during lipid peroxidation to their corresponding alcohols and to reduce free H_2O_2 to H_2O (Joy & Nair, 2008).

After 196h *postmortem*, the CAT enzyme showed higher means (P>0.05, Table 5) for ADE and ADE+B and was different from control and B, thus attributing the ability to reduce hydrogen peroxide (Góth et al., 2004) for animals that were fed vitamins ADE in meat 196h *postmortem*. CAT is the main regulator of hydrogen peroxide metabolism, and H_2O_2 in high concentrations is a toxic agent (Góth et al., 2004), so the H_2O_2 formed during superoxide dismutase activity is eliminated by catalase, which transforms into H_2O and O_2 (Domínguez et al., 2019), preventing the formation of the highly reactive hydroxyl radical (OH.-) (Chance et al., 1979).

The lipid oxidation measured in the form of MDA (1,3-propanedial) in the *longissimus lumborum* muscle (P<0.05) was significantly altered 360h *postmortem* according to the data obtained, the treatments ADE and ADE+B were shown to be more efficient in slowing down lipid oxidation. The values of MDA, which were above 1.5 and close to 2 denoting the rancidity of the meat (Spaziani et al., 2011) for the control and B treatments. Animals supplemented with ADE had reduced MDA, therefore, peroxidation lipid content decreased while the shelf life of meat from animals fed diets with vitamins E increased (Karami et al., 2011; Kasapidou et al., 2012; Ripoll et al., 2011). Although dietary supplementation has no significant impact on meat MDA levels 24h *postmortem,* the protective action of added vitamins may become evident during 360h *postmortem* storage.

The results showed that FRAP 24h *postmortem* obtained higher means (P<0.05) for the treatment supplemented with ADE and was different from the control, showing greater antioxidant activity for the animals that were fed with ADE vitamins. FRAP evaluates the total antioxidant potential, simultaneously evaluating the pro and antioxidant events in the cell, the ability of the meat to eliminate free radicals (Vu et al., 2018), it directly measures the reducing capacity of the substance, which is an important parameter for a compound is a good antioxidant (Benzie & Strain, 1996).

The increase in color stability due to supplementation with ADE and ADE+B may be due to a higher antioxidant activity (FRAP) that also occurred in meat 196h *postmortem*, as a

higher antioxidant activity leads to an increased ability to scavenge radicals free, protecting the phospholipid content against lipid oxidation, resulting in greater color stability (Kumar et al., 2015). Supplementation with ADE and ADE+B was able to influence this variable in meat at 24h, 196h and 360h *postmortem*.

The NO content showed a difference ($P<0.05$) 24h *postmortem*, obtaining higher values in the treatment with complex B in relation to ADE and ADE+B, which may indicate that the use of ADE could mitigate intracellular oxidation (Gülçin, 2012).

2.4. Conclusion

This study confirmed the antioxidant effects of vitamins ADE when added to the diet of Nellore cattle. In fact, dietary supplementation with ADE and ADE+B improved meat stability, provided greater antioxidant activity compared to the control and B-complex group, 196h and 360h *postmortem*. However, although ADE and ADE+B presented similar results, the use of B complex vitamins alone did not follow these results, therefore, the observed results must only be due to the action of ADE and perhaps VIT B may not have an effect because the animal produces these vitamins, supplementation is not necessary when seeking to improve meat quality.

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ATTACHMENT:

Table 1. Proportion of ingredients and nutrient composition of the experimental diets[1]

Item	Experimental Diets			
	Control	B	ADE	ADE+B
Ingredient, g/kg				
Corn Silage	300.4	300.4	300.4	300.4
Ground Corn	634.0	634.0	634.0	634.0
Soybean meal	38.5	38.5	38.5	38.5
Urea	9.9	9.9	9.9	9.9
Mineral premix[2]	16.0	-	-	-
B-blend mineral premix[2]	-	16.0	-	-
ADE mineral premix[2]	-	-	16.0	-
B+ADE mineral premix[2]	-	-	-	16.0
Chemical composition, g/kg				
Dry matter	560.2	560.2	560.2	560.2
Mineral matter	43.4	43.4	43.4	43.4
Organic matter	956.6	956.6	956.6	956.6
Crude protein	118.2	118.2	118.2	118.2
Ether extract	29.1	29.1	29.1	29.1
apNDF[3]	223.4	223.4	223.4	223.4
Non-fiber carbohydrates	594.6	594.6	594.6	594.6

[1]Control= No fat-soluble vitamin ADE and B-complex vitamin supplementation; B = diet with B-complex vitamin (biotin, niacin, and thiamine) supplementation; ADE = diet with fat-soluble vitamin (ADE) supplementation; ADE+B= diet with fat-soluble vitamin (ADE) and B-complex vitamin (biotin, niacin, and thiamine) supplementation.
[2]Ingredients per kg mineral: 172.5 g Ca; 20.8 g P; 31.25 g S; 20.8 g Mg; 31.25 g K; 68.75 g Na; 10.4 mg Co; 679 mg Cu; 8.35 mg Cr; 34.5 mg I; 1333 mg Mn; 8.35 mg Se; 2500 mg Zn, and 208 mg F. [3]Corrected for residual ash and residual nitrogenous

Table 2. Characteristics of pH, intramuscular fat and sarcomere length in meat from animals supplemented with vitamins.

Item	Experimental Diets[1]				SE	*P*-value
	Control	B	ADE	ADE+B		
pH_{24h}	5.79	5.53	5.52	5.76	0.109	0.1800
Intramuscular Fat	1.64b	1.66b	2.10a	2.26a	0.115	0.0005
Sarcomere length, µm	1.38	1.45	1.38	1.35	0.048	0.4694

[1]Control= No fat-soluble vitamin ADE and B-complex vitamin supplementation; B = diet with B-complex vitamin (biotin, niacin, and thiamine) supplementation; ADE = diet with fat-soluble vitamin (ADE) supplementation; ADE+B= diet with fat-soluble vitamin (ADE) and B-complex vitamin (biotin, niacin, and thiamine) supplementation.

Table 3. Color parameters in meat from animals supplemented with vitamins

Item	Experimental Diets[1]				SE	*P*-value
	Control	B	ADE	ADE+B		
24h postmortem						
Color Meat						
L*	38.39	39.16	38.31	38.00	1.324	0.9671
a*	16.29	15.7	16.51	16.25	0.421	0.5750
b*	14.4	14.41	14.52	14.58	0.658	0.9970
MMb	19.71	23.81	19.43	19.45	2.218	0.3744
DMb	9.51	9.84	8.24	8.68	1.355	0.8743
OMb	70.23	65.78	71.47	72.16	2.280	0.1598
Color Fat						
L*	74.1	70.95	71.6	71.61	0.872	0.0772
a*	5.27	6.33	5.96	4.90	0.463	0.1540
b*	18.55	18.15	19.28	17.88	0.614	0.4113
196h postmortem						
Color Meat						
L*	39.63	40.29	39.96	40.13	1.329	0.6404
a*	13.61	14.43	15.66	14.71	0.547	0.1950
b*	13.03	13.74	13.87	13.76	0.598	0.7431
MMb	29.98a	30.41a	20.83b	20.94b	3.371	0.0320
DMb	22.06a	14.37b	12.08b	13.47b	2.665	0.0465
OMb	51.40b	52.95b	66.77a	65.69a	3.737	0.0044
Color Fat						
L*	74.78	74.31	76.36	75.36	1.261	0.6810
a*	4.32	4.94	3.79	4.34	0.420	0.3071
b*	15.26	15.76	15.03	15.21	0.412	0.7877
360h postmortem						
Color Meat						
L*	41.94	41.74	40.60	40.81	1.264	0.8389
a*	13.74	13.12	13.34	14.09	0.440	0.4329
b*	13.4	13.39	12.92	13.33	0.513	0.8886
MMb	36.13a	30.40a	23.01b	26.97b	3.469	0.0081
DMb	14.73	17.47	13.95	12.32	1.758	0.4768
OMb	50.95b	49.58b	61.48a	61.36a	3.283	0.0011
Color Fat						
L*	74.86	75.95	76.56	76.21	0.899	0.5507
a*	3.73	4.18	3.84	3.67	0.285	0.5832
b*	15.01	15.23	15.04	14.73	0.311	0.7405

[1]Control= No fat-soluble vitamin ADE and B-complex vitamin supplementation; B = diet with B-complex vitamin (biotin, niacin, and thiamine) supplementation; ADE = diet with fat-soluble vitamin (ADE) supplementation; ADE+B= diet with fat-soluble vitamin (ADE) and B-complex vitamin (biotin, niacin, and thiamine) supplementation; L* - luminosity; a* - red intensity b* - yellow intensity; MMb – metmyoglobin; DMb – deoxymyoglobin; OMb - oxymyoglobin

Table 4. Characteristics of exudates losses, shear force, and myofibrillar fragmentation index (MFI) in meat from animals supplemented with vitamins

Item	Experimental Diet				SE	*P*-value
	Control	B	ADE	ADE+B		
24h postmortem						
Exudates losses, g kg -1	7.73	8.02	7.30	8.33	0.935	0.8839
Cooking losses, g kg-1	18.89	19.47	19.11	20.68	0.846	0.4449
Total losses, g kg-1	25.05	26.28	24.99	27.28	1.354	0.5750
Shear force, N	61.96	62.13	55.92	60.03	3.553	0.5139
MFI	38.52b	34.66b	61.46a	58.51a	4.867	0.0004
196h postmortem						
Exudates losses, g kg -1	7.76	7.34	7.35	7.11	0.496	0.9382
Cooking losses, g kg-1	19.69	21.81	21.55	20.71	1.350	0.6845
Total losses, g kg-1	25.93	27.82	27.27	27.71	1.414	0.8217
Shear force, N	53.27	53.56	49.69	49.52	3.357	0.7061
MFI	47.39b	48.90b	74.31a	69.09a	4.578	0.0001
360h postmortem						
Exudates losses, g kg -1	7.53	7.16	6.88	6.97	0.382	0.6362
Cooking losses, g kg-1	20.61	20.62	19.27	21.31	1.393	0.7847
Total losses, g kg-1	26.54	26.28	24.8	26.78	1.495	0.7979
Shear force, N	44.69	44.35	40.72	42.39	2.678	0.7198
MFI	55.44b	59.76b	75.39a	72.99a	4.535	0.0075

[1]Control= No fat-soluble vitamin ADE and B-complex vitamin supplementation; B = diet with B-complex vitamin (biotin, niacin, and thiamine) supplementation; ADE = diet with fat-soluble vitamin (ADE) supplementation; ADE+B= diet with fat-soluble vitamin (ADE) and B-complex vitamin (biotin, niacin, and thiamine) supplementation.

Table 5. Meat enzymatic activity from animals supplemented with vitamins

Item	Experimental Diets[1]				SE	*P*-value
	Control	B	ADE	ADE+B		
Muscle						
SOD, UI/mg	16.43	13.20	20.51	18.70	3.145	0.3890
GPx, UI/g	69.61	35.88	104.15	52.95	1.621	0.2064
CAT, UI/g	34.44	26.91	34.38	34.83	5.024	0.3827
MDA, µmol/g	0.75	0.85	0.54	0.61	0.104	0.2598
FRAP, µmol/g	0.65	0.59	1.03	1.00	0.149	0.0917
NO, µM/g	0.45	0.45	0.39	0.28	0.113	0.7147
24h postmortem						
SOD, UI/mg	6.45b	7.69ab	10.12a	7.77ab	0.857	0.0478
GPx, UI/kg	6.82a	6.66a	5.31b	5.20b	0.355	0.0023
CAT, UI/g	12.03	12.25	13.96	13.42	2.097	0.8999
MDA, µmol/g	0.97	0.65	0.57	0.61	0.061	0.1007
FRAP, µmol/g	0.34b	0.48b	0.65a	0.62a	0.073	0.0185
NO, µM/g	0.19ab	0.24a	0.15b	0.16b	0.205	0.0092
196h postmortem						
SOD, UI/mg	11.55	11.74	17.06	9.59	2.746	0.3772
GPx, UI/kg	19.83	19.99	19.98	17.33	3.275	0.9253
CAT, UI/g	20.52b	23.87b	40.58a	43.51a	5.485	0.0113
MDA, µmol/g	1.33	1.08	0.80	0.95	0.193	0.7297
FRAP, µmol/g	0.58b	0.74b	1.06a	1.01a	0.099	0.0104
NO, µM/g	0.47	0.42	0.28	0.35	0.073	0.2793
360h postmortem						
SOD, UI/mg	5.24	5.65	6.43	7.94	1.329	0.5338
GPx, UI/kg	14.72	13.25	19.7	19.76	1.392	0.6444
CAT, UI/g	36.39	36.39	46.41	49.8	9.206	0.6434
MDA, µmol/g	1.79a	2.04a	1.14b	1.10b	0.184	0.0017
FRAP, µmol/g	0.70b	0.75b	1.20a	1.01ab	0.113	0.0127
NO, µM/g	0.45	0.43	0.34	0.33	0.044	0.1686

*SOD= Superoxide dismutase; GPx= glutathione peroxidase; CAT= catalase; MDA= malondialdehyde; NO= Nitric Oxid; FRAP= Ferric Reducing Antioxidant Power Assay.

[1]Control= No fat-soluble vitamin ADE and B-complex vitamin supplementation; B = diet with B-complex vitamin (biotin, niacin, and thiamine) supplementation; ADE = diet with fat-soluble vitamin (ADE) supplementation; ADE+B= diet with fat-soluble vitamin (ADE) and B-complex vitamin (biotin, niacin, and thiamine) supplementation.

3. CHAPTER II

Effects of injectable vitamin-mineral supplementation on oxidative stability and meat quality parameters of ¾ Angus ¼ Nellore young males[1]

Jenifer Maira Lima Ramos[2]*, Mario Luiz Chizzotti[2]

[1] Manuscript prepared following the Meat Science guidelines.

[2] Departamento de Zootecnia da Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-000

Corresponding author: Ramos, Jenifer – E-mail: jenifer.ramos@ufv.br

Abstract

Vitamins A and E and microminerals such as Se, Mn, Zn and Cu promote an increase in the antioxidant capacity of muscle tissue and mitigating oxidative stress. The objective of this study was to evaluate the effects of injectable supplementation of complex vitamins A and E and micro-minerals Se, Mn, Zn and Cu, applied at weaning, castration and pre-slaughter or their combination, on meat quality, antioxidant status and lipid oxidation of castrated ¾ Angus ¼ Nellore cattle in the finishing phase. A total of 120 castrated ¾ Angus ¼ Nellore steers with an average age of 7 ± 1 month and an average body weight of 304.58 ± 33 kg were used. The experiment lasted 250 days and was conducted in a completely randomized design (DIC), with six treatments (N=20): (i) WCP - Application of vitamin-mineral complex (VMC) at weaning (W), castration (C) and pre-slaughter (P); (ii) WC0 - VMC at W and C + Application of saline solution (SS) at P; (iii) W0P - VMC at W + SS at C + VMC at P; (iv) 000- Control (SS at W, C and P); (v) 00P - SS at W and C + VMC at P; (vi) 0CP– SS at W + VMC at C and P. Quality analyzes were carried out (color, losses, shear force), activities of antioxidant enzymes (Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT)) and Malonaldehyde content (MDA) in the muscle *longissimus thoracis* and in the muscle *longissimus lumborum* cuts in 1h, 24h, 192h and 360h postmortem. In the *longissimus thoracis* muscle, the variables red intensity (a*) and Hue showed a difference 192h postmortem and Methamyoglobin (MMb) at 192 and 360h postmortem. In the *longissimus lumborum* muscle, the Hue, MMb and oxymyoglobin (OMb) variables were different 24h postmortem. The variables red intensity (a*), chroma and OMb showed a difference 192h postmortem and the variables red intensity (a*), yellow intensity (b*) and chroma showed a difference 360h *postmortem*. The shear force variable showed a difference 24h and 192h postmortem. antioxidant activity (CAT and FRAP) and MDA showed a difference 1h postmortem. SOD and FRAP 24h postmortem, FRAP and MDA 192h postmortem and CAT and FRAP 360h postmortem. In the *longissimus lumborum* muscle, FRAP showed a difference 24h and 360h postmortem, GPx and SOD 360h postmortem. Supplementation with the injectable vitamin-mineral complex reduces the oxidation of oxymyoglobin to metmyoglobin and provides greater antioxidant status and decreased lipid oxidation.

Keywords: Antioxidant, Colour, Minerals, Myoglobin, Oxidation, Shelf life.

3.1. Introduction

Increase the shelf life is one of the biggest challenges of the meat industry. It depends on oxidative processes caused by storage temperature, exposure to oxygen and light (Possamai et al., 2018). The possibility of extending meat shelf life by delaying oxidative deterioration is important to the meat industry market level (Luciano et al., 2009).

Meat oxidative stability is a result of the balance between antioxidant and pro-oxidant compounds (Domínguez et al., 2019). Thus, to extend meat shelf life, the use of antioxidants in animal diets can ensure a redox balance in cells and tissues (Surai et al., 2019).

Studies evaluating vitamin E in animal diets have demonstrated increased shelf life (Bellés et al., 2018; Calnan et al., 2019; Kasapidou et al., 2012; Possamai et al., 2018), due to reduced oxidation and increased of color stability. Another antioxidant that can be used is vitamin A (β-carotene), which cooperates with tocopherols in the ability to eliminate free radicals within the inner part of lipid membranes (Descalzo et al., 2005) .

In addition, some microminerals (Mn, Cu, Zn and Se) function as cofactors of antioxidant enzymes and offer another dietary strategy to improve meat quality and oxidation (Jiang & Xiong, 2016). Furthermore, the action between selenium and vitamin E can increase GPx production (Idamokoro et al., 2020), and Mn supplementation can increase SOD production (Arthington & Ranches, 2021).

Traditionally, the microminerals supplementation in occurs through oral dietary supply, which leads to high variability in the consumption of microminerals among animals (Manzano et al., 2012). Dietary supplementation may decrease absorption and contribute to the imbalance of micromineral supplementation status in ruminants (Genther & Hansen, 2014). Thus, the use of minerals via injection efficiently increases the micromineral status of animals compared (Genther & Hansen, 2014; Hartman et al., 2018), and it does not suffer interference from dietary antagonists (Arthington et al., 2014; Hartman et al., 2018). In addition, injectable supplementation eliminates individual variability in supplementation by applying a known amount to each animal, increasing oxidative stress control (Soldá et al., 2017; Teixeira et al., 2014; Vedovatto et al., 2019).

Muscle antioxidant capacity decreases with advancing *postmortem* time (Ke et al., 2017). Therefore, considerable antioxidant status must be achieved prior to slaughter in order to maximize protection against oxidation (Descalzo et al., 2005).

Pogge et al. (2012) reported that plasma levels Cu, Mn, Se and Zn were elevated 8 to 10 hours after the injection procedure. Therefore, the pre-slaughter mineral injection may be a viable and practical alternative for improving the antioxidant status of beef cattle.

In this sense, the present study hypothesizes that injectable supplementation of vitamin A and E complex and Copper, Zinc, Manganese, and Selenium microminerals complex improves the quality, antioxidant status and decreases the lipid oxidation of the meat of crossbred cattle in the finishing phase. The objective of this study was to evaluate the effects of injectable supplementation of vitamin A and E complex and Copper, Zinc, Manganese and Selenium microminerals complex, applied at weaning, castration and pre-slaughter or their combination, on the quality, oxidative stability and lipid oxidation of bovine beef in the finishing phase.

3.2.Material and methods

3.2.1. Facilities, animals, diets and experimental design

The experiment was conducted in a partner commercial feedlot. A total of 120 weaned crossbred castrated male bovines (¾ Angus ¼ Nellore) with an average age of 7 ± 1 month and average body weight of 304.58 ± 33 kg were used.

The experiment was carried out in a completely randomized design, in which the animals were randomly distributed into six treatments (N=20 in each group). The treatments consisted of different application moments of injectable vitamin and mineral supplementation: (i) WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; (ii) WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; (iii) W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; (iv) 000- Control (application of saline solution at weaning, castration and pre-slaughter); (v) 00P - Application of Saline Solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; (vi) 0CP– Application of saline solution at weaning + Application of vitamin-mineral complex at castration and pre-slaughter.

The experiment lasted a total of 250 days, the diets were the same for all animals. The vitamin-mineral complex was supplemented by injecting the Adapter Kit®MIN and Adapter®VIT Kit (Biogenesis Bagó), at a dosage of 1mL/75kg and 1mL/50kg, respectively.

Adapter® Min: 0.10 mg/kg copper; 0.4 mg/kg zinc; 0.10 mg/kg of manganese; and 0.05 mg/kg of selenium and Adapter Kit® Vit: 17.5 mg/kg of vitamin A and 0.5 mg/kg of vitamin E.

3.2.2. Harvesting, pH and temperature measurements

At the end of the experimental period, the animals were kept deprived of solids for 16 hours, stunned by a pneumatic gun, and bled following the Normative Instruction N° 9.013/MAPA 2017. After slaughter, the pH and temperature were measured in the *longissimus thoracis* muscle with a portable meat pH meter coupled with a thermometer (Model HI 99163 - Hanna Instruments). Each carcass was divided into two half carcasses, which were weighed, then chilled at 4°C for 24 hours. After the chilling period, the pH and temperature were measured again in the same spot. The pH meter probe calibration was performed in buffers at pH 4 and 7 at 4 °C before use.

3.2.3. Collection and analysis of muscle and meat

Following the chilling period, samples of the *longissimus lumborum and Thoracis* muscle were collected in each right half carcass, then sectioned into three portions, properly identified, and vacuum-packed. One portion of each sample was frozen immediately and the two remaining portions were aged at 4°C for seven and fourteen days before freeze storage.

3.2.4. Meat quality analysis

Three steaks of one inch (2.54 cm) thick were obtained from the frozen portions of the *longissimus lumborum and thoracis*. One steak was reserved for objective color analysis, another for estimating thawing and cooking losses, and then to evaluate shear force, and the third steak was used for the myofibrillar fragmentation index analysis. The analyses were carried out at the Meat Science Laboratory of the Animal Science Department at the Universidade Federal de Viçosa.

Steak samples were thawed for 16 hours at 4ºC for color evaluation. After thawing, steaks were removed from the package and exposed to air for reoxygenation for 30 minutes at 4ºC. CIELab readings L*, a*, and b* were taken using a HunterLab MiniScan EZ 45/0 LAV spectrophotometer, adjusted to the illuminant source D65 and for an observer 10º angle. Five

readings were performed for each sample at different points, and then the average value was used.

We use the equations described in (AMSA, 2012) to determine the hue angle and chroma of the sample:

$$\text{HUE} = \text{arctangent}\left(\frac{b}{a}\right)$$

$$\text{Chroma} = \sqrt{(a^2) + (b^2)}$$

The wavelength reflected from the instrument were recorded in the range of 400–700 nm at 10 nm intervals, and the reflectance ratio at 630 nm and 580 nm (R630/580) was directly used to assess color stability during display. The reflectance (R) at 473, 525, 572, and 700 nm was converted to reflex attenuation (A) using the equation: A = log (1/R), and the relative percentage of three redox forms of myoglobin was calculated following the AMSA (2012) equations:

$$\%\text{MMb} = \left(1.395 - \frac{\text{A572-A700}}{\text{A525-A700}}\right)$$

$$\%\text{DMb} = \left[2.35 \text{ x} \left(1 - \frac{\text{A473-A700}}{\text{A525-A700}}\right)\right] \text{x}100$$

$$\%\text{OMb} = 100 - (\%\text{MMb} + \%\text{DMb})$$

The exudation losses were performed according to the methodology described by Bruce et al. (2004), with modifications described by Silva et al. (2019). Steaks were weighed before and after thawing, which lasted 16 hours at a temperature of 5°C until they reached an internal temperature of 2 to 5°C. Losses were expressed as a percentage of the weight of the steak before and after the process: Loss (%) = [(weight before – weight after)/weight before] x 100.

The shear force was measured in the same steak used to estimate exudation losses. After cooking, the steaks were refrigerated for 16 hours at 4ºC. Then five cylindrical samples, 1.27 cm in diameter, were removed from each steak, parallel to the orientation of the muscle fibers, using a stainless-steel sharpened sampler. The cylindrical samples were sheared perpendicularly to the orientation of the muscle fibers, using a V-cut blade, with an angle of 60º and a thickness of 1.016 mm, and a fixed speed of 20 cm/min, coupled to the texture analyzer (G-R Electrical Manufacturing Company, Manhattan, KS, USA). The maximum forces used to cut the cylindrical samples were taken in the five cylindrical samples. The final shear force value (N) resulted from the average of the five cylindrical samples of each steak.

3.2.5. Evaluation of antioxidant activity and lipid peroxidation

A tissue homogenate (1mg/1mL) was prepared and used for the assay of the antioxidant activities of the superoxide dismutase (SOD), glutathione peroxidase (GPx), and Catalase (CAT) enzymes. The same homogenate was used to determine the concentrations of malondialdehyde (MDA) and antioxidant activity (FRAP - Ferric Reducing Antioxidant Power).

The preparation of the homogenate was carried out according to the methodology described by Walsh et al. (1993). The antioxidant activity of SOD and GPx enzymes was determined using test kits (Ransod and Ransel, respectively) supplied by Randox Laboratories. The SOD concentration was determined following the methodology described by Walsh et al. (1993). Protein concentrations were estimated by the method described by Bradford (1976) and enzyme activities were expressed in IU per g of soluble protein.

The catalase activity (CAT) was determined according to the methodology described by (Hadwan & Abed, 2016), with minor modifications. Hydrogen peroxide (H_2O_2) was used as a substrate. A 5 µL volume of sample was incubated with 100 µL of the reaction mixture containing H_2O_2 and phosphate buffer at pH 7.4. After 3 min of incubation, 150 µL of 32.4 mM ammonium molybdate ($(NH_4)6Mo7O_24.4H_2O$) was added. For each incubated sample, a blank solution was done using 5 µL of meat sample, 100 µL of phosphate buffer solution, and 150 µL of ammonium molybdate. The consumption of hydrogen peroxide was determined using a spectrophotometer at 374 nm (Thermo Scientific® model Multiskan GO). The values are expressed in U/mL CAT/min per g of soluble protein.

Tissue lipid peroxidation was evaluated by measuring MDA concentrations using the test for thiobarbituric acid reactive substances (TBARS) according to the methodology described by Buege & Aust (1978). The results are expressed in nmol equivalents of MDA per g of soluble protein.

The determination of antioxidant activity was measured by the FRAP (Ferric Reducing Antioxidant Power) methodology (Benzie & Strain, 1996). FRAP results were expressed in µmol equivalents per g of soluble protein.

3.2.6. Statistical analysis

Data were submitted to analysis of variance, considering significant differences when P-value was less than 0.05 using SAS 9.4 (Statistical Analysis System). The statistical model used was:

$Yij = \mu + Di + eij$

In which: μ = general constant; Di = effect of treatment i (fixed); eij = residual random effect. Tukey test was used to compare means when significant differences were detected. A 5% tolerance for type I error was adopted.

3.3. Results and discussion

Among the various parameters measured for beef color, the intensity of red (a*) is considered the most important because its decrease indicates oxidation of oxymyoglobin to metmyoglobin (Seyfert et al., 2006).

The color variables showed no difference in the *longissimus thoracis* muscle ($P>0.05$; Table 2) for 24h *postmortem* meat.

Within normal meat color ranges, high intensity of red (a*) and chroma values combined with relatively low intensity of yellow (b*) values are considered desirable because they represent a visually perceived vivid reddish color that consumers associate with fresh quality beef (Skaperda et al., 2021).

The *longissimus thoracis muscle* at 192h *postmortem* showed statistical difference ($P<0.05$, Table 1) for the intensity of red (a*), Hue angle and MMb variables. In which, intensity of red (a*) presented higher average values, while Hue angle and MMb presented lower averages for WCP and 0CP treatments. Demonstrating that when vitamin-mineral supplementation was performed combined at castration and pre-slaughter times (WCP and 0CP), OMb oxidation was significantly prevented, resulting in a significantly lower level of MMb at 8 days of aged in the *longissimus thoracis* muscle.

Moreover, an additional increase in hue matrix angle is related to a brownish, darker color that is not desirable by consumers (Skaperda et al., 2021). A similar pattern was observed in treatment control in the *longissimus thoracis muscle* at 192h *postmortem* and in the *longissimus lumborum muscle* at 24h postmortem ($P<0.05$; Tables 1 and 2).

Meat generally exhibits a cherry-red color as a result of the OMb formed by the Mb in the meat and oxygen derived from the air (Hoa et al., 2021). However, this desirable color gradually changes to an undesirable and unattractive color (so-called discoloration) due to MMb

formation (McMillin, 2008).

The *longissimus thoracis* muscle, with 192h and 360h *postmortem* there was a statistical difference (P<0.05) for the MMb variable, in which treatment control showed 192h and 360h *postmortem* higher mean values, indicating oxidation of oxymyoglobin to metmyoglobin and attributing a brownish tone to the meat (Seyfert et al., 2006).

Furthermore, variations in the color parameter that are influenced by the redox state of the heme pigment, may be related to *longissimus thoracis* muscle oxidation, as also reported by (Mancini & Hunt, 2005), since it accompanied the increase in MDA content (P<0.05, Table 6).

The Hue, OMb and MMb variables of the color of *longissimus lumborum* muscle at 24h *postmortem* showed significant differences (P<0.05; Table 2). In which, the treatment 000 presented higher mean values for the Hue and MMb variables, lower mean values of OMb. In the period of 192h *postmortem*, the variables intensity of red (a*), Chroma and OMb showed significant differences (P<0.05; Table 2) between the treatments performed.

Variables intensity of red (a*), Chroma and OMb showed difference (P<0.05, Table 2), between the treatments performed. The intensity of red (a*) and Chroma obtained the highest average values for the 0CP treatment.

On the other hand, the OMb obtained higher averages for the 0CP, WCP, 00P treatments and statistically differed from the WC0 and 000 treatments. Thus, demonstrating that injectable vitamin-mineral supplementation, performed at least in the pre-slaughter period, provides a decrease in the oxidation of oxymyoglobin to metmyoglobin (Seyfert et al., 2006).

The *longissimus lumborum* muscle at 360h *postmortem*, there was a statistical difference (P<0.05) for the intensity of red (a*), intensity of yellow (b*) and Chroma variables, in which the averages were higher for the 0CP and 00P treatments. The WCP treatment was different possibly because lipid oxidation was minimized by the higher antioxidant capacity (P<0.05, Table 7) without any effect on color preservation, as reported by Faustman et al. (2010).

The variables described in Table 3 for the *longissimus thoracis muscle* did not show statistical difference (P>0.05) at 24, 192 or 360h *postmortem*. However, the variable shear force (P<0.05) showed a difference at 24 and 192h *postmortem*, with treatment control having lower means, demonstrating that it was a more tender meat.

This possibly occurred because in the *postmortem* period, structural proteins undergo oxidation by ROS and remained more vulnerable to myofibrillar proteolysis, contributing to meat tenderness in cattle (Malheiros et al., 2019).

Furthermore, it is possible that caspases act in synergy with calpains to degrade

structural proteins oxidized by ROS, in addition to hindering the production of ATP, and consequently weakening muscle antioxidant defenses, paving the way for a massive production of ROS (Lana & Zolla, 2016), demonstrating a positive association between oxidation and meat tenderness (Malheiros et al., 2019).

Therefore, the results in the present study suggest that an increase in the oxidative damage of proteins in response to oxidative stress may have provided an increase in myofibrillar proteolysis, which allowed the development of the meat-tender phenotype (Lana & Zolla, 2016) for the animals that did not have injectable vitamin mineral supplementation at weaning, castration or pre-slaughter.

The variables related to losses and shear force of the *longissimus lumborum* muscle at different *postmortem* times (P>0.05; Table 4) did not differ between the treatments performed. Demonstrating that the cell biochemistry is different between the muscles of the same carcass and, therefore, the intrinsic properties of the muscles, such as mitochondrial concentration (Mitacek et al., 2019; Ramanathan & Mancini, 2018), types of fibers and their metabolism (Choi & Kim, 2009; Kirchofer et al., 2002; Picard & Gagaoua, 2020), have a great effect on the stability of *postmortem* meat color.

In muscle tissue there is the general endogenous antioxidant system consisting of (1) enzymatic antioxidants such as SOD, CAT and GPx, and (2) non-enzymatic antioxidants which include vitamins or their analogues (vitamins A and E), minerals (selenium and zinc). Together, enzymatic and non-enzymatic systems operate to neutralize the action of pro-oxidants in muscle tissues (Descalzo & Sancho, 2008).

In the present study, the variable GPx showed no difference (*P*>0.05, Table 5 and 6) in any *postmortem* period performed. This possibly occurred because vitamin E has a glutathione (GSH) sparing effect, so GPx uses GSH and when there is GSH sparing, GPx action is not affected (Hamid et al., 2011), the reason for this is that the Vitamin E may have taken over oxidative defense and thus reduced GPx induction (Singh et al., 1994).

In the *longissimus lumborum* muscle, the GPx variable showed a significant difference (P <0.05, Table 7) at 360h *postmortem*, with higher mean value for the WCP, 0CP, 00P and W0P treatment and lower mean value for the 000 treatment. Demonstrating that pre-slaughter injectable vitamin-mineral supplementation provides greater amounts of GPx, this was possibly due to the presence of selenium, as it is a structural and catalytic cofactor for GPx (Su et al., 2019). In addition, the presence of vitamin E may also have been important, as a synergistic interaction between selenium and vitamin E nutrients may increase GPx production (Idamokoro

et al., 2020).

Although CAT and SOD are coupled enzymes, they did not show the same activity pattern in the present study (Descalzo & Sancho, 2008). The SOD variable showed no difference (P>0.05, Table 6) in the *longissimus thoracis* muscle *at* 24h *postmortem* and in the *longissimus lumborum* muscle at 24 and 360h *postmortem* (P>0.05, Table 7). Demonstrating that injectable vitamin-mineral supplementation provides greater amounts of SOD, this was possibly due to the presence of Mn, as SOD is dependent on Mn (Arthington & Ranches, 2021).

The CAT variable showed difference (P>0.05, Table 6) in *longissimus thoracis* muscle at 1h and 360h *postmortem* (P< 0.05, Tables 5 and 6). Thus, attributing a greater ability to reduce hydrogen peroxide (Góth et al., 2004). However, there was no difference (P>0.05, Table 7) in the *longissimus lumborum* muscle in any *postmortem* period.

It is difficult to measure each antioxidant component separately. The assessment of total antioxidant potential is recently being used to determine their concentrations (Vu et al., 2018). Measurement of total antioxidant capacity is believed to be useful in determining the cumulative effects of all antioxidants (Athmanathan et al., 2002).

In the present study, FRAP showed higher mean values (P<0.05, Tables 5 and 6) for the WCP treatment in muscle at 1h, 24, 192 and 360h *postmortem* for *longissimus thoracis* muscle and 1h and 360h *postmortem* for *longissimus lumborum* muscle. Therefore, it can be assumed that the antioxidant compounds present in meat samples from animals undergoing WCP treatment exert a cooperative antioxidant activity (Merayo et al., 2020).

The MDA test is used as a lipid oxidation marker and predictor of perceived rancidity in meat. Spaziani et al. (2011) reported that MDA values that were above 1 and close to 1.5 mg MDA/kg denote rancidity to the product. In the present study, the observed MDA values for the 000 treatment are within this range or above, in the *longissimus thoracis* and *lumborum* muscle (Tables 5, 6 and 7).

In addition, the MDA variable showed a difference at 1h and 192h postmortem (P<0.05, Table 6) in *longissimus thoracis* muscle, in which the treatment 000 presented higher mean value than the other treatments. Therefore, use mineral-vitamin supplementation at least in the pre-slaughter period.

In general, vitamin-mineral supplementation can modulate the antioxidant system, improving enzymatic and non-enzymatic antioxidant defenses and decreasing oxidative stress indices.

3.4. Conclusion

Supplementation with the injectable vitamin-mineral complex, performed at least in the pre-slaughter period, reduces the oxidation of oxymyoglobin to metmyoglobin.

Supplementation with the injectable vitamin-mineral complex performed at weaning, castration and pre-slaughter provide a higher antioxidant status and a decrease in lipid oxidation in 1, 24, 192 e 360h postmortem.

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ATTACHMENT 2:

Table 1: Color parameters of the muscle *longissimus thoracis* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different *postmortem* times.

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
24h postmortem								
L*	43.39	42.27	43.59	42.56	43.51	41.77	0.807	0.3357
a*	14.47	15.56	14.71	15.42	13.87	14.86	0.665	0.5848
b*	14.69	14.25	14.71	15.28	14.47	14.52	0.459	0.6687
Chroma	20.64	20.84	20.81	21.73	20.07	20.79	0.734	0.7356
Hue	45.65	42.911	44.97	44.80	46.47	44.45	0.943	0.1515
MMb	42.25	31.84	35.43	35.87	40.40	36.63	4.008	0.6395
DMb	7.27	7.37	6.37	7.13	8.46	8.75	1.221	0.6010
OMb	50.49	60.79	58.20	57.00	51.14	54.62	3.900	0.4634
192h postmortem								
L*	46.12	42.42	45.00	43.42	45.80	43.81	0.995	0.0887
a*	14.21b	16.16a	15.27ab	14.72ab	14.65b	15.98a	0.504	0.0380
b*	15.46	14.54	15.13	15.01	14.91	15.14	0.297	0.4674
Chroma	21.03	21.76	21.51	21.05	20.91	22.02	0.485	0.2699
Hue	47.71a	42.01c	44.75ab	45.61ab	45.63ab	43.53bc	1.021	0.0097
MMb	40.05a	29.65c	33.53abc	37.58ab	35.24abc	31.49bc	2.477	0.0515
DMb	-5.19	-2.23	-4.45	-2.55	-4.53	-3.61	1.153	0.4379
OMb	65.14	72.59	70.92	64.97	69.29	72.27	2.731	0.1714
360h postmortem								
L*	43.65	41.31	41.27	42.02	42.99	40.30	0.903	0.0552
a*	13.99	15.19	14.67	15.12	14.12	14.92	0.411	0.1818
b*	15.40	15.46	15.07	14.92	15.02	14.75	0.387	0.6933
Chroma	20.81	21.69	21.06	21.26	20.61	21.00	0.453	0.6650
Hue	47.77	44.90	45.76	44.65	46.71	44.72	0.901	0.0762
MMb	39.90a	35.30bc	36.30abc	32.44c	38.27ab	34.79bc	1.675	0.0307
DMb	5.62	7.84	6.46	7.30	6.50	7.36	1.058	0.7060
OMb	52.92	55.76	57.24	60.26	54.06	56.52	2.301	0.2904

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter; L* - luminosity; a* - red intensity b* - yellow intensity; MMb – metmyoglobin; DMb – deoxymyoglobin; OMb - oxymyoglobin

Table 2: Color parameters of the muscle *longissimus lumborum* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different *postmortem* times.

	000	WCP	WC0	W0P	00P	0CP	SE	P-value
24h postmortem								
L*	40.39	38.73	38.64	37.70	39.00	39.20	0.979	0.6702
a*	14.26	16.63	15.85	15.75	16.01	15.70	0.509	0.1072
b*	13.58	14.11	13.42	13.32	14.27	13.22	0.366	0.5519
Chroma	19.87	21.82	20.79	20.65	21.20	21.06	0.556	0.2983
Hue	44.20a	40.37b	40.27b	40.22b	40.94b	41.85ab	0.808	0.0304
MMb	39.35a	28.69c	27.59c	31.46bc	28.76bc	35.48b	2.185	0.0004
DMb	11.98	10.52	10.75	11.44	10.07	10.82	1.064	0.8013
OMb	46.56b	60.78a	61.65a	57.10a	59.87a	55.24a	2.097	0.0006
192h postmortem								
L*	40.88	40.48	40.05	39.88	40.05	41.95	0.787	0.6554
a*	15.14ab	14.33b	14.51b	14.50b	15.56ab	16.63a	0.466	0.0158
b*	14.13	13.65	13.88	13.50	13.49	14.68	0.313	0.1496
Chroma	20.17b	20.27b	20.09b	20.07b	20.87ab	22.21a	0.443	0.0157
Hue	43.14	42.96	43.80	42.90	40.56	41.66	0.896	0.2275
MMb	36.56	36.97	39.08	37.36	32.16	30.25	2.306	0.0926
DMb	3.66	2.65	4.47	2.13	1.24	-0.17	1.317	0.2781
OMb	56.67b	68.15a	56.45b	62.72ab	67.51a	69.83a	2.568	0.0025
360h postmortem								
L*	40.18	40.58	39.70	38.63	38.42	39.17	1.164	0.6465
a*	15.67ab	14.70b	14.81b	15.37ab	16.10a	16.52a	0.394	0.0257
b*	15.12ab	14.17b	14.36b	13.99b	15.79a	16.10a	0.389	0.0031
Chroma	21.33bc	21.00c	20.64c	20.82c	22.57ab	23.11a	0.382	0.0008
Hue	44.65	42.90	44.24	42.27	44.49	44.20	0.912	0.4243
MMb	32.72	32.88	34.78	31.53	36.35	29.26	2.191	0.4587
DMb	10.47	10.39	11.52	11.03	10.60	11.28	0.769	0.8771
OMb	56.81	56.72	53.70	57.44	53.05	59.46	2.045	0.5105

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter; L* - luminosity; a* - red intensity b* - yellow intensity; MMb – metmyoglobin; DMb – deoxymyoglobin; OMb - oxymyoglobin

Table 3: Evaluation of exudative losses and shear force in the muscle *longissimus thoracis* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different postmortem times.

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
24h postmortem								
EL, g kg -1	1.25	1.34	0.81	1.20	0.99	1.38	0.269	0.6088
CL, g kg-1	11.45	11.10	11.61	11.58	9.84	10.03	0.806	0.3537
TL, g kg-1	12.56	12.29	12.35	12.63	10.72	11.27	0.922	0.5508
Shear force, N	35.01bc	49.92a	40.47ab	42.96ab	30.66bc	34.68bc	3.395	0.0027
192h postmortem								
EL, g kg -1	0.86	0.96	0.84	0.75	0.83	1.09	0.182	0.7071
CL, g kg-1	11.40	13.06	12.74	12.74	10.98	12.80	1.102	0.6501
TL, g kg-1	12.16	13.91	13.48	13.39	11.71	13.74	1.144	0.6349
Shear force, N	32.28b	45.14a	32.63b	41.37ab	34.29b	38.65ab	3.258	0.0529
360h postmortem								
EL, g kg -1	0.60	0.72	0.59	0.51	0.77	0.71	0.118	0.6040
CL, g kg-1	10.92	9.92	10.21	9.24	9.02	10.34	0.567	0.1692
TL, g kg-1	11.46	10.56	10.73	9.70	9.71	10.98	0.612	0.2500
Shear force, N	26.80	30.02	26.36	30.17	27.07	32.56	2.449	0.0574

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter; EL – Exudates Losses; CL – Cooking Losses; TL – Total losses.

Table 4: Evaluation of exudative losses and shear force in the muscle *longissimus lumborum* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different *postmortem* times.

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
24h postmortem								
EL, g kg -1	2.52	2.30	2.83	2.38	2.86	2.92	0.290	0.5526
CL, g kg-1	12.40	13.83	13.38	13.11	12.91	11.58	1.039	0.9531
TL, g kg-1	14.61	15.80	15.82	15.15	15.40	14.15	1.127	0.9180
Shear force, N	50.52	53.52	49.96	56.17	53.75	41.70	4.212	0.3838
192h postmortem								
EL, g kg -1	1.60	1.34	1.30	1.76	2.07	1.70	0.182	0.1338
CL, g kg-1	12.69	12.03	13.08	12.86	12.14	14.85	0.822	0.8047
TL, g kg-1	14.09	13.20	14.21	14.40	13.98	16.30	0.787	0.6794
Shear force, N	42.42	38.36	39.33	42.30	39.68	46.83	3.765	0.6635
360h postmortem								
EL, g kg -1	0.78	1.18	1.13	1.02	1.10	1.12	0.251	0.9665
CL, g kg-1	9.03	9.26	9.66	8.98	11.79	8.72	0.896	0.2726
TL, g kg-1	9.74	10.33	10.67	9.91	12.75	9.74	0.926	0.2809
Shear force, N	27.79	29.25	27.86	32.23	29.86	31.49	2.158	0.6600

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter; EL – Exudates Losses; CL – Cooking Losses; TL – Total losses.

Table 5: Activity of the antioxidant enzymes, Ferric Reducing Antioxidant Power Assay (FRAP) and Malondialdehyde (MDA) in the muscle *longissimus thoracis* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals 1h *postmortem*

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
SOD,UI/g	8.36	15.65	10.84	10.55	12.17	11.92	2.716	0.6242
GPx, UI/kg	15.34	35.24	24.55	26.70	36.22	43.57	9.542	0.3537
CAT	13.31b	28.09a	20.03ab	20.62ab	31.83a	32.97a	5.160	0.0534
FRAP	0.36b	0.88a	0.43b	0.57ab	0.59ab	0.84a	0.119	0.0117
MDA,mg/kg^{-1}	1.81a	0.76b	1.07b	0.95b	0.97b	0.97b	0.153	<.0001

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter. SOD - Superoxide Dismutase, GPx - Glutathione Peroxidase, CAT - Catalase

Table 6: Activity of the antioxidant enzymes, Ferric Reducing Antioxidant Power Assay (FRAP) and Malondialdehyde (MDA) in the muscle *longissimus thoracis* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different *postmortem* times.

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
24h postmortem								
SOD,UI/g	7.05c	19.51a	8.23c	14.63a	14.89ab	12.36bc	2.462	0.0170
GPx, UI/kg	9.02	27.89	17.01	19.69	27.70	31.92	10.992	0.6140
CAT	13.23	23.28	19.51	20.28	20.49	21.20	6.211	0.9044
FRAP	0.07c	0.54a	0.09bc	0.32abc	0.34ab	0.47a	0.086	0.0014
MDA,mg/kg	1.11	0.73	1.00	0.86	0.91	0.71	0.169	0.4435
192h postmortem								
SOD,UI/g	3.70	16.55	6.62	9.78	13.42	9.00	4.506	0.3013
GPx, UI/kg	10.06	40.56	20.95	28.21	33.64	31.65	8.905	0.2344
CAT	11.16	18.59	13.93	10.95	26.16	10.98	6.285	0.3824
FRAP	0.38b	0.88a	0.64ba	0.76a	0.87a	0.72a	0.095	0.0217
MDA,mg/kg	1.50a	0.76b	1.07ab	0.86b	0.99b	0.74b	0.143	0.0163
360h postmortem								
SOD,UI/g	6.48	14.27	9.37	12.84	11.80	11.05	2.463	0.2653
GPx, UI/kg	11.55	31.23	15.60	24.54	25.34	21.65	4.569	0.0795
CAT	12.71c	35.93a	20.25bc	22.01bc	29.02ab	24.71b	4.148	0.0143
FRAP	0.33c	1.11a	0.42bc	0.65bc	0.68b	0.53bc	0.120	0.0006
MDA,mg/kg	1.48	0.99	1.40	1.02	1.05	0.93	0.172	0.0937

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter. . SOD - Superoxide Dismutase, GPx - Glutathione Peroxidase, CAT - Catalase

Table 7: Activity of the antioxidant enzymes, Ferric Reducing Antioxidant Power Assay (FRAP) and Malondialdehyde (MDA) in the muscle *longissimus lumborum* cut of ¾ Angus ¼ Nellore cattle supplemented with vitamins A and E and micro-minerals at different *postmortem* times

	000	WCP	WC0	W0P	00P	0CP	SE	*P*-value
24h postmortem								
SOD,UI/g	6.55	14.18	9.46	12.61	11.37	18.14	2.947	0.1346
GPx, UI/kg	6.78	16.75	11.47	14.81	8.26	18.15	3.581	0.1799
CAT	14.70	20.40	15.05	20.85	17.68	20.60	3.231	0.5406
FRAP	0.08b	0.54a	0.15b	0.51a	0.34ab	0.29ab	0.941	0.0022
MDA,mg/kg	1.11	0.67	0.82	0.79	0.88	0.72	0.122	0.1366
192h postmortem								
SOD,UI/g	7.09	11.29	5.69	11.45	10.37	9.73	4.125	0.6053
GPx, UI/kg	26.50	34.94	22.33	38.95	25.50	27.36	13.954	0.8828
CAT	12.85	24.54	13.23	22.38	21.50	21.78	4,343	0.3156
FRAP	0.73	1.06	0.72	1.03	1.01	0.98	0.214	0.3211
MDA,mg/kg	1.16	0.68	1.07	0.84	0.89	0.84	0.415	0.6696
360h postmortem								
SOD,UI/g	4.87b	10.41a	7.84ab	10.19a	10.12a	10.69a	1.117	0.0036
GPx, UI/kg	15.14b	25.55a	17.29b	20.40ab	20.73ab	21.57ab	2.567	0.0519
CAT	14.60	22.05	16.04	17.43	18.02	20.21	2.011	0.0755
FRAP	0.29c	0.69a	0.39bc	0.54b	0.59a	0.66a	0.063	0.0001
MDA,mg/kg	1.43	0.88	1.11	0.99	0.92	0.95	0.148	0.1018

000 - Control (application of saline solution at weaning, castration and pre-slaughter;WCP - Application of vitamin-mineral complex at weaning, castration and pre-slaughter; WC0 - Application of vitamin-mineral complex at weaning and castration + Application of saline solution at pre-slaughter; W0P - Application of vitamin-mineral complex at weaning + Application of saline solution at castration + Application of vitamin-mineral complex at pre-slaughter; 00P - Application of saline solution at weaning and castration + Application of vitamin-mineral complex at pre-slaughter; 0CP- Application of saline solution at weaning + Application of vitamin-mineral complex in castration and pre-slaughter, SOD - Superoxide Dismutase, GPx - Glutathione Peroxidase, CAT – Catalase.