UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

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Effect of different selenium sources and levels on meat quality of Nellore cattle

Pirassununga
2018

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Thesis presented to the Faculty of Animal Science and Food Engineering of the University of São Paulo in partial fulfillment of the requirements for the degree of Doctor of Animal Science.

Specialization: Quality and Animal Productivity

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Pirassununga

2018

Ficha catalográfica elaborada pelo
Serviço de Biblioteca e Informação, FZEA/USP,
com os dados fornecidos pelo(a) autor(a)

Silveira da Silva, Janaina
SS587e Effect of different selenium sources and levels on meat quality of Nellore cattle / Janaina Silveira da Silva ; orientador Marcus Antonio Zanetti ; coorientador Alessandra Fernandes Rosa. -- Pirassununga, 2018.
100 f.

Tese (Doutorado - Programa de Pós-Graduação em Zootecnia) -- Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo.

1. carbonyl. 2. cholesterol. 3. feedlot. 4. HMG-CoA reductase. 5. malondialdehyde. I. Antonio Zanetti, Marcus, orient. II. Fernandes Rosa, Alessandra, coorient. III. Título.

DEDICATED

to GOD:
By His immense love and mercy, He took care of my life; He guided my steps and gave me strength at all times;

My family:
By encouraging, love, trust and care;

To my fiancé:
Murilo Trettel,
For all your love, affection, counsel, patience and protection;

"God is my strong fortress; and He sets the blameless in His way."

ACKNOWLEDGMENTS

First and above of all, I am thankful to God, Who is everything that I need.

My beloved Murilo Trettel, who always had patience in my stressful moments, took care of me with a lot of affection and love and often gave up his own obligations to help me.

The animals used in the experiment.

My advisor Prof. Dr. Marcus Antonio Zanetti, who accepted the challenge of advising me. I am thankful for his guidance, great support, and advice throughout my PhD research studies. It was a real privilege and an honor for me can receive and learn a little about his exceptional scientific knowledge.

My co-adviser Alessandra Fernandes Rosa who was very important to help me design the experiment and develop it well.

My friends Frederich Diaz Rodriguez and Rogério Cordeiro de Mira for all support, friendship and confidence.

Bianca Freire Bium and Thais de Oliveira, scientific initiation students, for all support and dedication.

Silvana Marina Piccoli Pugine, laboratory specialist, and Professor Mariza Pires de Melo for all support, dedication, confidence, and patience to help me with the analysis and availability of Laboratório de Quimica Biológica (LBQ).

José Aparecido Cunha, laboratory specialist, for selenium analysis and support.

My co-workers Renata, Susana and Roger for all support.

To Daniele Passarelli for help and availability in some analyses.

Professor Júlio de Carvalho Balieiro for his patience and willingness to teach and help me with statistical analysis.

I would express my hearted thanks and gratitude to all teaching staff, employers and students for assisting me:

Professor Catarina Abdalla Gomide; Saulo da Luz e Silva; Angélica Simone Cravo Pereira.

Students of LEACC laboratory, Cristina Tschorny Moncau, Bárbara Silva Vignato, Mikaele Alexandre Pereira.

Students of CEBER laboratory, especially Daniel Silva Antonelo, Madeline Rezende Mazon and Juan Fernandes Morales.

The section of Bovinocultura de Corte (Ismael, Ricardo, Valdir, and Gustavo) for the competence and great help in the execution of this project.

Bromatology laboratory (Rose, Rosilda, Raphael and João).

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship grant (CNPq/Brazil – Process nº142484/2015–6) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), which provided financial support for this study (Process nº 2015/07175-6).

Everyone who was important for my personal and professional education.

ABSTRACT

SILVA, J. S**. Effect of different selenium sources and levels on meat quality of Nellore cattle.** 2018. 100 f. Thesis (PhD) – Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga, 2018.

Selenium (Se) is an essential mineral with functions for both animals and humans. There are several regions in the world deficient in this mineral and studies have related Se supplemented with reducing cholesterol. Thus, the objective of this study was to evaluate the Se effect in different levels and sources in the diet finishing of Nellore cattle on the performance and meat quality. It was used 63 Nellore cattle (412 kg and ± 24 months of age) in a completely randomized design with two sources (sodium selenite and selenium-enriched yeast) and four supplementation levels (0; 0.3; 0.9 and 2.7 mg Se/kg DM). There were no changes in performance and carcass characteristics. The Se level reduced ($P<0.01$) lipid and proteins oxidation (TBARS and carbonyl) compared to the control treatment on retail display storage (0, 2, 4 and 6 days). Organic Se, regardless of level, provided Se 138% higher ($P<0.0001$) in meat and 22.6% higher ($P<0.0001$) in serum than inorganic Se. The activity of glutathione peroxides (GPx) in muscle was 288% higher for animals supplemented with selenium and consequently, the cholesterol concentration in L. dorsi was 10.2% lower ($P<0.001$). The serum HMG-CoA reductase concentration was 32.7% lower in animals receiving Se supplementation (organic or inorganic). In conclusion, Se supplementation in beef cattle diet is a way of naturally producing selenium-enriched meat and with better quality for human consumption.

Keywords: carbonyl, cholesterol, feedlot, HMG-CoA reductase, malondialdehyde, minerals

RESUMO

SILVA, J. S**. Efeito de diferentes fontes e teores de selênio sobre qualidade da carne de bovinos Nelore.** 2018. 100 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2018.

O selênio (Se) é um mineral essencial com funções para animais e humanos. Existem várias regiões do mundo deficientes neste mineral e estudos têm relacionado a suplementação de Se com a redução do colesterol. Assim, o objetivo deste estudo foi avaliar o efeito de diferentes teores e fontes de Se na dieta de terminação de bovinos Nelore sobre o desempenho e a qualidade da carne. Foram utilizados 63 animais da raça Nelore (412 kg e ± 24 meses de idade) em delineamento inteiramente casualizado, com duas fontes (selenito de sódio e selênio levedura) e quatro teores de suplementação (0; 0,3; 0,9 e 2,7 mg Se/kg MS) em confinamento, durante 84 dias. Não houve alterações no desempenho e nas características de carcaça. O teor de Se reduziu (P <0,01) a oxidação lipídica e proteica (TBARS e carbonila) em comparação ao tratamento controle durante o armazenamento em simulação de exposição no varejo (0, 2, 4 e 6 dias). O Se orgânico, independentemente do teor, forneceu valor de Se 138% superior (P <0,0001) na carne e 22,6% superior (P <0,0001) no soro em relação ao Se inorgânico. A atividade glutationa peroxidase (GPx) no músculo foi 288% maior nos animais suplementados com selênio e, consequentemente, a concentração de colesterol na carne foi 10,2% menor (P <0,001). A concentração sérica de HMG-CoA redutase foi 32,7% menor nos animais que receberam suplementação de Se (orgânico ou inorgânico). Foi concluído que a suplementação com Se na dieta de bovinos de corte é uma forma de produzir carnes enriquecidas com selênio naturalmente e com melhor qualidade para consumo humano.

Palavras-chaves: carbonila, colesterol, confinamento, HMG-CoA redutase, malondialdehyde, minerais

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LIST OF ABBREVIATIONS

a*: redness
ADG: average daily gain
b*: yellowness
BFT: back fat thickness
BW: body weight
CAT: catalase
Ca: calcium
CL: cooking loss
Co: cobalt
Cu: copper
DP: dressing percentage
DMI: dry matter intake
EROS: reactive oxygen species
G:F: gain to feed ratio
GPx: glutathione peroxidase
GR: glutathione reductase
GSH: reduced glutathione
GSSG: oxidized glutathione
Gr: Glutathione reductase
HCW: Hot carcass weight
HDL: high-density lipoprotein
HMGCR: HMG-CoA reductase enzyme
HMG-CoA: 3-hydroxy-3-methyl-glutaryl-CoA
IBGE: Instituto Brasileiro de Geografia e Estatística
kg: kilogram
L*: lightness
LDL: low-density lipoprotein
MDA: malondialdehyde
mg milligram
NRC: National Research Council
PVC: polyvinyl chloride
RD: Retail display

REA: ribeye area

SEM: standard error

SOD: superoxide dismutase

Se: selenium

SEM: standard error

SF: shear force

TBA: thiobarbituric acid

TCA: trichloroacetic acid

TBARS: 2-thiobarbituric acid reactive substances

TC: total cholesterol

TEP: 1,1,3,3 Tetraethoxypropane

TG: triglycerides

T3: triiodothyronine

T4: thyroxine

Zn: zinc

SUMMARY

CHAPTER I – INTRODUCTION

Red meat is a food of excellent nutritional quality, an important source of fundamental micronutrients such as B vitamins, essential minerals and high biological value protein, being a complete and fundamental food for human health. Brazil stands out in the production of meat, with beef cattle being a very important economic activity for the country. In the first quarter of 2018, the country slaughtered 7.72 million cattle and exported 346.16 thousand tons of fresh meat, which represented a turnover of US$ 1.3 billion (IBGE, 2018).

Although it represents an adequate source of nutrients, the consumption of red meat has been related to some possible harmful effects to health, such as greater risk of cancers and cardiovascular diseases. In addition, consumers are increasingly concerned not only with the nutritional value as well as with the quality of the product, checking for some aspects like tenderness, juiciness, meat flavor and color, which is one of the most important characteristics at the moment of purchase decision. Besides the factors cited, in recent times the consumer has also been concerned with the origin of the product.

Some of the characteristics in meat that decrease the consumer's acceptance are flavor´s loss natural, the presence of undesirable flavors and toxic compounds, all of them can be avoided with the use of antioxidant agents in cattle diet. Animal nutrition’s appropriate manipulation can improve both the efficiency of production’s system and, above all, reach the characteristics of consumer market that seeks to insert healthier foods into their diet. In this context, selenium as part of the enzyme glutathione peroxidase, acts as an antioxidant can improve some organoleptic meat characteristics, such as increase oxidative stability and even reduce the cholesterol content in the final product.

Besides that, Ferreira et al. (2002) evaluated the selenium content in several foods consumed in Brazil and from different regions, presumed that the Brazilian population is susceptible to selenium deficiency and that this fact aggravated among individuals with lower income. Therefore, the selenium’s supply for animals destined to human consumption is an interesting strategy to increase this element in meat and consequently in diet of Brazilian population.

Knowing the meat consumption importance for human health, the Brazil great position as supplier of this food and the lack of selenium in most Brazilian

soils. The objective of this work was to evaluate the effect of the supplementation of different levels and sources of selenium in the diet of Nellore cattle on the performance of the animals and the meat quality.

I.1 General objective

Evaluate the diet supplementation with different levels of organic (Se-yeast) and inorganic (selenite sodium) selenium influence on the meat quality in finishing cattle.

I.1.1 Specific objectives:

- Study the selenium transfer, in different levels and sources in the diet, to bovine meat.
- Check the differences between sources of selenium (sodium selenite and Se-yeast) in the animal performance and meat quality.
- Evaluate the Se effect on the meat oxidation and tenderness.
- Determine the selenium content in the diet that reduces cholesterol in the L. dorsi muscle.
- Check the selenium effect on reduced glutathione (GSH), oxidized glutathione (GSSG) and glutathione peroxidase (GPx).
- Evaluate the Se effect on cholesterol metabolism (HMG-CoA reductase).

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CHAPTER II – LITERATURE REVIEW

II.1 Importance of selenium

The essentiality of selenium was recognized in 1957 by Schwarz and Foltz, who identified that selenium has the capability to prevent hepatic necrosis in rats (McDowell, 2003). The second historical finding involving selenium was when Omaye and Tappel (1974) found a semi-logarithmic relationship between glutathione peroxidase (GPx) and the amount of selenium in diet in several poultry tissues.

Selenium is a component of several enzymes, of which the most important is glutathione peroxidase that functions as an antioxidant in tissues, catalyzing the decomposition of peroxides. Glutathione peroxidase (GPx) contains four selenium atoms and acts by catalyzing the transformation reaction of reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase (Gr) is responsible for the inverse reaction, transformation of GSSG to its GSH (FETTMAN, 1991).

Se is also involved in the metabolism of thyroid hormones, because the enzyme iodothyronine 5'-deiodinase that converts thyroxine (T4) to triiodothyronine (T3) is selenium-dependent (PAPPAS, 2008). This action is important in lipid metabolism, since T3 regulates the LDL's level (LDL-R) receptors, which are important for controlling blood cholesterol levels (MUKHOPADHYAY et al., 2003), in addition to T3 being more potent than T4.

According to McDowell (2003), selenium absorption in ruminants is inferior to that absorbed by monogastrics, due to the formation of insoluble compounds in the rumen. The minimum selenium requirement for beef cattle is 0.1mg/kg dry matter (DM) (NRC, 1996) and the maximum estimated concentration to avoid toxicity problems is 5 mg / kg DM in the NRC (2005).

Selenium concentration in bulks is generally low (exception of some seleniferous areas in the world), so intake from the natural content of plants and dietary components is insufficient to meet the nutritional requirement of this element, therefore, selenium supplementation in mineral mixtures is indispensable in most areas (GIERUS, 2007).

According to Combs Junior (2001), the estimate is that 0.5 to 1.0 billion people have a lack of Se. In most areas of Brazil, daily human consumption of selenium is below the recommended levels, as selenium deficiency exists in almost all Brazilian soils, except for the northern region (MAIHARA et al., 2004).

Thus, due to the importance of both the animal and humans, the development of research aimed at increasing this element in the final product is important for livestock and for the public health area.

II.2 Cholesterol synthesis

Cholesterol is the precursor of steroid hormones, vitamin D, bile salts and is part of the cell membrane (STOJEVIĆ, 2008). Cholesterol biosynthesis can be regulated indirectly by the reduction of reduced glutathione (GSH) and an increase of oxidized glutathione (GSSG), being that the increased GSSG/GSH decreases the activity of the enzyme HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA), reducing the cholesterol synthesis (KIM; CHAO; ALLEN, 1992). The HMG-CoA reductase needs thiol groups such as GSH to become biochemically active, while disulfides such as GSSG decrease the enzyme activity (ARMSTRONG et al., 2001).

Knowing the mechanism of cholesterol biosynthesis and the selenium's function as a component of GPx, some studies demonstrated that selenium supplementation reduces cholesterol concentration. Netto et al. (2014) observed decreasing the reduced form of glutathione (GSH), increasing the oxidized form (GSSG) in the liver and a cholesterol concentration decreased in the meat of Brangus bulls receiving 2 mg of selenite sodium/kg of DM. Like Correa et al. (2013a) working with Nellore cattle supplemented with 2.5 mg Se/kg DM also observed a significant reduction in cholesterol in both blood and meat. Kang; Bansal and Mehata (1998) working with rats on high-fat diets observed that serum cholesterol levels were lower in animals treated with Se. As well as Cases et al. (1999), Kang; Mehata and Bansal (2000a) also working with rats and Kang; Bansal and Mehata (2000b), working with rabbits.

Despite many studies related the effect of selenium on the cholesterol metabolism, to our knowledge, there are no studies evaluating the effect of selenium supplementation on the concentration of HMG-CoA reductase enzyme

(HMGCR) in bovine. Nassir et al. (1997) found an increase in HMG-CoA reductase activity in the liver of Se-deficient rats with high cholesterol levels. Dhingra & Bansal (2006) observed that Se supplementation reduce the gene expression of HMG-CoA reductase in rats.

Due to the importance of meat intake as a source of nutrient for humans, the possibility of manipulating the diet of animals with Se supplementation aiming to reduce the cholesterol content of meat is very interesting.

II.3 Inorganic and organic selenium

Selenium supplementation can be performed with two chemical forms, organic and inorganic. Inorganic selenium is the most used in mineral supplementation, with sodium selenite being the most common form. However, organic selenium is more expensive and used in high-performance diets, mainly found as selenomethionine (SeMet) and selenocysteine (SeCis), which are sulfur amino acid analogs (GIERUS, 2007).

Several factors such as the chemical form of the mineral, diet components, selenium status and animal species, may influence the bioavailability and distribution of selenium in the body. Negative interactions can occur among minerals, one of the advantages attributed to organic minerals is that they do not suffer as much as inorganics with undesirable interactions with other elements, although there are some discrepant results.

Apparent Se absorption can be reduced due to increased sulphate intake, that results in reduced plasma concentrations of Se (IVANCIC JÚNIOR; WEISS, 2001). The absorption capacity of inorganic Se in ruminants is low because rumen microorganisms reduce most inorganic selenium from the diet to the elemental selenium form (Se0), which has low solubility and therefore is excreted in the faeces (KIM; VAN SOEST, COMBS, 1997). In organic selenium sources, due to the form similar to sulfur amino acids, the selenium incorporation into proteins occurs in substitution for sulfur amino acids, improving absorption (SUZUKI, 2005). Different metabolism systems are used to absorb chemical forms of selenium, characterizing inorganic forms by a lower bioavailability than organic forms (WEISS, 2005).

The absorption of Se in the selenite form occurs by diffusion, while in Selenate and SeMet it occurs by active transport, selenate being absorbed similarly to sulfate, with sodium dependent transporters located in the distal portion of the small intestine, and SeMet similarly to methionine with sodium-dependent amino acids (VENDELAND et al., 1994). SeMet is the most retained in tissue proteins than SeCys and inorganic Se forms (SURAI, 2006).

Some studies have shown that the selenium's muscular concentration is higher in animals supplemented with Se-yeast in relation to sodium selenite. Paiva (2006) compared the supplementation of two organic selenium sources (Se-yeast and Se-methionine) with sodium selenite at three levels (0.2, 0.8 and 1.4 mg Se / kg) in diets supplied to Suffolk lambs for a period of 84 days. The Se concentration in serum, liver and kidney; glutathione peroxidase activity in liver and metabolic selenium balance were similar between Se sources, at the levels used in the experiment. However, the organic source (Se-yeast) provided the highest selenium content in the animals muscle, thus increasing the selenium amount in lamb meat.

Juniper et al. (2008) studied the effect of supplementation with different selenium sources: sodium selenite (0.30 mg Se / kg DM) and selenium yeast (0; 0.30 and 0.50 mg Se / kg DM) for male bovines, castrated, for a period of 112 days. Authors did not observe a difference between organic and inorganic selenium sources on the meat oxidative stability (TBARS). However, they found higher Se content and higher GPx activity in the muscle tissue of animals receiving diets containing Se-yeast.

Rossi et al. (2015) evaluated the effect of Se source in final fattening period of Charolais heifers (162 heads, 517 ± 61 days of age). The heifers were supplemented from the beginning of the fattening period with 0.2 mg Se / kg DM of ration in the sodium selenite form and in the last 60 days of fattening were divided into two groups with different Se sources (selenite sodium and selenium-yeast). The authors did not observe effect of Se source on growth, meat centesimal composition, thawing loss and losses by dripping or cooking. However, Se-yeast supplementation improved the selenium's content in the animals meat.

These results confirm the different value between organic and inorganic Se form, indicating that the Se organic form is incorporated into the body's proteins, such as muscle tissue, in place of methionine.

II.4 Oxidation and antioxidants

In the cell metabolism, with the oxygen reduction in oxidative phosphorylation, occurs the reactive oxygen species formation (EROS) that are involved in degenerative processes because they have the characteristic of oxidizing molecules, which can cause irreversible damage to the cells (MEIRELLES, 2009) .

The increase in oxidant species generation causes an increase in the reactive oxygen species, which can lead to a pro-oxidant state, favoring oxidative lesions and possibly resulting in cell death (GUTTERIDGE, 1993). Pro-oxidants help in the highly oxidant species generation such as peroxyl radical, hydroxyl radical, hydrogen peroxide and nitric oxide, which are responsible for the induction of proteins and lipids oxidation (BUTTERFIELD et al., 1998).

Oxidation is a process that affects the meat conservation, because it induces modifications in lipids and proteins altering its organoleptic and nutritional properties (INSANI et al., 2008). Thus, it causes some problems such as undesirable flavors, coloration loss, nutrients destruction and toxic compounds formation, decreasing the consumer’s acceptance (KANNER, 1994).

The color loss is related to the fact that oxidation affects the amount of iron free and bound to the heme pigment of myoglobin protein (ESTÉVEZ; VENTANA; CAVA, 2006). The myoglobin oxidation and the oxygen consumption lead to the metamioglobin development that results in the meat browning (MARTINAUD et al., 1997).

Another characteristic of the meat quality affected by oxidation is the tenderness degree, because oxidation increases the resistant bonds between myofibril proteins (LUND et al., 2007). In contrast, there are antioxidant enzymes that are an efficient system to protect oxidative tissues. The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (TEREVINTO et al. 2015).

The meat oxidative stability depends on the balance between antioxidants and pro-oxidants (MERCIER; GATELLIER; RENERRE, 2004). After slaughter, the alterations resulting from the muscle conversion into meat weaken the defense antioxidant systems and potentiate the oxidation (HARRIS et al. al., 2001).

One strategy to improve the meat oxidative stability is to add antioxidants in animal diet. Selenium as an essential component of the GPx enzyme can improve the meat oxidative stability products.

II.4.1 Lipid Oxidation

Lipid oxidation is the result of a series of biochemical events resulting from the free radicals action on the cell membranes of lipids, with radicals formation such as alkoxyl and peroxyl. Thus, they cause the unpleasant odor and taste (warmed-over-flavor) development (CURI et al., 2002).

Unsaturated lipids and pro-oxidant components presence increase susceptibility to oxidation in meat and the free radical chain reaction can be initiated by exposing food to light, heat, ionizing radiation, metal ions or metalloprotein catalysis (MEIRELLES, 2009).

Lipid oxidation triggers reactions that culminate in the release of degradation products of fatty acids, such as aldehydes (FERREIRA; MATSUBARA, 1997). Malondialdehyde is a 3-carbon dialdehyde with carbonyl groups at the C-1 and C-3 positions. It can be found in several foods, but in fatty ones, its concentration depends on the degree of fatty acid unsaturation, on the presence of metals, on the pH and on the cooking time and temperature to which they are subjected (PIKUL; LESZCZYNSKI; KUMMEROW, 1989; ULU, 2004).

Besides that, malondialdehyde is considered the major byproduct of lipid oxidation, and as a bifunctional aldehyde, it is highly reactive and can interact through DNA and protein cross-linking, promoting chromosomal aberrations and reduced protein synthesis capacity, respectively (ADDIS, 1986; PEARSON et al., 1983).

The TBARS technique is a method to evaluate the meat lipid oxidation level using as a principle the quantification of malondialdehyde (SANTÉ-LHOUTELLIER; ENGEL; GATELLIER, 2008).

The free radicals formed in the lipid oxidation process can react or interact with the food, being of great importance as well as for nutritional quality, human health such as the reduction in the shelf life and the consequent economic aspects.

Selenium supplementation can provide a positive effect on lipid metabolism improving some meat characteristics. Correa et al. (2013b), observed lipid oxidation reduction, measured by TBARS, in the muscle (*L. dorsi*) of Nellore cattle supplemented with 2.5 mg of selenium and 500 IU of vitamin E.

However, the results are inconsistent; some studies have not observed effect of the selenium supplementation on lipid oxidation. Juniper et al. (2008) did not observe alterations in the oxidative stability of the bovine meat receiving 0.5 mg selenium / kg of DM in the diet. Like Vignola et al. (2009), who also did not find significant differences for lipid oxidation (TBARS) in lambs supplemented for 63 days with 0.45 mg Se / kg DM in relation to the control group, with detail that, after 9 days of storage there were differences between groups supplemented with Se and control.

II.4.2 Protein oxidation

Meat consists of three types of proteins, the myofibrils which constitute 60.5% of the total proteins and are represented by actin and myosin. The sarcoplasmic proteins which are 29% of the total and are represented by enzymes and myoglobin; and finally the proteins of the connective tissue (collagen and elastin) that represent 10.5% of the total (VUORELA et al., 2005).

According to Lund et al. (2011), the products formed in the proteins oxidation are dependent on the amino acids involved, in the way the process is initiated depending on the type of pro-oxidant species present in the meat. In the reaction of reactive oxygen species and other oxidizing agents, such as metamioglobin with proteins, carbonyl formation occurs and the reduction of sulfhydryl groups, also known as thiols, occurs (XIONG, 2000).

The quantification of carbonyl groups (aldehydes and ketones) and thiols groups, called sulfhydryl groups, is one of the ways to evaluate protein oxidation (SILVA, 2014). According to Vuorela et al. (2005), the carbonyl groups formation is the most important alteration of oxidized proteins.

As only oxidation of lipids was seen as a cause in food deterioration, this was studied profoundly, while protein oxidation for a long time was a neglected fact, because proteins were not believed to be also targets of reactive oxygen species (EROS) (LUND et al., 2011).

However, the meat protein oxidation is responsible for many changes that affect the quality of the meat (SANTÉ-LHOUTELLIER; ENGEL; GATELLIER, 2008). According to Mercier; Gatellier; Renerre (2004), one of these changes is the fragmentation, aggregation and decrease of protein solubility due to amino acid changes. Thus, it can reduce the digestibility and consequently the meat nutritional value (MORZEL et al., 2006). Besides, Rowe et al. (2004) found that the early muscle proteins oxidation has a negative effect on meat tenderness, even in steaks with 14 days of maturation.

Wang et al. (2009) observed that supplementation with 0.3 mg Se-yeast / kg DM for broiler chickens was able to reduce the amount of carbonyls and malondialdehyde in the meat of their male offspring (poultry) slaughtered at 21 days. In cattle, evaluation of the Se supplementation effect on meat protein oxidation is still scarce. Rossi et al. (2015) cited the importance of studies to verify the mechanisms involved in the Se organic supplementation effects on the potential impact on the postmortem softening and protein oxidation process.

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CHAPTER III - PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT QUALITY OF NELLORE CATTLE SUPPLEMENTED WITH SUPRANUTRITIONAL DOSES OF SODIUM SELENITE OR SELENIUM-ENRICHED YEAST

Original manuscript submitted to Animal (Cambridge) journal (ANIMAL-18-60214R1), in August 28th, 2018.

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Short title: High selenium levels diets for beef cattle

III. Abstract

The enrichment of meat with Selenium is important to improve the intake of selenium by humans. The effects of supranutritional doses of sodium selenite or selenium-enriched yeast on performance, carcass characteristics and meat quality were evaluated using 63 Nellore cattle in a completely randomized design with two sources (sodium selenite and selenium-enriched yeast), three levels (0.3, 0.9 and 2.7 mg Se/kg DM) and control treatment (without addition of selenium). Final body weight (BW), average daily gain (ADG), dry matter intake

(DMI) and gain to feed ratio (G:F) at the end of 84 days of supplementation were not influenced by treatments (P>0.05). Hot carcass weight (HCW), dressing, pH, ribeye area (REA), back fat thickness (BFT) and marbling score were also not influenced by treatments (P>0.05). Additionally, TBARS concentration, colour, shear force (SF), cooking loss and drip loss remained unchanged (P>0.05). The selenium concentration in the meat of animals receiving organic selenium was higher (P<0.001) than that of animals receiving sodium selenite, at all levels (0.3; 0.9 and 2.7 mg/kg DM). The meat of animals receiving 2.7 mg of organic Se/kg of DM presented concentration of 372.7 µg Se/kg in the L. dorsi muscle, and the intake of 150 g of this meat by humans provides approximately 100% of the recommended Se intake (RI) (55 µg Se/day for adults). Therefore, the use of supranutritional doses of 2.7 mg Se/kg of DM, regardless of source, is a way of naturally producing selenium-enriched meat without compromising performance, carcass characteristics and quality of Nellore bovine meat.

Keywords: antioxidant, *Bos indicus*, feedlot, growth, minerals

III. Implications

Selenium is an essential mineral with functions for both animals and humans. There are several regions in the world deficient in this mineral, so the production of selenium-enriched meat is a way to increase the intake of this mineral by humans. However, the use of high selenium doses in *Bos indicus* is still scarce. It was observed that supplementation with sodium selenite or selenium-enriched yeast, with values up to 27 times the requirement (National Research Council, 2000), is a way to naturally produce selenium-enriched beef for human consumption, without compromising the performance and characteristics of animal carcasses.

III.1. Introduction

Healthy diet is an essential part of a healthy lifestyle and these diets are gaining popularity (Fašiangová et al., 2017). In this context, adequate animal nutrition is essential not only to improve the efficiency of the production system, but also to increase the quality of the final product and to meet the needs of the increasingly demanding consumer beef market. Among the nutrients that can be used in animal production, selenium may improve the nutritional value and the quality characteristics of meat (Surai, 2006). In addition, selenium content in several foods produced and consumed in most regions of Brazil are generally low (Ferreira et al., 2002), so the daily human consumption in most areas of the country is lower than recommended levels (Maihara et al., 2004). Approximately 1.1 billion people worldwide are selenium deficient (White et al., 2012).

For both animals and humans, selenium is an essential trace mineral and deficiency is still a global problem in many countries (Zhang et al., 2010). Selenium is involved in several functions such as cognitive process and thyroid hormone (Rayman, 2012), protection against cancer, regulation of inflammatory mediators, lowering the risk of cardiovascular diseases and improving the lipid profile (Maranhão et al., 2011; Cominetti et al., 2012; Colpo et al., 2013), bone homeostasis and protecting against bone diseases (Zhang et al., 2014). Fašiangová et al. (2017) reported that eggs enriched with Se may represent a functional food with the possibility of being used to increase the intake of selenium by humans and, therefore, the content of selenium in the human body.

The recommended selenium dose for beef cattle supplementation regardless of source is 0.1 mg/kg DM (National Research Council, 2000) and the maximum estimated concentration to avoid toxicity problems is 5 mg/kg DM

(National Research Council, 2005). Selenium can be supplemented in the inorganic or organic forms, and some studies have shown higher bioavailability of organic selenium in relation to inorganic for cattle (Cozzi et al., 2011; Sgoifo Rossi et al., 2015). Furthermore, several authors have observed higher Se deposition in bovine muscles receiving organic source compared to the inorganic source (Lawler et al., 2004; Juniper et al., 2008b; Cozzi et al., 2011; Pereira et al., 2012). Therefore, the supply of supranutritional doses (greater than 0.1 mg/kg of DM) of selenium for beef cattle may be useful in increasing this element in meat and consequently in the diet of the population living in areas with deficiency of this mineral. Despite there are many studies on the effect of selenium neither of them investigated the best selenium level and source at the same time. In addition, the production of animal protein of better nutritional quality through natural processes such as biofortification, respecting the toxic limit for the animal, is important for contributing to the quality of life of the population. Thus, the aim of this study was to evaluate the effects of supranutritional doses of sodium selenite or selenium-enriched yeast on the performance, carcass characteristics and meat selenium of Nellore cattle. The hypothesis of this research is that supranutritional selenium's levels have no effect on animal performance and carcass characteristics, but it increases the concentration of selenium in the meat considerably.

III.2. Material and methods

III.2.1 Animal and feeding

In total, 63 Nellore (*Bos indicus*) bulls (412 ± 19 kg BW; 24 months old) were weighed, tagged and housed in a feedlot system with individual pens at the

Department of Animal Sciences of the College of Animal Sciences and Food Engineering - University of Sao Paulo, Pirassununga, SP, Brazil. Initially, all animals were submitted to a 14-day adaptation period, when the concentrate levels were progressively increased. At the end of the adaptation period, animals (9/treatment) were submitted in a 2×4 factorial arrangement. Factors were two Se sources (inorganic: sodium selenite and organic: selenium-enriched yeast), four levels (0; 0.3; 0.9; 2.7 mg/kg of DM). The selenium-enriched yeast used was Alkosel 3000 (Alkosel® 3000, Lallemand Animal Nutrition) with 3000 mg Se/kg of product. Diets were formulated according to (National Research Council, 2000) recommendations; control diet had 0.063 mg of Se/kg of DM. Animals were fed with total mixed common diet offered daily at 07.00 a.m. and 03.00 p.m containing 70% concentrate and 30% roughage (Table 1), during 84 days. Every two days, leftovers were collected, weighed and sampled for dry matter determination. The feed supply was adjusted every two days based on the amount of leftovers from the previous days, to ensure 5% to 10% refusals. Animals were weighed after the complete 16-hour fasting period, at baseline (day 0) and after 28, 56 and 84 days of feeding. Dry matter intake (DMI), average daily gain (ADG) and gain to feed ratio (G: F) were calculated from the feed intake data and BW measurements.

Tabela 1. Dietary ingredients and chemical composition (DM basis) of the basal diet.

Items	Content
Ingredients (%)	
Corn silage	30.00
Corn grain	56.84
Soybean 45%	10.64
Urea	1.34
Mineral mixture[1]	1.18
Chemical composition (%)	
CP	17.00
RDP	10.34
TDN[2]	79.22
EE	2.88
Selenium (mg/kg)	0.063

RDP = rumen digestible protein; TDN = total digestible nutrient.

[1] Trace mineral mixture content (per kg): zinc. 2230 mg; calcium. 180 g; sodium. 74 g; manganese. 470 mg; copper. 1550 mg; cobalt. 5 mg; iodine. 47 mg; monensin sodium. 2300 mg.

[2] Calculated as described by Weiss; Conrad and Pierre (1992).

Selenium levels treatments: organic or inorganic selenium at the amount of 0.3, 0.9 and 2,7 mg/kg was added on basal diet.

III.2.2 Carcass data collection

At the end of feeding, animals were slaughtered at slaughterhouse located at the University of Sao Paulo according to humane slaughter procedures as required by Brazilian law. Hot carcass weight (HCW) and dressing per cent (DP) were immediately determined after evisceration. After cleaning, carcasses were kept in cold storage (0 - 4°C) until *rigor mortis* completion. Temperature and pH measurements were performed between the 12th and 13th ribs using digital pH meter 1 and 24 h after slaughter (Hanna Instruments model HI99163; Hanna Instruments, São Paulo, Brazil).

After 24 h of chilling, the left half of carcasses were sawn between the 12th and 13th ribs to expose an area for back fat thickness (BFT) and ribeye area

(REA) measurements using a ruled grid with scale in cm^2. During boning, three samples were collected from the *L. dorsi* muscle at the 13^{th} rib towards the head in the left half of the carcass. One sample was subdivided in 5 g samples (in triplicate) and immediately frozen in liquid N_2 for TBARs and selenium analysis. One sample (2.5 cm thick) was used for ageing period of one day and analysis of colour, cooking loss and shear force. Another sample was used for drip loss by the standard bag method, it was used a sample cut (40 – 50g) stored at 4±1°C in individual closed plastic bags, after 2 days of storage, the cuts were removed from the bag and weighed to estimate drip loss, expressed as a percentage relative to the initial weight.

The colour was measured with a portable colorimeter (mod. CM-2500D, Konica Minolta Sensing, Inc, Tokyo, Japan), using the L*, a*, b*, a CIE Lab system scale with D_{65} light source, observation angle of 10° and cell opening of 30 mm. The marbling score (intramuscular fat deposition) was visually determined with the aid of standard USDA cards. Tenderness and cooking loss were determined according to American Meat Science Association recommendations (AMSA, 2015). Cooking loss was expressed as the weight change percentage of the sample before and after cooking. The tenderness was determined from six cylinders (1.27 cm of diameter) with the fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade, using a texture analyser (TMS-Pro model, Food Technology Corporation) equipped with a Warner-Bratzler blade and test speed of 200 mm/min. The shear force of each sample was considered as the average value of the six cylinders.

III.2.3 Analytical procedures

Dry matter (DM), ash, crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), nonstructural carbohydrate (NSC), neutral detergent-insoluble crude protein (NDIN) and lignin (L) determinations were performed according to methodology described by Silva and Queiroz (2009). Selenium was determined using the fluorimetric method as described by Olson et al. (1975), using the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) bovine liver standard (1577c) as a quality control.

The oxidative stability of lipids in meat samples was measured by the TBARS (substances reactive to 2-thiobarbituric acid) test, according to Vyncke (1975). Five grams of the samples were homogenized with 15 mL of 7.5% trichloroacetic acid (TCA) solution containing 0.1% EDTA (g/mL) and 0.1% propylgalate (g/mL), for 30 seconds at 25,000 rpm in homogenizer (IKA brand, model Turrax T18) and filtered with Whatman n.1 filter paper. The filtrate obtained was incubated 1:1 with 0.02 M thiobarbituric acid (TBA) in boiling water for 40 minutes. The absorbances of the tests were measured at wavelengths of 532 and 600 nm in a spectrophotometer (Multiskan FC, Thermo Scientific, USA). The absorbance of the sample was considered as the difference between the absorbance at 532 nm and the absorbance at 600 nm, which corrected possible turbidity of the sample. TBARS values were calculated from the standard curve with different dilutions of TEP (1,1,3,3 Tetraethoxypropane) between 0.02 and 1.1 µg/mL and expressed as mg malondialdehyde (MDA) per kg of sample.

III.2.4 Statistical analysis

A completely randomized design was used in a 2x4 factorial arrangement, with two selenium sources (organic or inorganic), four Se levels (0; 0.3; 0.9 and 2.7 mg/kg DM). Individual bull was considered an experiment unit. The statistical model used was:

$Y_{ijk}= \mu + S_i + L_j + SL_{ij} + \boldsymbol{\varepsilon}_{ijk}$

where: Y_{ijk} = observed dependent variable; μ= overall means; S_i = effect of sources (inorganic or organic); L_j = effect of levels (0; 0.3; 0.9 and 2.7 mg/kg DM);

SL_{ij} = interaction in selenium source and levels; $\boldsymbol{\varepsilon}_{ijk}$ = residual effect.

Performance, carcass traits and meat quality data were evaluated using the GLM procedure of SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). Sources, levels and sources × levels interaction were considered as fixed effects. Means were compared by PDIFF test, and differences were considered statistically significant when $P \leqslant 0.05$.

III.3. Results

There was no effect ($P>0.05$) of Se source, level or interactions on either BW, ADG or G:F (Table 2). The mean live weight of animals at the end of the feedlot was 572 ± 4.81 kg and the mean daily weight gain was 1.77 ± 0.06 kg/day, as expected with the diet formulation. The average dry matter intake during the experimental period was 11.35 ± 0.26 kg or 2.30 ± 0.04% LW and the feed efficiency (kg of weight gain/kg of dry matter intake) was 0.156 ± 0.003. There was no significant effect ($P>0.05$) of source and selenium level on the carcass characteristics evaluated (Table 3). The mean hot carcass weight (HCW) was

334 ± 3.3 kg; dressing of 58.4 ± 0.3%; drip loss of 3.7 ± 0.37%; pH of 6.47 ± 0.04 1h after slaughter; pH of 5.6 ± 0.04 24h after slaughter; ribeye area (REA) of 77.7 ± 1.6 cm^2; back fat thickness (BFT) of 6.2 ± 0.56 mm and marbling score of 460 ± 11.4. The meat quality characteristics evaluated were not influenced (P>0.05) by treatments (Table 4). There was a significant effect of the source x level interaction (P<0.0003) for Se concentration on muscle. Organic selenium, regardless of level, provided higher Se concentration in meat (*Longissinus dorsi* muscle) in relation to inorganic Se (Figure 1).

Tabela 2. Performance of feedlot Nellore cattle supplemented with different selenium sources and levels.

Item	Sources			Levels				
	I	O	SEM	0.0	0.3	0.9	2.7	SEM
Initial BW (kg)	412.2	412.9	3.146	416.9	408.1	409.7	415.6	4.448
Final BW (kg)	573.0	572.2	3.399	571.1	564.2	572.6	579.2	4.808
ADG (kg/day)	1.775	1.776	0.039	1.724	1.740	1.813	1.823	0.056
0-28 day	1.752	1.880	0.078	1.778	1.758	1.790	1.938	0.109
28-56 day	1.934	1.959	0.062	1.952	1.971	1.988	1.875	0.087
56-84 day	1.557	1.461	0.052	1.402	1.415	1.604	1.610	0.073
DMI (% BW)	2.287	2.323	0.032	2.232	2.317	2.318	2.353	0.045
DMI (kg/day)	11.26	11.45	0.184	11.03	11.27	11.39	11.71	0.261
0-28 day	10.43	10.68	0.228	10.29	10.46	10.44	11.03	0.322
28-56 day	11.28	11.44	0.214	11.16	11.38	11.33	11.57	0.303
56-84 day	12.12	12.28	0.172	11.69^{B}	12.02^{AB}	12.48^{A}	12.61^{A}	0.243
G:F	0.158	0.155	0.002	0.156	0.154	0.159	0.156	0.004
0-28 day	0.167	0.175	0.006	0.172	0.167	0.170	0.175	0.008
28-56 day	0.171	0.170	0.004	0.174	0.173	0.175	0.162	0.005
56-84 day	0.119	0.129	0.004	0.121	0.118	0.130	0.128	0.006

I= inorganic; O= organic; BW= body weight; ADG = average daily gain; DMI = dry matter intake; G:F = gain to feed ratio; SEM= standard error; n=63 animals.
Values in the row with different superscripts differ significantly at P<0.05.

Tabela 3. Carcass characteristics of Nellore bulls receiving different selenium sources and levels.

	Sources			Levels				
Item	I	O	SEM	0.0	0.3	0.9	2.7	SEM
HCW (kg)	332.14	335.88	2.173	335.24	328.17	332.79	339.85	3.027
Dressing (%)	58.13	58.70	0.196	58.69	58.19	58.12	58.65	0.278
pH 1h	6.46	6.48	0.031	6.41	6.51	6.43	6.54	0.044
pH 24h	5.64	5.60	0.028	5.65	5.61	5.60	5.61	0.039
REA (cm^2)	77.57	77.83	1.156	77.22	78.08	79.36	76.14	1.635
BFT (mm)	5.94	6.39	0.040	5.89	6.06	6.28	6.44	0.610
Marbling score[1]	452	467	8.016	467	468	457	446	11.364

I= inorganic; O= organic; HCW = hot carcass weight; REA = ribeye area; BFT= backfat thickness; SEM= standard error; n=63 animals; [1]100–199 = Practically Devoid. 200–299 = Traces. 300–399 = Slight. 400–499 = Small. 500–599 = Modest. 600–699 = Moderate.

Tabela 4. Meat quality of Nellore bulls receiving different selenium sources and levels.

	Sources			Levels				
Item	I	O	SEM	0.0	0.3	0.9	2.7	SEM
TBARs	0.147	0.133	0.006	0.148	0.150	0.129	0.133	0.008
Lightness (L*)	32.37	32.59	0.441	31.85	33.00	32.56	32.52	0.624
Redness (a*)	13.93	13.73	0.259	13.55	13.65	13.99	14.15	0.367
Yellowness (b*)	12.39	12.02	0.389	11.88	12.03	12.27	12.64	0.551
Shear force (N)	47.47	50.18	0.141	49.76	51.71	45.05	48.79	0.211
Cooking loss (%)	29.96	30.00	0.527	29.80	30.75	29.28	30.10	0.745
Drip loss (%)	3.445	4.001	0.266	3.530	3.921	3.801	3.655	0.377

I= inorganic; O= organic; TBARs = tiobarbituric acid reactive substances; SEM= standard error; n=63 animals.

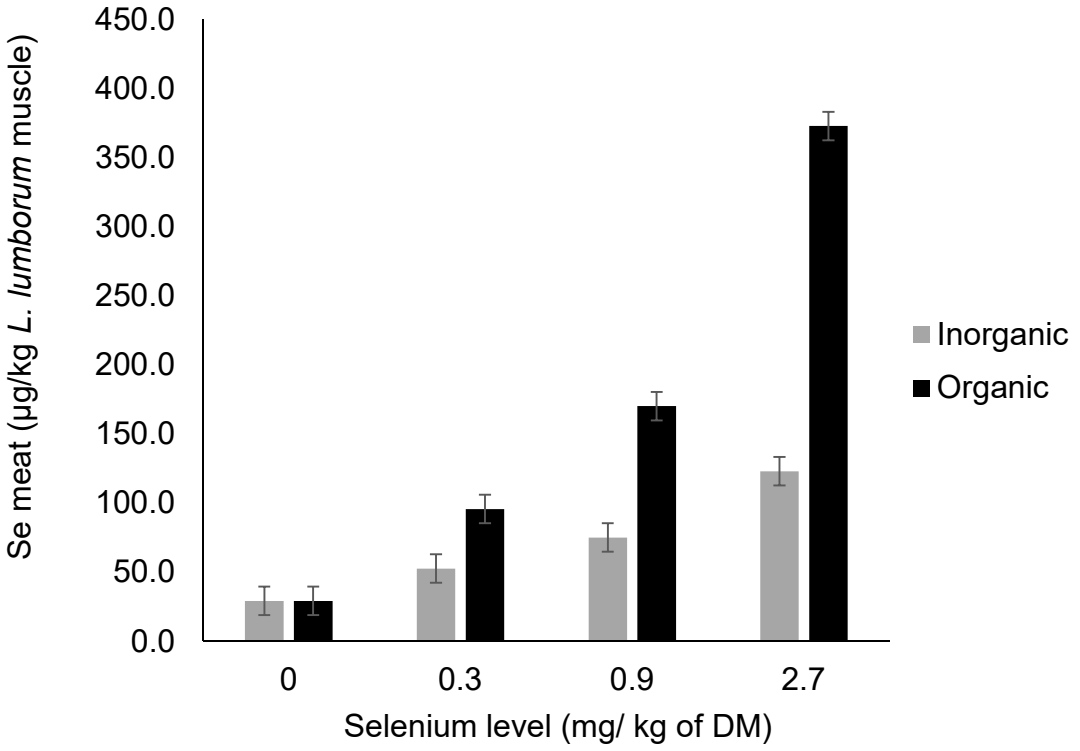

Figure 1. Selenium in meat (longissimus lumborum muscle) of Nellore supplemented with different selenium levels (0; 0.3, 0.9 and 2.7 mg/kg DM) and sources (organic or inorganic).

III.4. Discussion

III.4.1 Performance

The general performance was not influenced (P>0.05) by treatments, and previous studies have also reported lack of effect of supranutritional selenium supplementation on performance of feedlot cattle (Juniper et al., 2008b; Cozzi et al., 2011; Sgoifo Rossi et al., 2015; Muegge et al., 2017), calves (Skřivanová et al., 2007) and lambs (Vignola et al., 2009). At the first feedlot period, the ADG difference between Se levels was relatively large, but there was no significant difference due to the high standard error that is normal in an initial period of feedlot where the animals usually have compensatory gain, the same did not occur in subsequent periods. The present study confirms the hypothesis that the

supply of supranutritional selenium doses (approximately twenty-seven times greater than the National Research Council requirement (2000) does not compromise the performance of feedlot cattle. The results are consistent with studies that have also used selenium doses well above those recommended by the European Union and the United States of America (Lawler et al., 2004; Taylor, 2005; Juniper et al., 2008a).

At the end of the feedlot period, animals receiving 0.9 and 2.7 mg of Se/kg of DM had DMI 7.3% higher than the control treatment. Muegge et al. (2017) observed higher dry matter intake in steers receiving 3 mg/day of organic selenium in relation to inorganic selenium, but there was no significant difference in response compared to control treatment (0.1 mg/kg DM). In the present study, there was no difference in DMI between sources; and only the effect of Se level was observed. Additionally, although there was no significant difference, treatments with high selenium doses (0.9 and 2.7 mg Se/kg DM) resulted (P = 0.073) in daily gain 14.6% higher than the control treatment, a result that proves that the use of high selenium levels does not affect the performance of animals. Netto et al. (2014) observed higher DMI, ADG and G:F in Brangus cattle supplemented with 2.0 mg sodium selenite for a period of 101 days compared to the control group (0.06 mg Se). In the present study, the supplementation period was only 84 days and a trend (P=0.073) of better ADG was observed in treatments with high selenium doses (0.9 and 2.7 mg Se/kg DM). This result reinforces the possibility that the feedlot period may have been too short to significantly influence ADG (5% significance level). Other studies with shorter experimental periods such as those carried out by Juniper et al. (2008a), 75 days of feedlot and Taylor (2005), with 56 days of supplementation, also showed no

effect on the performance of animals that received high dietary selenium levels. Although there are many other studies on the effect of organic and inorganic selenium, the results are inconsistent and neither of them studied use different doses and sources together.

III.4.2 Carcass characteristics

Other studies also observed no effect of selenium supplementation on carcass characteristics. Crossbreed steers receiving supranutritional doses (> 2.8 mg Se/kg DM) of selenate or Se-methionine from wheat or hay did not show differences in HCW, REA, BFT or marbling score (Lawler et al., 2004). As with Belgian Blue bulls receiving selenium-enriched barley (1.73 mg/kg), no differences were observed in HCW, dressing, pH after 1 and 24 hours in relation to control treatment with 0.58 mg Se/kg of DM (Mehdi et al., 2015). The mean pH values (after 1 and 24 hours) were 6.5 and 5.6, respectively. These values are in agreement with the natural decrease that occurs due to the accumulation of lactic acid originated from the degradation of glycogen contained in muscles. As reported by Vignola et al. (2009), there was no significant influence on the pH of lambs supplemented with sodium selenite or yeast. Sgoifo Rossi et al. (2015) also found no effect of Se on the pH values of meat from Charolais heifers in the period of 1-8 days of storage. The lack of significant effect on carcass characteristics is consistent with the performance result and reinforces the observation that the supply of up to 2.7 mg of organic or inorganic Se for 84 days does not affect the development of Nellore cattle terminated in feedlot.

III.4.3 Meat quality

The degree of lipid oxidation of meat was measured by TBARS (thiobarbituric acid reactive substances), expressed in malondialdehyde content (MDA) per kg of meat, one of the major of the secondary oxidation products of polyunsaturated fatty acids. Malondialdehyde, as a bifunctional aldehyde, is highly reactive, and can interact through DNA and protein cross-links, promoting chromosomal aberrations and reducing protein synthesis capacity (Addis, 1986). Due to its antioxidant function, selenium is of great importance in the antioxidant defence system of cells, which can reduce lipid oxidation and avoid changes in colour, formation of toxic compounds and presence of undesirable aromas in meat.

In the present study, TBARS concentrations (mg MDA equivalent/kg of meat) in *Logissimus dorsi* muscle samples were not significantly ($P > 0.05$) influenced by Se supplementation. During handling, processing and cold storage of fresh meat, reactive oxygen species (ROS), such as free radicals and peroxides, are responsible for catalysing lipid oxidation (Descalzo et al., 2005). The results of this study refer to fresh meat (24 hours after slaughter), therefore, less exposed to oxidative processes. It could be observed that the mean TBARS value was 0.14 mg MDA/kg of meat, a result that is much lower than the maximum acceptance limit for the sensory perception of oxidation, which according to Campo et al. (2006) is 2 mg of MDA/kg of meat.

Other authors have also reported lack of effect of selenium supplementation on lipid oxidation of meat even under display-life exposure. Juniper et al. (2008b) observed no difference in TBARS values of *Longissimus dorsi* samples stored in modified atmosphere (75: 25, O2: CO2) for 10 days. Similarly, Skřivanová et al. (2007) using calves also reported lack of effect of

supplementation with 0.5 mg of selenium/kg of DM on the TBARS values of muscle stored for up to 6 days, when compared to control treatment. The authors pointed out that only the addition of Se in the diet may be insufficient to increase oxidative stability, and the combined treatment of addition of Se and vitamin E presented better results.

Additionally, Vignola et al. (2009) reported that *Longissimus dorsi* muscle samples from lambs supplemented with sodium selenite or selenium yeast (0.3 or 0.45 mg/kg DM) presented a difference ($P<0.05$) in TBARS values compared to control treatment, but only at 9 days after slaughter and when values were corrected for fat content (expressed as mg MDA per kg of fat). We believe that the inclusion of selenium in the diet can reduce lipid oxidation when meat is exposed to the adverse conditions that contribute to oxidation (high storage period, presence of light and oxygen, storage temperature, type of packaging and others).

The colour characteristics of meat were not altered by supplementation with different selenium levels and sources, a result that agrees with the lack of effect also in the pH value. Similar results were also reported with Se-enrichment diet on meat colour of bulls (Cozzi et al., 2011; Mehdi et al., 2015). Shear force ranged from 45 to 52 N and was not influenced ($P> 0.05$) by selenium levels and sources. This result is different from that reported by Cozzi et al. (2011) who observed improvement in meat tenderness (after 6 and 11 days of ageing under vacuum package) of Charolais bulls fed with selenium-enriched yeast in relation to animals receiving sodium selenite. However, Sgoifo Rossi et al. (2015) found only a trend ($P=0.076$) for better meat tenderness at 48 hours post-mortem with the use of selenium-enriched yeast instead of sodium selenite. Mehdi et al. (2015)

did not observe differences in the tenderness of meat stored for six days of Belgian Blues bulls receiving Se-enriched barley Se in relation to control animals. The results of the effect of selenium on meat tenderness are divergent, possibly due to differences in the content and source used, experiment duration, animals used and time after slaughter. Drip loss (3.4 to 4.0%) or cooking loss (29.28 to 30.75%) were not affected (P>0.05) by Se level and/or source. Cozzi et al. (2011) and Mehdi et al. (2015) also found no significant effects of Se supplementation on cooking loss. The meat quality results were not influenced by selenium supplementation.

III.4.4 Selenium in meat

The inclusion of 2.7 mg of organic Se/kg of DM in bovine diet resulted in 372.7 µg Se/kg in the *L. dorsi* muscle, providing 55.9 µg of Se in 150 g of beef, or approximately 100%, of Se intake recommendation (IR) that according to WHO and FAO, (2001) is 55 µg of Se/day for adults. While meat from animals supplemented only with 0.3 mg of organic Se/kg of DM resulted in an average of 95.4 µg Se/kg of meat, providing approximately 14.3 µg of Se in 150 g of meat (only 26.1% of Se IR). Meat is one of the main sources of selenium in the human diet. However, in order to ingest meat from animals receiving selenium content of up to 0.3 mg/kg DM, it would be necessary to add selenium-rich foods to obtain daily intake above at least 40 µg Se/day.

Values of 360 to 430 µg of selenium/kg of meat were observed in the region of Pará (Brazil), where soil selenium levels are higher and some foods are also rich in selenium, such as Brazil nuts (Lemire et al., 2010). While in other regions of Brazil, selenium levels in meat are low, Pereira et al. (2012) in a study with Nellore steers receiving sodium selenite or organic selenium, with level of

0.21 mg/kg DM, verified values of 120 to 150 µg Se/kg of meat (inorganic and organic selenium, respectively). Zanetti et al., (2015) observed values of 39 and 262 µg Se/kg of meat in animals without selenium supplementation and supplemented with 2.5 mg of organic Se/kg of DM, respectively. Additionally, a study carried out in several states in the south-eastern region of Brazil observed Se content in meat between 19 and 97 µg Se/kg (Ferreira et al., 2002). Results similar to those verified in the present study also confirmed the need to increase selenium supplementation in the diet of animals as a strategy to produce selenium-enriched meat.

Despite the concern with human exposure to excessive selenium levels in the diet, the consumption of meat enriched with Se using 2.7 mg Se/kg of ration would have to be higher than 1.0 kg/day to reach the maximum allowable limit of 400 µg Se/day (WHO and FAO, 2001). In addition, organic selenium is much less toxic than inorganic selenium. The use of organic selenium provides higher selenium concentrations in meat in relation to the inorganic source. Therefore, it is more efficient in the production of selenium-enriched meat, a strategy that must be economically evaluated and that may be necessary to increase the human intake of selenium, a mineral that has shown to be important for health but is deficient in several regions of the world.

Based on the results of this research that studied sources and levels at same time, different from the others research, it is possible to conclude that the use of supranutritional doses up to 2.7 mg Se/kg , organic or inorganic does not compromise performance, carcass characteristics or quality of Nellore bovine meat. Therefore, it could be a way to naturally produce selenium-enriched meat.

III. Acknowledgements

The authors would like to thank the São Paulo Research Foundation (FAPESP/Process 2015/07175-6), which provided financial support for this study, and the National Council of Scientific and Technological Development (CNPq/Brazil–Process 142484/2015–6) that granted scholarship to the first author.

III. Declaration of interest

No potential conflict of interest relevant to this article is reported.

III. Ethics statement

All procedures used in this experiment involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines, approved by the College of Animal Sciences and Food Engineering (FZEA) under protocol number 2790110815.

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CHAPTER IV - MEAT QUALITY AND OXIDATIVE STABILITY OF NELLORE BEEF CATTLE FED WITH DIFFERENT SELENIUM SOURCES AND LEVELS

Original manuscript submitted to Meat Science journal (MEATSCI_2018_743), in September 24th, 2018.

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IV- Abstract

The effects of selenium (Se) on meat quality and oxidative stability of lipids and proteins from Nellore beef cattle were evaluated using 63 non-castrated animals in a completely randomized design with two sources (sodium selenite and selenium-enriched yeast), four levels (0; 0.3; 0.9 and 2.7 mg Se/kg DM). Lipid oxidation was determined in meat samples exposed to vacuum packaging (VC) and retail display (RD) conditions using the TBARS (thiobarbituric acid reactive substances) test. Protein oxidation was determined in meat exposed to retail display using carbonyl test. There was no effect ($P>0.05$) of treatment on the vacuum packaging variables and meat quality. Se level reduced ($P<0.05$) lipid

and proteins oxidation (TBARS and carbonyl) compared to the control treatment on retail display storage. In conclusion, Se supplementation, regardless of source, improved the oxidative stability of lipids and proteins in samples exposed to retail display conditions, demonstrating the antioxidant effect of this mineral.

Keywords: antioxidant, *Bos indicus*, carbonyl, feedlot, minerals, TBARS

IV. 1 Introduction

Oxidative stability of meat is an important factor to keep nutritional quality, extend shelf life, reduces toxicity and increase the market value of meat and its products. Besides nutritional deterioration, the meat oxidation generates toxic compounds, which are deleterious to human health. Some of these oxidation products have been found to promote inflammatory conditions in the gut and are linked to start carcinogenic processes (Estévez & Luna, 2017). In Brazil, the major way used for sell meat is in expanded polystyrene (EPS) trays covered with plastic wrap (Possamai et al., 2018). During the storage and retail display, meat is sensitive to environmental factors like oxygen, light, handling conditions and oxidation process, that can cause modifications of muscle lipids and proteins then, affects the organoleptic and nutritional properties of meat and meat products (Insani et al., 2008).

In this context, antioxidant compounds can neutralize the oxidative responses, extending shelf life and improve meat quality, important measures for commercial industry and human health. Among of the antioxidants, Se is known as an integral part of the enzyme glutathione peroxidase (GSH-Px) that has a function to prevent oxidative damage to body tissues (catalyzes the reduction of

hydrogen peroxide and organic peroxides) (Skřivanová, Marounek, De Smet, & Raes, 2007).

Many studies that use of about 0.3 mg of Se/kg of DM have been shown no effect on meat oxidation in cattle (Juniper, Phipps, Ramos-Morales, & Bertin, 2008; O'Grady, Monahan, Fallon, & Allen, 2001), calves (Skřivanová et al., 2007) and lamb (Ripoll, Joy, & Muñoz, 2011; Vignola et al., 2009). In addition, among the Se sources, studies had shown higher bioavailability of organic Se in relation to inorganic (Cozzi et al., 2011; Sgoifo Rossi et al., 2015). However, neither of them investigated the best Se level and source at the same time. Besides that, there is little known about the effect of Se in protein oxidation during storage of bovine meat.

The objective of this study was to investigate whether different selenium sources and levels affect the quality and the oxidative stability (lipid and protein) of meat from Nellore beef cattle during storage (vacuum package and retail display).

IV. 2 Material and methods

All procedures used in this experiment involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines, approved by the College of Animal Sciences and Food Engineering (FZEA) under protocol number 2790110815.

IV. 2.1 Animal and feeding

In total, 63 Nellore (*Bos indicus*) bulls (412 ± 19 kg BW; 24 months old) were weighed, tagged and housed in a feedlot system with individual pens at the Department of Animal Sciences of the College of Animal Sciences and Food Engineering - University of Sao Paulo, Pirassununga, SP, Brazil. Initially, all animals were submitted to a 14-day adaptation period, when the concentrate levels were progressively increased. At the end of the adaptation period, animals (9/treatment) were submitted in a 2×4 factorial arrangement. Factors with two Se sources (inorganic: sodium selenite and organic: selenium-enriched yeast), four levels (0; 0.3; 0.9 and 2.7 mg/kg of DM). The selenium-enriched yeast used was Alkosel 3000 (Alkosel® 3000, Lallemand Animal Nutrition) with 3000 mg Se/kg of product. Diets were formulated according to recommendations of the National Research Council (2000) except to selenium level (control diet had 0.063 mg of Se/kg of DM). Animals were fed with total mixed common diet offered daily at 07.00 a.m. and 03.00 p.m, containing 70% concentrate and 30% roughage (Table 1), during 84 days. Every two days, leftovers were collected, weighed and sampled for dry matter determination. The feed supply was adjusted every two days based on the amount of leftovers from the previous days, to ensure 5% to 10% refusals.

Table 1. Dietary ingredients and chemical composition (DM basis) of the basal diet.

Items	Content
Ingredients (%)	
Corn silage	30.00
Corn grain	56.84
Soybean 45%	10.64
Urea	1.34
Mineral mixture[1]	1.18
Chemical composition (%)	
CP	17.00
RDP	10.34
TDN[2]	79.22
EE	2.88

CP = crude protein; RDP = rumen digestible protein; TDN = total digestible nutrient; EE = ether extract. [1] Trace mineral mixture content (per kg): zinc. 2230 mg; calcium. 180 g; sodium. 74 g; manganese. 470 mg; copper. 1550 mg; cobalt. 5 mg; iodine. 47 mg; monensin sodium. 2300 mg. [2] Calculated as described by Weiss, Conrad, & St. Pierre, (1992).
Selenium levels treatments: organic or inorganic selenium at the amount of 0.3, 0.9 and 2,7 mg/kg was added on basal diet.

IV. 2.2 Sample collection

At the end of feeding period, animals were slaughtered at slaughterhouse located at the University of Sao Paulo according to humane slaughter procedures as required by Brazilian law. During slaughter, samples of liver were collected (about 5 g, in triplicate) of each animal and immediately frozen in liquid N_2 for TBARS analysis.

Immediately after slaughter, the carcasses were stored at 0 - 2 ºC for *rigor mortis* development and resolution. They remained in cold storage for a 24 hour period. To control the quality of meat, the pH and the temperature were determined 1 and 24 hours *post mortem* in the *Longissimus lumborum* (LL) muscle between the 12th and the 13th right carcass ribs. The LL muscles were removed from the carcasses for aging period evaluations (vacuum packaging) and retail display analysis.

IV. 2.3 Storage

IV. 2.3.1 Vacuum packaging

Four meat samples (approximately 2.5 cm thick) of each animal were vacuum packed in high barrier flexible film (200mm x 310mm, code B530FZ; Cryovac) and stored in a refrigerator (2.0 ± 1.0°C) for 1, 14, 28 or 42 d. At the end of each period, it was measured the pH, color, cooking loss (CL), shear force (SF) and collected samples (about 5 g, in triplicate) for TBARS analysis.

IV. 2.3.2 Retail display

Three meat samples 24 hs *post-mortem* (RD 0) and 14 days (RD 14) of ageing of each animal were placed into identified expanded polystyrene trays, with a purge absorbent (SECA MEAT- PAD, Techno Paper - Brazil) and covered with polyvinyl chloride (PVC) film (thickness = 6.8 ± 0.6 mm; and oxygen permeability (PO_2) of 650 $cm^3m^{-2}h^{-1}$ at 23 ºC) and placed in a refrigerated display counter (model vega vertical, 125 LX; Auden) at 4°C for 6 days. On specific days (2, 4 and 6), samples of each animal placed in trays were removed from the refrigerated display counter and evaluated for pH, color, and then three subsamples (5 g each) were collected and frozen in liquid N_2 for TBARS and carbonyl analysis.

IV. 2.4 Analytical procedures

IV. 2.4.1 pH measurement

Temperature and pH measurements were performed between the 12^{th} and 13^{th} ribs using digital pH meter (Hanna Instruments model HI99163; Hanna

Instruments, São Paulo, Brazil) 1 and 24 h after slaughter. The pH also was measured before vacuum package analysis (1, 14, 28 and 42 d).

IV. 2.4.2 Color

For color analysis beef samples were exposed for 30 min (Trater & Hunt, 2003) to air and then the color was measured with a portable colorimeter (mod. CM-2500D, Konica Minolta Sensing, Inc, Tokyo, Japan), using the L*, a*, b*, a CIE Lab system. The measurements were taken in three different positions. The colorimeter was calibrated according to the manufacturer's recommendations, operating with the following specifications: optical geometry 45/0, 30 mm opening diameter, observation angle of 10° and illuminant D65.

IV. 2.4.3 Tenderness and cooking loss

Tenderness and cooking loss were determined according to American Meat Science Association recommendations (AMSA, 2015). Cooking loss was expressed as the weight change percentage of the sample before and after cooking. The tenderness was determined from six cylinders (1.27 cm of diameter) with the fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade, using a texture analyser (TMS-Pro model, Food Technology Corporation) equipped with a Warner-Bratzler blade and test speed of 200 mm/min. The shear force of each sample was considered as the average value of the six cylinders.

IV. 2.5 Oxidative stability measurements

IV. 2.5.1 Lipids

The lipid oxidation was measured according to the method of Vyncke (1975) and expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde (MDA) kg^{-1} meat.

IV. 2.5.1 Proteins

The protein oxidation was determined by the carbonyl protein assay according to Oliver, Ahn, Moerman, Goldstein, & Stadtman (1987), modified by Vuorela et al. (2005).

IV. 2.6 Statistical analysis

A completely randomized design was used in a 2x4 factorial arrangement, with two Se sources (organic or inorganic), four Se levels (0; 0.3; 0.9 and 2.7 mg/kg DM). Individual bull was considered an experiment unit (n= 9 animals/treatment).

The pH, color variables (L*, a*, b*), cooking loss, tenderness, TBARS and carbonyl were evaluated by SAS (Statistical Analysis System, version 9.3) using the MIXED procedure with different times of vacuum ageing (1, 14, 28 and 48 days) or retail display (2, 4 and 6 days). Sources, levels, time, and doubles and triples interactions were considered as fixed effects. Means were compared by PDIFF test, and differences were considered statistically significant when $P \leqslant 0.05$. Pearson correlation was applied between lipid and protein oxidation.

IV. 3 Results

IV. 3.1 Vacuum packaging

As show in Table 2, Se source and levels not influenced ($P>0.05$) quality characteristics and oxidative stability from Nellore meat samples, while the aging time affected these characteristics ($P<0.05$; Table 3). Muscles pH ranged between 5.40 - 5.53, ‘L*’ values 32.48 – 38.92, ‘a*’ values 13.83 – 15.44, ‘b*’

values 12.21 – 16.73. As expected, the shear force reduced according to the ageing days (P<0.05), while cooking loss and TBARS increased as long of ageing.

Table 2. pH, color (L*, a*, b*), cooking loss, shear force and TBARS according to selenium source and level, of meat samples exposed to vacuum packaging conditions (means values and standard errors).

Item	Sources		SEM	Levels				SEM
	I	O		0.0	0.3	0.9	2.7	
pH	5.467	5.478	0.008	5.464	5.467	5.481	5.477	0.011
L*	36.80	36.52	0.206	36.48	36.54	36.82	36.81	0.288
a*	14.86	15.12	0.130	14.83	15.15	15.15	14.84	0.185
b*	14.67	14.46	0.174	14.46	14.80	14.64	14.37	0.247
CL (%)	29.69	29.73	0.267	30.07	30.01	29.46	29.31	0.377
SF (N)	38.21	39.76	0.613	40.19	39.92	38.14	37.70	0.860
TBARS	0.257	0.235	0.008	0.263	0.249	0.236	0.236	0.012

I= inorganic; O= organic; SEM: standard error, L*: lightness, a*: redness, b*: yellowness, CL: cooking loss, SF: shear force, TBARS: thiobarbituric acid reactive substances, n= 63 animals.

Table 3. pH, color (L*, a*, b*), shear force, cooking loss and TBARS in *L. lumborum* of Nellore cattle, according to time storage in vacuum packaging (means values and standard errors).

Item	Time				SEM	p-valor	Effect
	1	14	28	42			
pH	5.53	5.49	5.40	5.46	0.01	<0.0001	Q
L*	32.48	37.31	38.10	38.92	0.30	<0.0001	Q
a*	13.83	15.36	15.34	15.44	0.18	<0.0001	Q
b*	12.21	14.29	15.04	16.73	0.20	<0.0001	L
SF (N)	48.1	38.9	33.2	35.2	0.08	<0.0001	Q
CL (%)	29.61	28.46	29.28	30.62	0.42	0.0018	Q
TBARS	0.140	0.147	0.363	0.333	0.01	<0.0001	L

SEM: standard error, L*: lightness, a*: redness, b*: yellowness, CL: cooking loss, SF: shear force, TBARS: thiobarbituric acid reactive substances, n= 63 animals.
L: effect linear; Q: effect quadratic.

IV. 3.2 Retail display

The significance values (P) for effect of Se sources, levels, time, and double and triple interactions of meat samples exposed to retail display (RD 0 and RD 14) conditions are shown in Table 4. Se sources and levels had no effect (P>0.05) in the meat color exposed to retail display (RD0 and RD14). However, regardless of source, there was effect of Se level (P<0.05) in lipid and proteins oxidation (TBARS and carbonyl). Besides that, all characteristics were affected by retail display time.

Table 4. Significance values (P) for source, level, time, and double and triple interactions of meat samples exposed to retail display (RD 0 and RD 14) conditions.

Storage	Item	Source	Level	Time	S*L	S*T	L*T	S*L*T
RD 0	L*	ns	ns	<.0001	ns	ns	ns	ns
	a*	ns	ns	<.0001	ns	ns	ns	ns
	b*	ns	ns	<.0001	ns	ns	ns	ns
	TBARS	ns	0.0073	<.0001	ns	ns	ns	ns
	Carbonyl	ns	0.0092	<.0001	ns	ns	ns	ns
RD 14	L*	0.0974	ns	<.0001	ns	ns	ns	ns
	a*	ns	ns	<.0001	ns	ns	ns	ns
	b*	ns	ns	<.0001	ns	ns	ns	ns
	TBARS	ns	0.0169	<.0001	ns	ns	ns	ns

L*: lightness, a*: redness, b*: yellowness, TBARS: thiobarbituric acid reactive substances.
S*L= sources x levels interactions; S*T= sources x time interactions; L*T= levels x time interactions; S*L*T= sources x levels x time interactions, n=63 animals.

Se level reduced (P<0.05, Table 5) lipid and proteins oxidation (TBARS and carbonyl) compared to the control treatment, regardless of source. TBARS values increased with time of storage in retail display (P<0.01; Figure 1). All color variables (L*, a*, b*) showed linear or quadratic associations with time of storage (P<0.01, Figure 2) in samples exposed to retail display (RD0 and RD14).

Table 5. Color variables (L*, a*, b*) and TBARS according to selenium source and level, of meat samples exposed to retail display conditions (means values and standard errors).

	Item	Sources		SEM	Level				SEM
		I	O		0	0.3	0.9	2.7	
RD 0	L*	33.74	33.89	0.293	33.91	33.82	33.57	33.94	0.416
	a*	13.21	13.27	0.168	13.05	13.36	13.34	13.19	0.237
	b*	13.08	13.04	0.249	13.09	13.14	13.00	13.03	0.348
	TBARS	0.498	0.471	0.012	0.533^{a}	0.474^{b}	0.484^{b}	0.447^{b}	0.017
	Carbonyl	0.980	0.960	0.020	1.058^{a}	0.943^{b}	0.928^{b}	0.949^{b}	0.029
RD 14	L*	36.46	35.80	0.24	36.13	35.99	36.18	36.21	0.342
	a*	12.08	11.78	0.196	11.86	12.01	12.31	11.55	0.277
	b*	11.53	11.19	0.234	11.40	11.21	11.66	11.18	0.330
	TBARS	0.448	0.447	0.020	0.49^{a}	0.45^{ab}	0.43^{b}	0.42^{b}	0.017

I= inorganic; O= organic; S= sources; L= levels; S*L= sources x levels interactions; SEM: standard error, L*: lightness, a*: redness, b*: yellowness, TBARS: thiobarbituric acid reactive substances; RD: retail display (0 and 14 days of ageing), n= 63 animals.

^{a,b} Means in the same row with a different letter differ significantly at P<0.05.

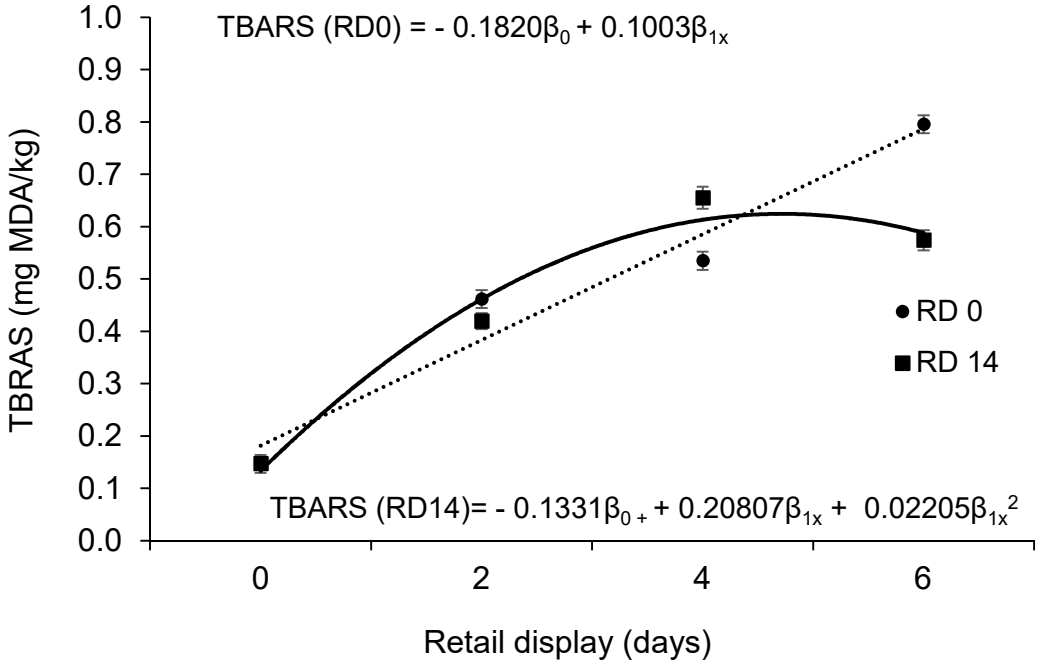
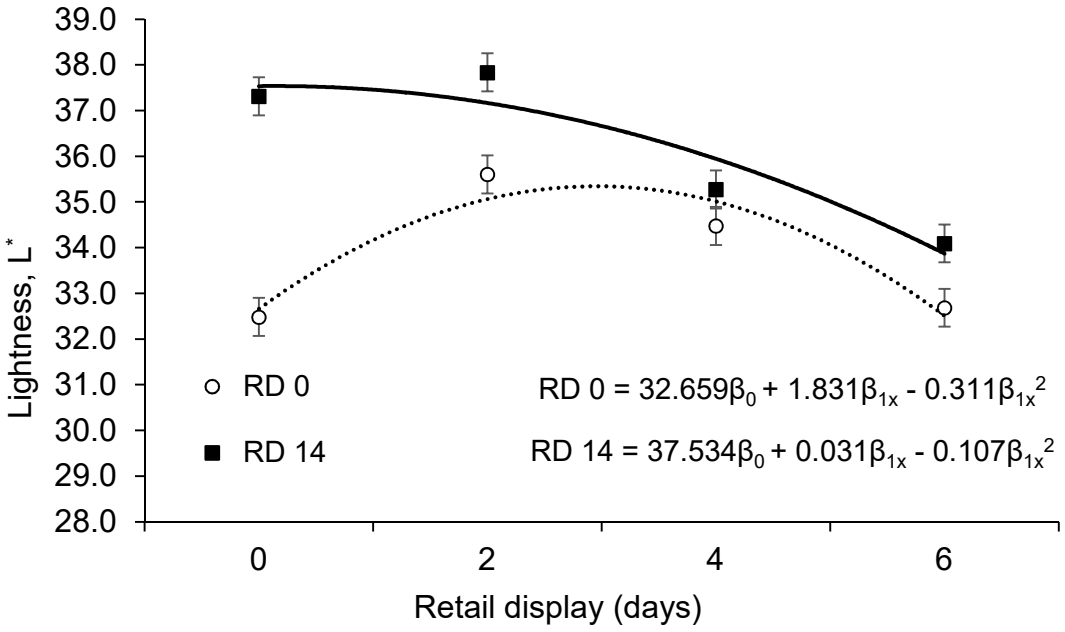
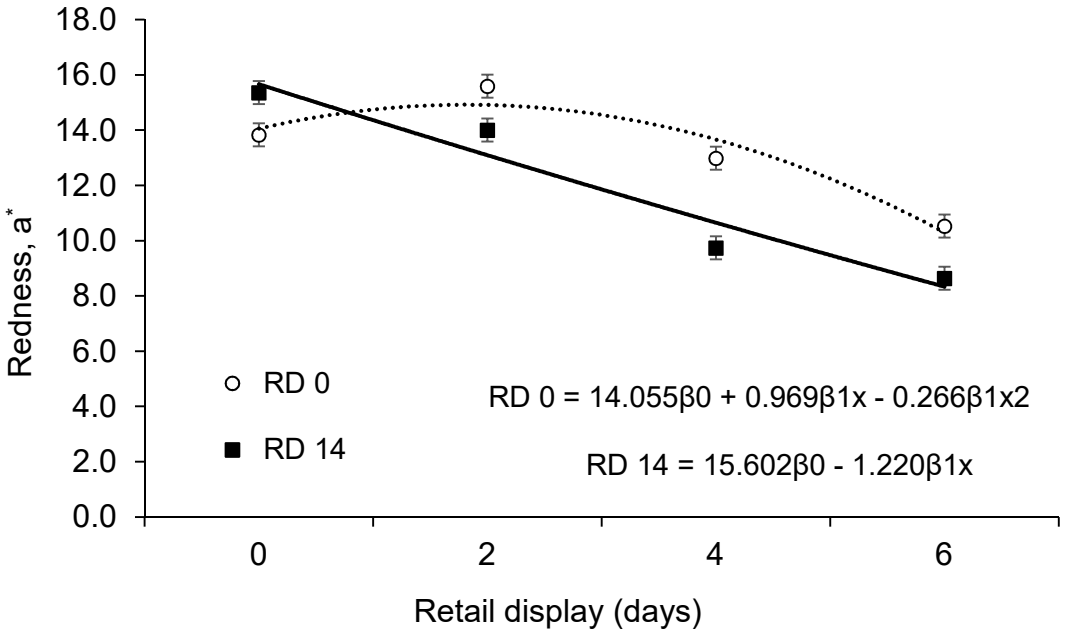


Figure 1. Means of thiobarbituric acid-reactive substance (TBARS) concentration in meat samples, as a function of time of retail display (RD, 0 and 14 days of ageing).


39.0
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
Lightness, L*
RD 0
RD 14
RD 0 = 32.659β0 + 1.831β1x - 0.311β1x2
RD 14 = 37.534β0 + 0.031β1x - 0.107β1x2
0
2
4
6
Retail display (days)


18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Redness, a*
RD 0
RD 14
RD 0 = 14.055β0 + 0.969β1x - 0.266β1x2
RD 14 = 15.602β0 - 1.220β1x
0
2
4
6
Retail display (days)

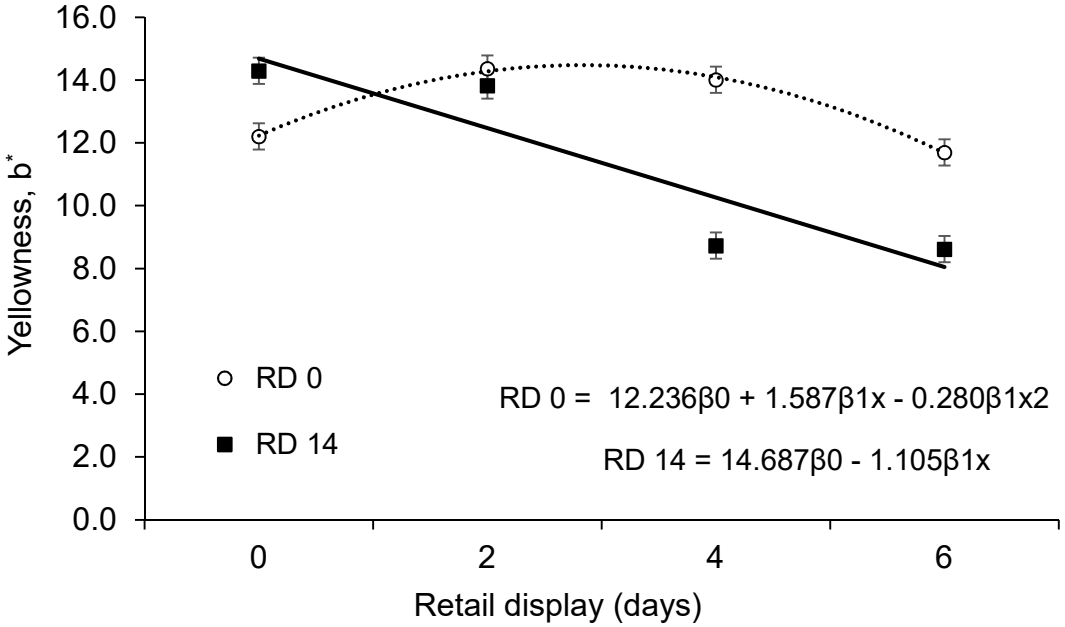


Figure 2. Least square means (±SEM) of L*, a*, b* in Nellore cattle meat samples exposed to retail display (RD0 and RD14) conditions.

Lipid and protein oxidation correlation was significant (P<0.0001, Figure 3) in meat exposed to retail display (RD0) at 0 and 6 days.

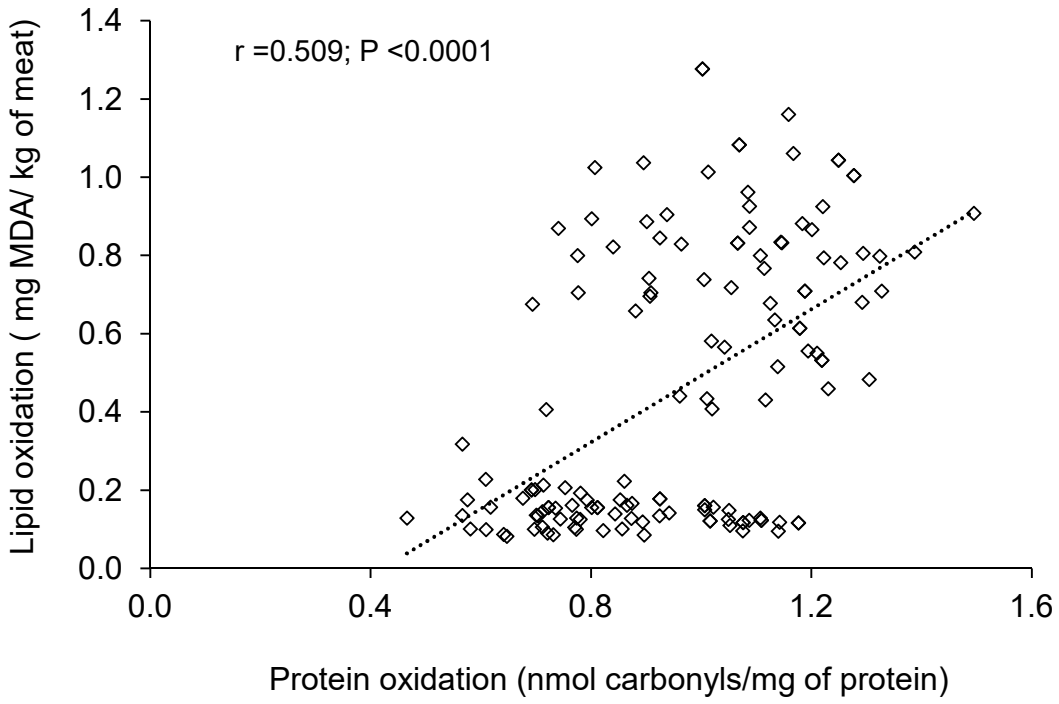


Figure 3. Pearson correlation between protein (carbonyls) and lipid oxidation (TBARS) in Nellore cattle meat samples at 0 and 6 days of retail display (RD0) conditions.

IV. 4 Discussion

IV. 4.1 Vacuum packaging

In general, pH values were normal (pH= 5.47) and not affected the other meat quality characteristics. The absence of the effect of Se source and level on pH is consistent with other studies in finishing cattle (O'Grady et al., 2001; Sgoifo Rossi et al., 2015). In contrast, Cozzi et al. (2011), reported a higher pH in meat of animals fed with organic Se (pH= 5.60) compared with that fed with sodium selenite or switched (pH= 5.51) from it to Se yeast in the last 70 days, but this difference was observed just with 6 days of ageing under vacuum package and at 11 days there was no difference detected.

Color (L*, a*, b*), the most important parameter for evaluating meat quality, during of vacuum packing storage were 35.54; 13.9; 12.99, respectively. Values within the range ideal for beef according to Muchenje et al. (2009). However, different results were observed by Cozzi et al. (2011), who reported higher lightness (L*) in meat bulls fed organic Se than that NaSe, after 6 and 11 days of vacuum packaged ageing. The temperature and amount of time of the ribeye during blooming may affect the rate of bright red color development (Trater & Hunt, 2003). The different results can be because of the different time of exposure to air between the experiments.

The tenderness, characteristic attributed to a person's perception of meat, such as softness to tongue, resistance to tooth pressure and adhesion (Muchenje et al., 2009), varied few from 37.5 to 40.2 N. Several of inherent and environmental factors can alter the meat tenderness and other meat quality characteristics (e.g. breed, *ante-mortem* stress conditions, slaughter processes and chilling conditions, age, sex, muscle type, marbling, nutrition) (Frylinck, O'Neil, du Toit, Strydom, & Webb, 2015). Regardless of the Se treatment, the prolonged ageing time of meat samples in vacuum package leading to a decreased shear force. In general, higher oxidation has been reported to a decrease in beef tenderness due to lower protease activity and inducing myofibrillar protein crosslinking (Lund, Heinonen, Baron, & Estévez, 2011). In this study, it was not observed this effect but there was a tendency (P=0.09) to improve meat tenderness according to Se level. Sgoifo Rossi et al. (2015) also reported a tendency to reduce shear force (P=0.076) in Charolaise beef heifers meat with organic Se supplementation. Lund, Heinonen, Baron, & Estévez (2011) suggest that the oxidative conditions inducing the formation of disulfide cross-link

proteins that can influence meat tenderness. However, further investigations are necessary to clarify the role of Se in this process.

Lipid oxidation (TBARS, mg of malondialdehyde/kg of meat) in vacuum packing was not influenced by Se source and level. TBARS concentration increased by 128% at 42 days of aged (0.334) in relation to the value of 1 day (0.147). In agreement, Cozzi et al. (2011) and O'Grady, Monahan, Fallon, & Allen (2001) did not observe Se effect on lipid oxidation in vacuum packaging conditions. The vacuum package conditions did not allow us to verify whether Se was capable of reducing lipid peroxidation possibly due to the low concentration of oxygen.

IV. 4.2 Retail display

Neither level of Se supplementation or source influenced color (L*, a*, b*) data in the current study. In line with these results, Mehdi et al. (2015) reported that Se enrichment in cereal not influenced (P>0.05) the color of Belgian Blue meat under retail display conditions. Similarly, Vignola et al. (2009) reported no effect of Se source in lamb meat color during 9 days of retail display and Skřivanová, Marounek, De Smet, & Raes (2007) adding Se-enriched yeast in the diet of calves, also not observed influence in meat color. Taylor et al., (2008) reported that beef steers supplemented with supranutritional Se (± 2.9 mg/kg of DM) did not have different attributes (color and drip loss) to those from unsupplemented (± 0.4 mg/kg of DM) animals despite having greater Se contents in muscle. In contrast, Sgoifo Rossi et al. (2015) reported improvement lightness in Charolaise meat supplemented with organic Se compared with the sodium selenite group during 8 days of storage.

TBARS values (mg malondialdehyde/kg meat) increased with time of storage of retail display (RD0), after two days (d 2, TBARS= 0.46), the concentration increase more than three times in relation to fresh meat (d 0, TBARS= 0.15). However, the major values (0.79) observed in this experiment did not reach the limit for the acceptability of oxidized meat, that is 2 mg of MDA/kg proposed by Campo et al. (2006). Juniper, Phipps, Ramos-Morales, & Bertin (2008) observed no significant ($P>0.05$) difference in meat TBARS concentrations keeping until 10 d *postmortem*, with increasing Se supplementation (from 0.16 to 0.5 mg/kg, sodium selenite or Se yeast) in diets of beef cattle. Similarly, Skřivanová, Marounek, De Smet, & Raes (2007) reported none effect of Se-yeast (0.50 mg/kg feed) on the oxidative stability (TBARS) of *Longissimus thoracis et lumborum* of calves in the relation to control. In contrary, the present experiment observed, during of retail display (RD0 and RD14) period, about 11.5% lower TBARS concentration in treatments receiving Se, regardless of source, than the control treatment. That fact, confirms the selenium antioxidant function to prevent oxidative damage to body tissues, by reducing hydrogen peroxide and organic peroxides (Arthur, 2001).

In addition to lipid oxidation, the degree of protein oxidation also is considered a major cause of quality meat deterioration (Lund, Heinonen, Baron, & Estévez, 2011). In despite that in the last year, there was an increase in studies devoted to protein oxidation, although still are few in relation to lipid oxidation. The reasons may be due the high complexity of the chemistry behind the oxidation of food proteins, the lack of specific methodologies for assessing P-OX in food systems and the belief that other biochemical phenomena such as lipid

oxidation or microbial spoilage explained all deleterious changes occurred in food system (Estévez, 2011).

In the present study, there was effect of Se level ($P<0.05$) on protein oxidation (carbonyls, nmol/mg of protein) in *L. lumborum* samples of Nellore cattle exposed to retail display (RD 0). Se supplementation, regardless of source, reduced about 11% carbonyls values (0.94 nmol/mg of protein) in relation to the control treatment (1.06 nmol/mg of protein). In the same line, study about influence of feeding system on lipids and proteins oxidation, and antioxidant enzymes activities of meat from Aberdeen Angus steers, it was observed bigger GPx activity and lower amount of carbonyl in the feedlot system, what could be due to high Se level in grain and concentrate in comparison with pasture (Terevinto, Cabrera, & Saadoun, 2015). To our knowledge, there is no literature available on the effect of Se supplementation on carbonyl concentrations in beef cattle meat.

Protein oxidation may be induced due to some hydroperoxides or other oxidative compounds, according to Souza et al. (2013) it is possible that lipids oxidation being the first to occur and later protein oxidation possibly by the gradual formation of the ROS from the lipid oxidation. Then, the present work studied lipid and protein oxidation correlation and observed that there was a significant correlation ($P=0.0001$) but not high ($r=0.509$). Results are in agreement with Insani et al. (2008) who found a poor correlation between protein and lipid oxidation. This result stands out the importance to analyze protein oxidation in meat regardless of lipid oxidation.

IV. 5 Conclusion

This study showed the Se supplementation, regardless of source, were capable of inhibiting (lipid and protein) undesirable reactions throughout the storage time of meat from Nellore cattle in retail display condition, without negative effect on the others meat quality characteristics.

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CHAPTER V - SELENIUM SOURCES AND LEVELS ON CHOLESTEROL METABOLISM IN NELLORE CATTLE

V. Abstract

The effects of different selenium sources and levels on cholesterol metabolism in Nellore beef cattle were evaluated using 63 non-castrated animals, in a completely randomized design with two sources (sodium selenite and selenium-enriched yeast), four levels (0; 0.3; 0.9 and 2.7 mg Se/kg DM) for 84 days. Glutathione peroxidase (GPx), reduced glutathione (GSH), oxidized glutathione (GSSG) and Se were determined in liver at end of the experiment. Total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) and Se were determined in serum at different times (0, 28, 56 and 84 days). Also was determined GPx activity and cholesterol in muscle, and HMGCR in serum. There was a significant effect of source and level interaction ($P < 0.0001$) for GPx in liver. GSSG and Se increased linearly according to Se level. The GPx activity in the meat of animals fed with 2.7 mg Se/kg of DM was 288% higher than control treatment. The cholesterol in the meat of animals selenium supplemented was 10.2% lower than control treatment ($P<0.05$). Selenium serum increased with the supplementation and time, and there was a significant triple interaction between source x level x time ($P < 0.05$). HMGCR concentration in animals supplemented with Se, regardless of source, was 32.7% lower in relation to control treatment ($P<0.0003$). In conclusion, Se supplementation, regardless of source, reduced cholesterol in serum and meat due reduced in HMG-CoA reductase so improving the meat quality.

Keywords: antioxidant, feedlot, HMG-CoA reductase, meat, Se-enriched

V.1 Introduction

Lifestyle changes have brought modern man the challenge of healthy eating. Among healthy disorders, cardiovascular diseases (CVDs) remains a major cause of morbidity and mortality worldwide (ROTH et al., 2017). Several studies have been tried to understand how each food affect the developing of cardiovascular disease risk. Among the foods, the red meat consumption has been related to increased cardiovascular risk primarily by causing dyslipidemia. However, red meat consumption is important to contribute with several important nutrients to the diet, such as minerals (including iron and zinc), vitamins (including D, E and B12) and essential amino acids.

Recent findings demonstrated that red meat does not significantly increase cardiovascular risk when it is assumed in recommended doses, in fact, the major issues are the amount of visible fat and preservative in red meat (BRONZATO; DURANTE, 2017). Besides that, studies in meat production are being developed with the focus shifting from on quantity to quality. One of the possible strategies is into animal nutrition area by incorporating healthy ingredients in animal diets. At this point, the mineral selenium is known by a vital role in many of biochemical and physiological processes, including antioxidant defense systems, immune function, and thyroid hormone metabolism (HATFIELD et al., 2014).

Selenium is an essential micronutrient for animals and humans and selenium deficiency is a worldwide problem (RAYMAN, 2012; ZHANG et al., 2010). In many regions of Brazil the selenium contents in soil, plants, and animals are usually low, in this way it was found low selenium level in the Brazilian population, excluding that from the north (MAIHARA et al., 2004). Several reports in humans health indicated that food sources containing high amounts of

selenium (naturally or artificially) improve lipid profiles, with significant reductions in total cholesterol (CARVALHO et al., 2015; COMINETTI et al., 2012; MARANHÃO et al., 2011; RAYMAN et al., 2011). On the other hand, Zhang et al., (2016) observed that results with selenium supplementation like role in cardiovascular disease (CVD) have been inconsistent and controversial, occurring a significant benefit of CVD only within selenium range of 55–145 μg/L of blood. One of the potential effects of selenium is supported by the ability of selenoproteins, such as glutathione peroxidase and selenoprotein S, to combat the oxidative modification of lipids, inhibit platelet aggregation, and reduce inflammation (BLANKENBERG et al., 2003).

In an earlier study of our team, we observed an increased GSSG/GSH ratio and a reduction in cholesterol concentration in Brangus bovine muscle, supplemented with 2 mg sodium selenite/kg DM (NETTO et al., 2014). However, selenium supplementation can be done in main forms: inorganic mineral, including sodium selenite or selenate (inorganic) and organic forms such as Se-enriched yeast. Although organic forms have shown higher bioavailability than inorganic for cattle (COZZI et al., 2011; SGOIFO ROSSI et al., 2015). As the selenium effect on cholesterol was studied with a single source, it is important to study the main sources together and to identify the selenium effect in cholesterol biosynthesis.

Despite many studies related the effect of selenium on the cholesterol metabolism, to our knowledge, there are no studies evaluating the effect of selenium supplementation on the concentration of HMG-CoA reductase enzyme (HMGCR) in beef cattle. Nassir et al. (1997) found an increase in HMG-CoA reductase activity in the liver of Se-deficient rats with high cholesterol levels.

Dhingra & Bansal (2006) observed that selenium supplementation reduce the gene expression of HMG-CoA reductase in rats. Therefore, understand the selenium effect on cholesterol metabolism in cattle and determining the optimal selenium source and level in cattle diet can be an important strategy to attend the demands of the consumer market to producing meat with better quality and Se-enriched. Thus, the aim of this study was to evaluate the effects of different selenium sources and levels on cholesterol metabolism in Nellore cattle.

V. 2 Material and methods

All procedures used in this experiment involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines, approved by the College of Animal Sciences and Food Engineering (FZEA) under protocol number 2790110815.

V. 2.1 Animals and feeding

In total, 63 Nellore (*Bos indicus*) bulls (412 ± 19 kg BW; 24 months old) were weighed, tagged and housed in a feedlot system with individual pens, at the Department of Animal Sciences of the College of Animal Sciences and Food Engineering - University of Sao Paulo, Pirassununga, SP, Brazil. Initially, all animals were submitted to a 14-day adaptation period, when the concentrate levels were progressively increased. At the end of the adaptation period, animals (9/treatment) were submitted in a 2×4 factorial arrangement. Factors with two Se sources (inorganic: sodium selenite and organic: selenium-enriched yeast) and four levels (0; 0.3; 0.9 and 2.7 mg/kg of DM). The selenium-enriched yeast used was Alkosel 3000 (Alkosel® 3000, Lallemand Animal Nutrition) with 3000 mg

Se/kg of product. Diets were formulated according to recommendations of National Research Council (2000). Animals were fed with total mixed common diet offered daily at 07.00 a.m. and 03.00 p.m, containing 70% concentrate and 30% roughage (Table 1), during 84 days. Every two days, leftovers were collected, weighed and sampled for dry matter determination. The feed supply was adjusted every two days based on the amount of leftovers from the previous days, to ensure 5% to 10% refusals.

Table 1. Dietary ingredients and chemical composition (DM basis) of the basal diet.

Items	Content
Ingredients (%)	
Corn silage	30.00
Corn grain	56.84
Soybean 45%	10.64
Urea	1.34
Mineral mixture[1]	1.18
Chemical composition (%)	
CP	17.00
RDP	10.34
TDN[2]	79.22
EE	2.88

RDP = rumen digestible protein; TDN = total digestible nutrient. [1] Trace mineral mixture content (per kg): zinc. 2230 mg; calcium. 180 g; sodium. 74 g; manganese. 470 mg; copper. 1550 mg; cobalt. 5 mg; iodine. 47 mg; monensin sodium. 2300 mg. [2] Calculated as described by Weiss; Conrad and Pierre (1992).
Selenium treatments: organic or inorganic selenium at the amount of 0.3, 0.9 and 2,7 mg/kg was added on basal diet.

V. 2.2 Sample collection

Blood samples were collected from each bulls before morning feeding via jugular venipuncture into vacutainer tubes at 0, 28, 56, 84 days of experiment,

then centrifuged at 1100xg for 10 min at 4ºC to obtain serum immediately frozen for total cholesterol (TC), LDL and HDL cholesterol, triglycerides and Se analysis. At the end of feeding period, animals were slaughtered at slaughterhouse located at the University of Sao Paulo according to humane slaughter procedures as required by Brazilian law. During slaughter, samples of *Longissimus lumborum* (LL) muscle were collected (in triplicate) and immediately frozen in liquid N_2 for glutathione peroxidase (GPx) analysis. Liver samples also collected (in triplicate) for oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione peroxidase (GPx) and Se analysis.

The carcasses were stored at 0 - 2 ºC for *rigor mortis* development and resolution and remained in cold storage for a 24 hour period. After this period *Longissimus lumborum* (LL) muscle samples of right carcass (between 12ª and 13ª ribs) were collected (in triplicate) for cholesterol analysis.

V. 2.3 Analytical procedures

The evaluation of the GSH and determination of the GSH / GSSG relation was performed through the methodology described by Tietze (1969). Total glutathione was measured after tissue homogenization in 5% sulfosalicylic acid by the recycle method in the presence of dithionitrobenzoic acid (DTNB), NADPH and glutathione reductase (GR). Thionitrobenzoic acid (TNB) formation, which is a colored product absorbed at 412 nm, it is proportional to the amount of GSH (and GSSG) of the samples and monitored by spectrophotometer (model DU800, Beckman Coulter brand) in kinetics for 3 minutes.

GSSG was determined by the direct derivatization of GSH by homogenizing another sample of the same tissue in the presence of N-ethylmaleimide (NEM 12.5nM), followed by alkaline hydrolysis (pH=11) using

KOH and waiting five minutes. The pH was then corrected to 7 using HCl and the samples were centrifuged at 13000 rpm for 15 minutes and absorbance read at 412 nm for 3 minutes at 25°C in a spectrophotometer (model DU800, Beckman Coulter brand).

Glutathione peroxidase (GPx) activity in liver or muscle were determined using the spectrophotometric method based on a decline in the concentration of NADPH at 340 nm per 3 minutes at 37ºC, according to Paglia & Valentine (1967). The protein of samples was determined and GPx was expressed in nmol/min/mg protein.

Selenium was determined using the fluorimetric method as described by Olson, Palmer, & Cary (1975), using the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) bovine liver standard (1577c) as a quality control.

Cholesterol analyses in muscle were performed using the enzymatic method according to Saldanha, Mazalli, & Bragagnolo (2004). The calibration curve was built based on a standard cholesterol solution (1.006 mg/ 100 mL), with concentrations ranging between 0.01 and 0.05 mg/mL. The absorbance was read and compared to a blank sample at 499 nm.

The HMG-CoA Reductase (HMGCR) was determined with ELISA Kit (MyBioSource, Inc., San Diego, CA, USA) employs Double Antibody Sandwich Technique. This assay is based on characteristics of the tested antigen with more than two valances, which can identify coated antibody and detection antibody at same time. Assay procedure followed standard protocol of MyBiosource assays. Each well of the microtiter plate is precoated with specific antibodies. The curve points, standards, and serum samples (100 µL) were added to each well, sealed

with adhesive tapes and incubated at 37°C for 90 min. Uncombined antibodies and impurities were washed (with 350 uL of washing buffer per well) for twice in an automatic plate-washing machine (Wellwash 4MK, Thermo Scientific, USA). Biotinylated Bovine HMGCR antibody liquid (100 µL) was added to each well (except blank) for to binds to the captured antigen, sealed with adhesive tapes and incubated at 37°C for 60 min. ELISA plate was washed three times for to remove the unbound detection antibody. Enzyme-conjugate liquid (100 µL) was added to each well (except blank), sealed with adhesive tapes and incubated at 37°C for 30 min. ELISA plate was washed five times. Color Reagent liquid (100 µL) was added to each well and incubated in dark at 37°C for 30 min. Color Reagent C (100 µL) was added to each well and read OD (450 nm) within 10 min in an Microplate Reader (Multiskan FC, Thermo Scientific, USA). For to determine the HMGCR antigen concentration (ng/mL), the OD was compared to an OD standard curve generated using a blank and a known antigen concentrations (0.312 to 5 ng/mL).

V. 2.4 Statistical analysis

A completely randomized design was used in a 2x4 factorial arrangement, with two selenium sources (organic or inorganic), four Se levels (0; 0.3; 0.9 and 2.7 mg/kg DM). Individual bull was considered an experiment unit (n=9 animals/treatment). The data of glutathione peroxidase (GPx), reduced glutathione (GSH), oxidized glutathione (GSSG) and Se were determined in liver evaluated using the GLM procedure. Total cholesterol (TC), HDL and LDL cholesterol, triglycerides (TG) and Se were evaluated by SAS (Statistical Analysis System, version 9.3) using the MIXED procedure with different times (0, 28, 56 and 84 days) of the experiment. GPx and cholesterol in muscle, and HMGCR in

serum were evaluated using the GLM procedure of SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). Sources, levels, time, and doubles and triples interactions were considered as fixed effects. Means were compared by PDIFF test, and differences were considered statistically significant when $P \leqslant 0.05$.

V. 3 Results

V. 3.1 GPx, GSH, GSSG, GSH/GSSG and Se in liver of Nellore cattle

The significance values (P) for effect of selenium sources, levels and interactions in liver samples are shown in Table 2. There was a significant effect of source and level interaction ($P<0.0001$) for the glutathione peroxidase activity (GPx). Inorganic Se did not show any difference between the levels, whereas, in the organic Se, supplementation with 0.9 and 2.7 mg Se/kg of DM provided higher activity of GPx in relation to 0.3 mg and these were higher than the control treatment (Table 3). GSH was not influenced by treatment ($P>0.05$), however, GSSG increased linearly ($P<0.0001$, Table 4) according to Se level altering significantly GSH/GSSG ratio ($P<0.0001$). Selenium liver concentration increased linearly with the Se level in the diet ($y = 0.0583 + 0.4254x$, $R^2=0.92$, $P<0.0001$), regardless of source. Animals fed with 2.7 mg Se/kg DM had Se liver values (1.215 mg/kg of DM) 23 times higher than the control treatment (0.053 mg/kg of DM).

Table 2. Significance values (P) for the source, level and interactions of the glutathione and selenium in the liver of Nellore cattle.

Item	Level	Source	S*L
GPx	<0.0001	0.0011	0.0189
GSH	0.1253	0.9387	0.5651
GSSG	<0.0001	0.5695	0.0726
GSH/GSSG	<0.0001	0.3579	0.1856
Se	<0.0001	0.2579	0.6095

GPx= glutathione peroxidase; GSH= reduced glutathione; GSSG= oxidized glutathione; n= 63 animals.
S*L= sources x levels interactions.

Table 3. Glutathione peroxidase activities (GPx; nmol/min/mg of protein) in liver of Nellore cattle.

Se level (mg/kg)	Inorganic	Organic
0.0	616.28 ± 14.16 ^{Aa}	616.28 ± 14.16 ^{Ac}
0.3	637.58 ± 6.84 ^{Aa}	670.13 ± 11.32 ^{Ab}
0.9	652.10 ± 8.61 ^{Ba}	749.12 ± 18.96 ^{Aa}
2.7	625.41 ± 8.02 ^{Ba}	745.47 ± 18.72 ^{Aa}

Values are means ± SE;
^{a,b,c} Lower case letters on the column indicate significant differences (P<0.05).
^{A,B} Capital letters on the line indicate significant differences (P<0.05).

Table 4. The GSH, GSSG, GSH/GSSG ratio and Se concentration in the liver of Nellore cattle fed control diet or diet supplemented with different selenium sources and levels.

Item	Sources		SEM	Levels				SEM
	I	O		0.0	0.3	0.9	2.7	
GSH[1]	3.62	3.62	0.063	3.61	3.80	3.50	3.57	0.090
GSSG[1]	0.038	0.037	0.001	0.026^{d}	0.033^{c}	0.043^{b}	0.047^{a}	0.001
GSH/GSSG	101.05	105.06	2.80	138.87^{a}	115.22^{b}	82.82^{c}	75.32^{c}	3.965
Se[2]	0.453	0.493	0.034	0.053^{d}	0.218^{c}	0.407^{b}	1.215^{a}	0.031

GSH = reduced glutathione; GSSG = oxidized glutathione; Se = selenium; I= inorganic; O= organic; n= 63 animals.
[1]= µmol/g of protein; [2]= mg/kg.
^{a,b,c} Means in the same row with a different letter differ significantly at P<0.05.

V. 3.2 Cholesterol and glutathione peroxidase (GPx) in meat

Selenium levels had effect (P<0.0001) on glutathione peroxidase and cholesterol in meat samples (Table 5). The GPx activity in the meat of animals fed with 2.7 mg Se/kg of DM was 288% higher than control treatment. There was no difference between 0.3 and 0.9 mg Se/ kg of DM Se level, however, the GPx average was 207% higher than control treatment. Cholesterol decreased with the increasing levels of selenium (P<0.001), regardless of source, animals supplemented with Se (44.81 mg of cholesterol/ 100 g of meat) had cholesterol 10.2% lower than control treatment (49.92 mg of cholesterol/ 100 g of meat).

Table 5. Total cholesterol and glutathione peroxidase in *L. lumborum* muscle samples according to selenium source and level (means values and standard errors).

Item	Sources		SEM	Level				SEM
	I	O		0	0.3	0.9	2.7	
TC	46.33	45.84	0.50	49.92[a]	45.10[b]	45.22[b]	44.12[b]	0.41
GPx[1]	59.84	58.08	4.51	21.38[c]	62.02[b]	69.40[b]	83.03[a]	4.03

I= inorganic; O= organic; TC = total cholesterol; GPx= glutathione peroxidase; SEM: standard error, n= 63 animals.

[1]= nmol of NADPH/min/mg of protein

[a,b] Means in the same row with a different letter differ significantly at P<0.05.

V. 3.3 Total cholesterol and fractions, Se and HMG-CoA in serum

The significance values (P) for effect of selenium sources, levels, time, and double and triple interactions in serum are shown in Table 6. There was a significant effect of level (P<0.05) and time (P<0.0001) for the total cholesterol. There was a significant effect of source (P<0.05) and time (P<0.0001) for the LDL.

Table 6. Significance values (P) for source, level, time of the experiment, and double and triple interactions of total cholesterol and fractions in serum of Nellore cattle.

Item	Level	Source	Time	S*L	S*T	L*T	S*L*T
TC	0.0396	0.0680	<.0001	0.1403	0.9608	0.9762	0.9695
LDL	0.0947	0.0360	<.0001	0.3951	0.9040	0.9899	0.9881
HDL	0.1583	0.8987	<.0001	0.0605	0.6820	0.9860	0.7821
TG	0.4386	0.3137	<.0001	0.5573	0.1373	0.2072	0.7415
Se	<.0001	<.0001	<.0001	0.0016	<.0001	<.0001	0.0296

TC = Total cholesterol; LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very low density lipoprotein; TG = triglycerides; Se = selenium.
S*L= sources x levels interactions; S*T= sources x time interactions; L*T= levels x time interactions; S*L*T= sources x levels x time interactions, n=63 animals.

There was a significant effect of Se level (P<0.05) and time (P<0.0001) on the serum cholesterol (Table 7 and Table 8), the level of 2.7 mg/kg of DM reduced 8% the cholesterol concentration. The low-density lipoprotein (LDL) was influenced by selenium source (P<0.05) and time (P<0.0001). High-density lipoprotein (HDL) and triglycerides (TG) were no influenced by treatments, there was the effect of time (P<0.0001).

Table 7. Total cholesterol and fractions in Nellore cattle serum fed with different selenium sources and levels.

Item (mg/dL)	Sources		SEM	Levels				SEM
	I	O		0.0	0.3	0.9	2.7	
TC	116.68	110.52	1.877	118.22^{a}	116.69^{a}	110.78^{ab}	108.71^{b}	2.634
LDL	52.91^{A}	47.44^{B}	1.353	52.49	52.00	50.05	46.17	1.929
HDL	61.68	61.48	1.042	62.38	62.79	58.63	62.51	1.462
TG	15.04	15.66	0.387	15.19	15.33	16.07	14.82	0.549

TC = Total cholesterol; LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very low density lipoprotein; TG = triglycerides; I= inorganic; O= organic; n= 63 animals.
^{a,b,c} Lower case letters within the levels indicate significant differences (P<0.05).
^{A,B} Capital letters within the sources indicate significant differences (P<0.05).

Table 8. Total cholesterol and fractions in Nellore cattle serum during the experimental period.

Item (mg/dL)	Time				SEM	p-valor	Effect
	0	28	56	84			
TC	99.75^{d}	110.18^{c}	118.48^{b}	126.00^{a}	2.634	<.0001	L
LDL	44.35	49.14	48.61	58.61	1.914	ns	-
HDL	52.96^{c}	60.34^{b}	67.55^{a}	65.46^{a}	1.356	<.0001	Q
TG	12.86	16.48	13.21	19.54	0.566	ns	-

TC = Total cholesterol; n= 63 animals.
L: effect linear; Q: effect quadratic.

There was a significant triple interaction between source x level x time (P <0.05) for selenium serum concentration over the experimental period (Fig. 1). Organic selenium supplementation provided a higher Se serum concentration than the inorganic source. For the organic source, the best level was 2.7, followed by the others. In the inorganic source, the best level was also 2.7, however, there was no difference between 0.3 and 0.9 and these were superior to the control treatment.

There was a significant effect (P<0.0003) of selenium level (Figure 2) in serum HMGCR concentration, regardless of source. Se supplementation (0.36 ng/mL of serum) reduced by 32.7% in relation to control treatment (0.54 ng/mL of serum).

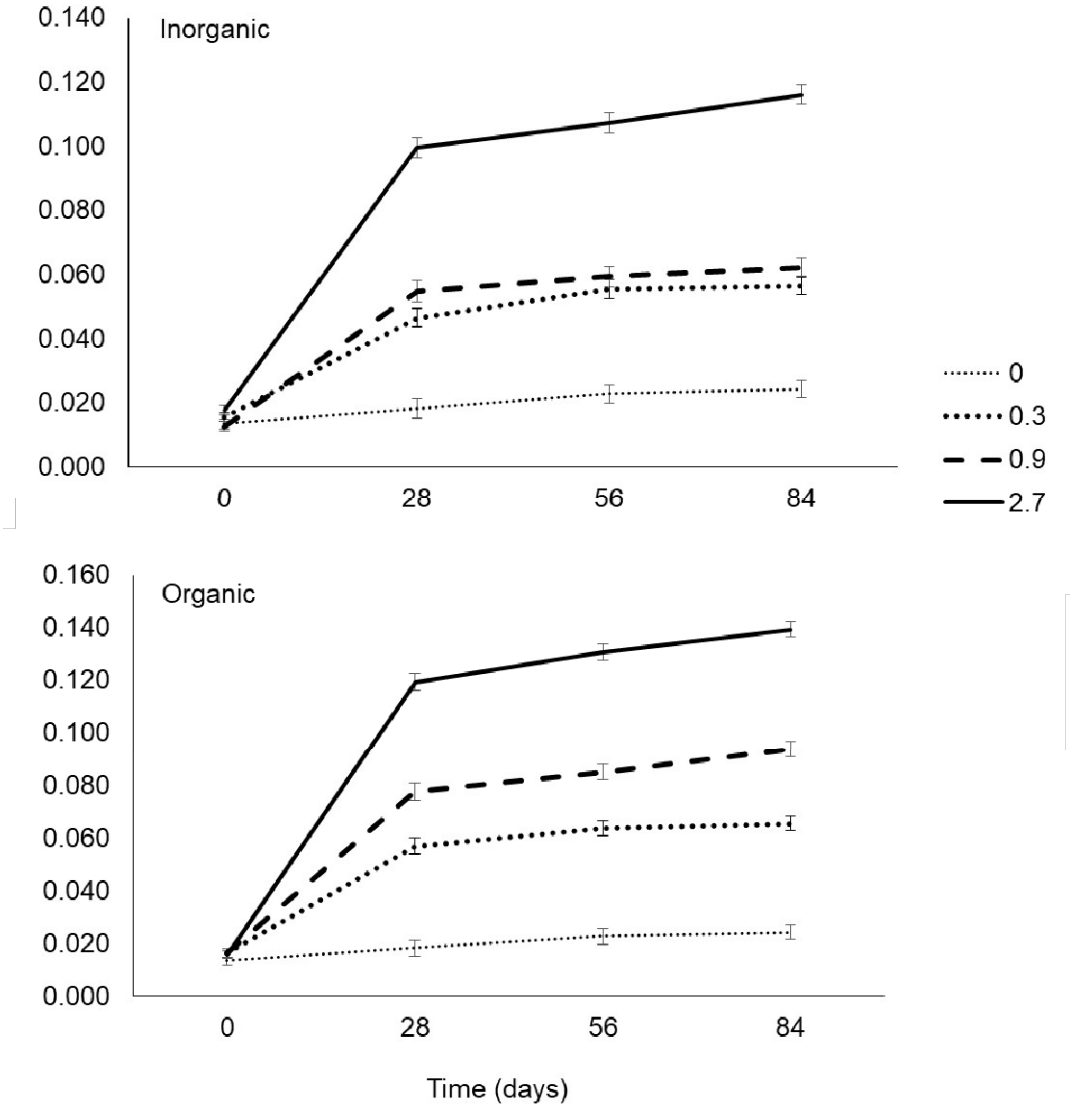

Figure 1. Selenium concentration (mg/kg) in serum of Nellore cattle receiving different selenium sources (organic and inorganic) and levels (0; 0.3; 0.9; 2.7 mg/kg of DM) over the experimental period.

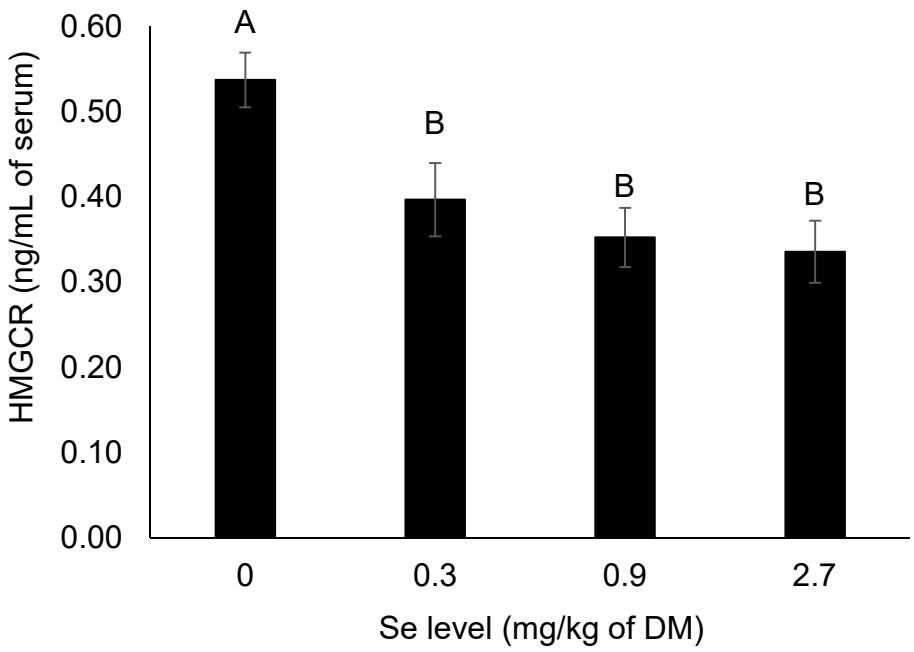

Figure 2 - HMGCR mean (ng/mL) in serum of Nellore supplemented with different selenium levels (0; 0.3; 0.9 and 2.7 mg/kg DM).

V. 4 Discussion

V. 4.1 GPx, GSH, GSSG, GSH/GSSG and Se in liver of Nellore cattle

Several documents reported that organic Se addition in animal feed results in higher GPx activity, and also that GPx can be used to evaluate the Se status of cattle (MEHDI; DUFRASNE, 2016). The average liver GPx activity was between 616 and 749 nmol/min/mg of protein. Animals receiving 0.9 and 2.7 of organic Se had higher GPx, however, inorganic Se showed no difference in different supplementation levels and GPx was lower than organic. According to Bermingham et al. (2014) selenium enriched food (like yeast) is more effective to increasing GPx than a single source. Since Secysteine is a precursor of GPx synthesis, then, the percentages of liver GPx are affected by selenium dietary forms that are metabolized differently. Yan & Johnson (2011) reported that liver GPx activity responded to dietary Se supplementation in rats. Alimohamady et al.

(2013) observed significantly improved in GPx activity in lambs supplemented with different selenium sources and levels. Gunter; Beck; Hallford (2013) verified that cows supplemented with organic and inorganic selenium showed higher GPx activity in erythrocytes compared to cows in the control group. According to Hall et al. (2014) GPx increased by 21% during the first 14 days of lactation in Se-yeast supplemented cows. Netto et al. (2014) observed higher liver GPx activity in bovine liver supplemented with inorganic Se (2 mg/kg DM). Therefore, the literature results between selenium forms and selenoprotein activity are conflicting (BERMINGHAM et al., 2014) and there are few works with GPx activity values in the liver of cattle.

The liver is the central organ of Se metabolism (COMBS, 2015), the Se liver concentrations found in this study, increased linearly with the dietary Se level, regardless of source. JUNIPER et al. (2008) also observed no significant difference in liver Se concentration of cattle that had been offered selenium supplemented diets by selenium-yeast or sodium selenite at the same level (0.30 mg of Se/kg of DM). The animals receiving 2.7 mg/kg of DM, regardless of source, had 1.215 mg Se/kg of liver. Despite the high Se values found in the liver, all concentrations are below that could be considered toxic for the animals according to Puls (1988).

V. 4.2 Glutathione peroxidase and cholesterol (GPx) in meat

There was a linear level effect ($P<0.01$) to the graded Se addition to the diet on meat GSH-Px activity at 0 d postmortem. The results are in agreement with Juniper et al. (2008) that also reported linear dose effect and similar values of GPx activity in *longissimus dorsi* of beef cattle. The cholesterol concentration average found in this study was 46.09 mg/100 g of meat, close to result to that

obtained by Costa et al. (2002) and Rule et al. (2002) working with cattle finished in confinement. Cholesterol biosynthesis can be regulated indirectly by the reduction of reduced glutathione (GSH) and an increase of oxidized glutathione (GSSG) because the increased GSSG/GSH ratio causes a decreases in the activity of the enzyme HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA), reducing the cholesterol synthesis (KIM; CHAO; ALLEN, 1992). In this experiment, there was observed an increase of 288% in the biosynthesis of glutathione with a consequent 10.2% of reduction in meat cholesterol concentration. The earlier study of our team also observed an increased GSSG/GSH ratio and a reduction in cholesterol concentration in Brangus bovine muscle supplemented with 2 mg sodium selenite/kg DM (NETTO et al., 2014). Our research found that using low selenium level and organic or inorganic source can reduce cholesterol in meat, selenium supplementation can be a good strategy to produce meat cattle with better human health benefits.

V. 4.3 Total cholesterol and fractions, Se and HMG-CoA in serum

As expected, cholesterol concentrations increased throughout the experimental period with the consumption of rich energy diet (composed main by corn and soybean). Earlier studies have suggested the role of selenium in reducing cholesterol. El-Demerdash; Nasr (2014) reported that Se significantly decreased the levels of cholesterol in rats. Asadi et al. (2017) observed that dietary supplementation of selenium decreased serum and yolk cholesterol content in Laying Hen. Kang; Bansal; Mehta (2000) cited that selenium seems to have a hypocholesterolemic effect where supplementation with Se decreased total cholesterol in rabbits. Hall et al. (2014) also reported lower serum cholesterol concentrations in cows receiving Se-yeast. However, neither of them evaluate

the direct effect of selenium by quantification of HMG-CoA reductase (cholesterol biosynthesis enzymes).

The HMGCR mean at the end of the feedlot was 0.41 ng of HMGCR/mL of serum. Decreased HMG-CoA reductase activity causes a reduction in cholesterol synthesis due to decreased carbon flux through the mevalonate pathway (RUSSELL, 1992). To our knowledge, this is the first study examining the association of selenium status with the amount of HMGCR in cattle. In rats, it was observed that selenium supplementation reduce the gene expression of HMG-CoA reductase (DHINGRA; BANSAL, 2006). This change in the expression of HMG-CoA reductase justifies our results of the decreased amount of HMG-CoA reductase with the selenium supplementation.

Nowadays the Statins are the main kind of drugs largely used for cholesterol reduction in the blood's human and their action way is thought HMG-CoA activity inhibition. Statins bind to the catalytic domain of HMG-CoA reductase, preventing its action (MCFARLAND et al., 2014). Selenium act in the enzyme production, a different mechanism of statins, besides that, statins have several side effects (THOMPSON et al., 2016). The result observed in bovine can occur in human as well, because the metabolic way for cholesterol synthesis is the same. If this true, selenium could be an interesting strategy to substitute or reduce statins levels for cholesterol control.

V. 5 Conclusion

In conclusion, Se supplementation, regardless of source, reduced cholesterol in serum and meat due reduced in HMG-CoA reductase. The Se

supplementation has the potential to increase selenium and reducing cholesterol in beef produced for human consumption with many health benefits.

Acknowledgements

The authors would like to thank the São Paulo Research Foundation (FAPESP/Process 2015/07175-6), which provided financial support for this study, and the National Council of Scientific and Technological Development (CNPq/Brazil–Process 142484/2015–6) that granted scholarship to the first author.

Declaration of interest

No potential conflict of interest relevant to this article is reported.

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