Lipid and selenium sources on fatty acid composition of intramuscular fat and muscle selenium concentration of Nellore steers

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ABSTRACT - The objective of this study was to evaluate the effect of lipid and selenium sources in diets for finishing Nellore steers on the fatty acid composition and selenium concentration of the longissimus muscle. Fifty Nellore steers (body weight = 458±39 kg) were assigned to one of six dietary treatments: 1) diet containing sunflower seed and inorganic selenium; 2) sunflower seed and organic selenium; 3) whole cottonseed and inorganic selenium; 4) whole cottonseed and organic selenium; 5) soybeans and inorganic selenium; and 6) soybeans and organic selenium. Diets were formulated with the same amount of nitrogen and calories and supplied once daily to steers in collective pens, with three animals per pen, for 120 d. At the end of the trial, steers were slaughtered and samples of the longissimus muscle were collected for fatty acid and selenium analysis. Effect of selenium sources was detected for selenium concentration in the longissimus muscle. Organic selenium had higher concentrations in the meat compared with inorganic selenium. The total saturated, monounsaturated and polyunsaturated fatty acids did not differ between the sources of lipids and selenium. For selenium sources, no differences were observed between the concentrations of polyunsaturated fat. Also, no differences in C18:2 cis-9 trans-11 concentrations were noted; however, steers fed sunflower seed presented greater proportions of this fatty acid in the meat. The results indicated that the use of sunflower seed, cottonseed or soybeans and organic or inorganic selenium in feedlot diets to Nellore cattle does not alter the great part of the fatty acid profile of the longissimus muscle. However, the inclusion of sunflower seed in the diet increases the meat CLA cis-9, trans-11, which is desirable and beneficial for the health of consumers.

Key Words: antioxidant, cattle, longissimus, soybean seed, sunflower seed, whole cottonseed

Introduction

Several research studies have been conducted to alter the beef fatty acid profile resulting in healthier product for human consumption. The fatty acid composition of beef is dependent on several factors such as animal, breed, age, sex and nutritional diet (Tume, 2004).

Results from the literature have demonstrated that it is possible to change the fatty acid profile of beef by feeding animals with ingredients containing high levels of unsaturated fatty acids (UFA) that are partially or completely protected from ruminal modification (Doureau & Ferlay, 1994; Silva et al., 2009). Previous studies have demonstrated that feeding grains rich in UFA increased their concentration in beef cattle meat (Felton & Kerley, 2004; Madron et al. 2002; Yang et al., 1999).

The increase in the UFA content of meat leads to an increase in the lipid oxidation process due to the greater susceptibility of UFA to this process. The action of antioxidants is to minimize the detrimental lipid oxidation by neutralizing or scavenging free radicals (Barros et al., 2008).

In an attempt to protect the fatty acids from oxidation, many antioxidant substances have been studied. Selenium (Se) is a mineral which plays a key role in antioxidant enzymes (e.g. glutathione peroxidase) that protect cellular membranes from oxidative damage. The organic Se has been shown to have higher absorption rate than the inorganic Se (Underwood & Suttle, 2001).

In recent years, Brazil has emerged as an important part in the international beef market. Most of its meat comes from Zebu cattle (mainly Nellore breed) that are usually finished on forage pastures or for short periods of feedlot and slaughtered at low levels of external and intramuscular fat. There is a lack of information about the effects of using different lipid and selenium sources on the fatty acid composition of the longissimus muscle (LM) of Nellore steers fed for short periods on feedlot and slaughtered at low levels of body fat.
Therefore, the objective of this study was to evaluate the effects of lipid and selenium sources on the LM fatty acid composition and selenium concentration of finished Nellore steers.

**Material and Methods**

All animal procedures described in this experiment were conducted following the Institutional Animal Care and Use Committee Guidelines of Universidade de São Paulo.

Fifty-four Nellore steers (30 months old and body weight = 458±39 kg) were assigned in a completely randomized design with a 3 × 2 factorial arrangement.

The diets were formulated with the same amount of nitrogen and calories differing in lipid source (sunflower seed, whole cottonseed or soybeans) and Se source (inorganic or organic). The inorganic and organic Se was provided with sodium selenite mineral salt and Sel-Plex mineral salt, respectively (Table 1).

The animals were allotted in 18 pens, with 3 animals per pen, and fed one of the six diets: diet containing sunflower seed and organic Se; sunflower seed and inorganic Se; whole cottonseed and organic Se; whole cottonseed and inorganic Se; whole soybeans and inorganic Se; and whole soybeans and organic Se. The inorganic Se (sodium selenite 44.5%) and organic Se (Sel-PlexTM, Alltech Inc.) were pre-mixed in corn meal and added to the diets to reach 0.21 mg/kg of dry matter (DM), according to the recommended by NRC (2000).

The animals were slaughtered after 120 days of feeding at the slaughter house of Universidade de São Paulo, in accordance with Humanitarian Slaughter Guidelines as required by Brazilian laws.

After twenty-four hours of chilling at 2 °C, carcasses were ribbed between the 12th and 13th ribs and carcass backfat thickness was measured at ¾ of the lateral end of LM using an electronic ruler. A 2.5 cm thick LM sample was taken from each animal, vacuum packaged and frozen at -18 °C for subsequent analyses.

Selenium analysis were determined fluorometrically by the method of Olson et al. (1975). The digestion time increased from 15 to 30 min past the appearance of perchloric acid fumes to better assure complete oxidation of all forms of selenium to selenite.

A sample of LM was lyophilized and used for ether extract (AOAC, 2000) and fatty acid composition determinations. Lipids were extracted for fatty acid composition, according to the Hara & Radin (1978) methodology with modifications. Five grams of LM were mixed to 28 mL of hexan/propanol (3:2 vol/vol), homogenized for 1 min, vacuum filtered, and received a sodium sulphate (67 mg mL^−1) solution up to 50% of the volume, filtered and vortex-agitated for 30 seconds. The supernatant was transferred to a tube with 2 g of sodium sulphate and insufflated with N₂ and was left to rest for 30 minutes. After that, the liquid was transferred to 10 mL glasses, insufflated with N₂, protected and kept under -20 °C until dry with N₂ for methylation.

The extracted lipids were hydrolyzed and methylated according to the methodology described by Christie (1982), with modifications. Around 40 mg of lipids were transferred to a tube with 2 mL of hexane followed by addition of 40 µL of methyl acetate. The tube was agitated with vortex and 40 µL of methylation solution (1.75 mL of metanol/0.4 mL of 5.4 mol/L of sodium metoxide) were added. After the mixture was agitated for 2 min, followed by 10 min resting, and 60 µL of a finished solution was added (1 g of oxalic acid/30 mL of diethyl ether), and agitated during 30 sec, 200 mg of calcium chloride were added and it was left to rest for 1 hour. After, the sample was centrifuged at 3,200 rpm for 5 min under 5 °C and the supernatant was collected and stored in flasks for reading.

Fatty acid methyl esters were determined by gas chromatograph (ThermoFinnigan, Termo Electron Corp., MA, USA) equipped with a flame ionization detector and a 100 m Supelco SP-2560 (Supelco Inc., PA, USA) fused silica capillary column (100 m, 0.25 mm and 0.2 µm)

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Table 1 - Ingredients and chemical composition of diets (g/kg), on a dry matter (DM) basis

<table>
<thead>
<tr>
<th>Ingredients, g/kg DM</th>
<th>Lipid source</th>
<th>Sunflower seed</th>
<th>Whole cottonseed</th>
<th>Whole soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>400.0</td>
<td>400.0</td>
<td>400.0</td>
<td></td>
</tr>
<tr>
<td>Ground corn grain</td>
<td>150.0</td>
<td>150.0</td>
<td>150.0</td>
<td></td>
</tr>
<tr>
<td>Sunflower seed</td>
<td>130.0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Whole cottonseed</td>
<td>-</td>
<td>167.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Whole soybean</td>
<td>-</td>
<td>-</td>
<td>130.0</td>
<td></td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>302.5</td>
<td>268.0</td>
<td>252.5</td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>5.8</td>
<td>2.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mineral¹</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Monensin</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Nutrients, g/kg DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein²</td>
<td>123.0</td>
<td>122.0</td>
<td>133.0</td>
<td></td>
</tr>
<tr>
<td>Total digestive nutrients³</td>
<td>715.9</td>
<td>737.0</td>
<td>715.9</td>
<td></td>
</tr>
<tr>
<td>Ether extract⁴</td>
<td>62.0</td>
<td>61.0</td>
<td>60.1</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>2.10</td>
<td>2.10</td>
<td>2.20</td>
<td></td>
</tr>
</tbody>
</table>

¹ Mineral salts: 1) Sodium selenite mineral salt, composition: Ca - 136.0 g/kg; P - 65.0 g/kg; Na - 183 g/kg; Cl - 282.2 g/kg; Mg - 3.5 g/kg; S - 20 g/kg; Mn - 157.15 g/kg; Zn - 1,000 g/kg; Fe - 4,982 g/kg; Cu - 600 g/kg; Co - 40 g/kg; I - 60 g/kg; Se - 40 g/kg; F - 489.739 g/kg.

² Sel-Plex mineral salt, composition: Ca - 120 g/kg; P - 65 g/kg; Na - 183 g/kg; Cl - 282.2 g/kg; Mg - 3.4 g/kg; S - 20 g/kg; Mn - 145.92 g/kg; Zn - 1,000 g/kg; Fe - 4,978 g/kg; Cu - 600 g/kg; Co - 210 g/kg; I - 60 g/kg; Se - 40 g/kg; F - 489.77 g/kg. The mineral salts were premixed in corn meal and added to the diets.

³ Total dry matter.

⁴ Fatty acid methyl esters were determined by gas chromatograph (ThermoFinnigan, Termo Electron Corp., MA, USA) equipped with a flame ionization detector and a 100 m Supelco SP-2560 (Supelco Inc., PA, USA) fused silica capillary column (100 m, 0.25 mm and 0.2 µm).
film thickness). The column oven temperature was programmed at 70 °C for 4 min, 170 °C (13 °C min⁻¹), and 250 °C (35 °C min⁻¹) for 5 min. The gas fluxes were 1.2 mL min⁻¹ for carrier gas (He); 45 mL min⁻¹ for make-up gas (N₂); 40 mL min⁻¹ for hydrogen and 450 mL min⁻¹ for synthetic flame gas. One µL sample was injected in split mode at 1/21. Injector and detector temperatures were 250 and 300 °C, respectively.

Fatty acids were identified by comparing the relative retention times of fatty acid methyl esters peaks with a reference compound (CRM-164, Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium). Fatty acids were expressed as percentage of overall fatty acids identified.

Effects of lipid and selenium sources and interactions on fatty acid and selenium muscle composition were evaluated by analysis of variance using the command PROC MIXED of software SAS (Statistical Analysis System, version 9.1) according to the model:

\[ Y_{ij} = \mu + T_i + S_j + TS_{ij} + e_{ijk} \]

In which \( Y_{ij} \) = the dependent variable; \( \mu \) = the overall mean; \( T_i \) = fixed effect of lipid sources (sunflower seed, whole cottonseed and soybeans); \( S_j \) = fixed effect of selenium sources (organic and inorganic); and \( e_{ijk} \) = residual error.

For slaughter and carcass weights, the initial live weight was used as a covariate.

The effects of treatment according to the diet were determined with averages calculated by command LSMEANS and submitted to the t-Test. A significance level of 0.05 was used.

**Results**

There were no significant interactions between lipid and selenium sources for carcass traits (Table 2; P>0.05). Effect of lipid or selenium sources was observed for carcass backfat thickness and ether extract (Table 2; P>0.05). In addition, no effect of lipid was observed for the selenium concentration (Table 2; P>0.05). However, effect of selenium sources were detected for selenium concentration in LM, as well as for effect of lipid or selenium sources for slaughter and carcass weights (Table 2; P<0.01).

The majority of the individual fatty acids were not affected by either lipid or selenium source (Table 3). However, the sunflower seed diet increased the concentration of C17:0 iso (P<0.05) and the conjugated linoleic acid (CLA) isomer C18:2 cis-9, trans-11 (P<0.0001) when compared with treatments whole cottonseed and soybeans (Table 3). The concentration of monounsaturated C24:1 in the treatments sunflower seed and whole cottonseed were similar, but greater than in the treatment with soybeans as lipid source.

Organic selenium treatments increased the concentration of C17:0 (P<0.05) and C24:1 (P<0.05) and decreased C18:1 trans-16 (P<0.05; Table 3).

Higher concentrations of C18:1 c11 and C18:1 c12 (P<0.01) were noted for the sunflower seed treatment when compared with whole cottonseed in organic selenium diets. On the other hand, in inorganic selenium diets, the sunflower seed treatment showed smaller concentrations (P<0.01) of these fatty acids when compared with whole cottonseed. The treatment with soybeans did not differ from others and from either organic or inorganic source for C18:1 c11 and C18:1 c12.

The C18:1 c13 concentration was not affected by lipid source within organic selenium diets, but with inorganic diets, treatments whole cottonseed and soybeans presented higher levels (P<0.05) than the sunflower seed treatment, in which this fatty acid was not detected. The C20:5 concentrations were affected by lipid source in both organic and inorganic selenium diets.

Concentrations of C18:1 c11, C18:1 c12 and C18:1 c13 were lower for organic selenium in whole cottonseed diets (P<0.01; P<0.01 and P<0.05, respectively; Table 4). Similar results were observed for C18:1 c11 and C18:1 c13 in the diets with soybeans (P<0.05 and P<0.01, respectively). The

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**Table 2 - Least squares means, standard error of the mean and probabilities of carcass traits from feedlot-finished Nellore steers fed different lipid and selenium sources**

<table>
<thead>
<tr>
<th>Traits</th>
<th>Lipid source</th>
<th>Standard error</th>
<th>Selenium</th>
<th>Standard error</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole cottonseed</td>
<td>Soybean grain</td>
<td>Sunflower seed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L×Se</td>
</tr>
<tr>
<td>Slaughter weight, kg</td>
<td>593.3a</td>
<td>575.6a</td>
<td>562.7b</td>
<td>7.20</td>
<td>586.7a</td>
</tr>
<tr>
<td>Carcass weight, kg</td>
<td>361.1a</td>
<td>351.0a</td>
<td>342.7b</td>
<td>4.70</td>
<td>356.0</td>
</tr>
<tr>
<td>Backfat thickness, mm</td>
<td>8.3</td>
<td>9.3</td>
<td>8.4</td>
<td>0.72</td>
<td>8.7</td>
</tr>
<tr>
<td>Ether extract, g/kg</td>
<td>3.8</td>
<td>3.8</td>
<td>3.5</td>
<td>1.09</td>
<td>3.7</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.14</td>
<td>0.14</td>
<td>0.13</td>
<td>0.008</td>
<td>0.12b</td>
</tr>
</tbody>
</table>

Means in the same row within lipid or selenium source are different according to significance level by Student’s T test.

L×Se - interaction between lipid and selenium sources.

1 Longissimus muscle (original matter basis).
sunflower seed diets with organic selenium indicated higher concentrations of C18:1 c11 and C18:1 c12 (P<0.05).

In the treatments with whole cottonseed and soybeans, there was no difference of C20:5 concentration between organic and inorganic selenium, but in treatment sunflower seed, the C20:5 level was greater in organic selenium diets (P<0.05).

The lipid source did not affect the total of saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acid concentrations, or even the MUFA:SFA and PUFA:SFA ratios. Similarly, the total ω-3, ω-6 and ω-6:ω-3 ratio were not affected by treatments.

### Discussion

Lawler et al. (2004) observed no differences in meat lipids of cattle fed sources of selenium (Se). This was expected, because the action of selenium should occur as a protector of the oxidation of unsaturated fatty acids. However, the use of different lipid sources in diets for feedlot steers did not affect carcass weight and backfat thickness (Radunz et al., 2009; Oliveira et al., 2011).

In the present study, the data indicated that organic selenium had higher concentrations in the meat compared with inorganic selenium. These results were similar to those...
found by Cary et al. (1973), Lawler et al. (2004) and Taylor, (2005). In addition, Ehlig et al. (1967) compared sources of selenite and selenium methionine (0.4 mg Se/d) and observed that supplementation with selenium methionine resulted in higher selenium concentrations in lamb muscles. Furthermore, Allaway (1973) and van Ryssen et al. (1989) reported greater incorporation of Se in the skeletal muscle of sheep consuming up to 1.0 ppm of Se as an organically bound Se source versus sodium selenite. This may have occurred due to selenium methionine, which is used for the selenium cysteine synthesis and can be incorporated directly into the muscle tissue, because a significant fraction “escapes” from rumen hydrolysis and is absorbed in the intestine (Koening et al., 1997) and incorporated into muscle proteins (Hildebrand, 1992), and the excess of inorganic selenium absorbed and not used in the synthesis of selenium proteins is excreted (Itoh & Suzuki, 1997). This difference between sources of selenium observed in this study may be due to the fact that organic minerals are absorbed through mechanisms similar to the absorption of amino acids and peptides, thus avoiding common problems associated with the absorption of minerals in inorganic form. Therefore, selenium from organic sources can be incorporated into muscle protein, unlike what occurs with inorganic sources, such as sodium selenite.

The indicators of carcass lipid content in this study were unaffected (P>0.05) by lipid and selenium sources. Thus, most of the fatty acid composition data were not likely confounded by differences in total carcass fat content across treatments. Saturated fatty acids are most abundant in intramuscular fat, corresponding to 45 to 48% of the total fatty acids, whose main representatives are C14:0, C16:0 and C18:0 (Scollan et al., 2006). In this study, the values were considered appropriate, between 43% and 45%, which makes meat healthier from the standpoint of human health as the hypercholesterolemic effect of SFA. This positive response might be associated with lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids (MacDonald, 2000). However, no difference was observed for C14:0 with the use of lipid source for beef cattle, in other studies (Oliveira et al., 2011).

Monounsaturated fatty acids have a neutral or slightly hypcholesterolemic effect (Penny & Shaomei, 1997). The values observed in this study indicated that animals fed cottonseed diets produced healthier meat. The MUFA found in most vegetable oils is oleic acid. Currently, the recommendation of oleic acid is 20% of the total caloric intake and it is considered one of the factors responsible for the beneficial effects of Mediterranean populations who have abundant olive oil in the diet which is rich in oleic acid (Simopoulos, 2006; Schmid et al., 2006). The C18: 2 cis-9 trans-11 values of the present study were within the normal range of variation for beef cattle (0.2 to 1.0% of total fatty acids) according to Raes et al. (2004).

The increase in CLA concentration is of great interest to human health and was analyzed in this study. The cis-9, trans-11 and trans-10, cis-12 (CLA isomers) have anticarcinogenic and antiinflammatory activities, respectively (Pariza et al., 2001); however, other isomers can also offer benefits through the modulation of fat metabolism (MacDonald, 2000). Although it is interesting for the lipogenic action of trans-10, cis-12, no significant amounts of this isomer was found in beef (Medeiros et al., 2005; Silva et al., 2009), while cis-9, trans-11 corresponds to 57 to 85% of the total value of CLA (Lawson et al., 2001; Mir et al., 2004,) and can be absorbed or formed by incomplete biohydrogenation to acid (trans-11 octadecenoic acid). After absorption, this acid can be converted to CLA cis-9, trans-11 by stearyl-CoA desaturase enzyme (SCD) or delta-9 desaturase (Santora et al., 2000).

According to Felton & Kerley (2004), fat diets have a tendency to increase the proportions of PUFA in the kidney fat without changing the composition of subcutaneous or intramuscular fat, like the findings in this study. This might occur due to the activity of delta-9-desaturase in the tissues, converting SFA (stearic acid) into MUFA (oleic acid). The most abundant PUFA in the diet is linoleic acid, corresponding to 84-89% of the total PUFA. Linoleic acid is an 18-carbon fatty acid ω-6 series with two unsaturations. The recommendation for PUFA intake is approximately 10% of total energy intake because of the reduced risk of coronary heart disease, compared with a diet rich in saturated fat (Simopoulos, 2002).

For meat lipid composition, it is important to consider the type of PUFA n-3, because of the protection afforded by these isomers against cardiovascular disease by reducing LDL-cholesterol in plasma and other chronic diseases, as well as changes in the immunological and mental states (Mahan & Escott-Stump, 2005). Concerning linoleic acid n-6, it is important to emphasize that its actuation is essential in dermal processes, mental retardation, reproductive failure and polydipsia (Mahan & Escott-Stump, 2005).

To be considered a healthy diet for humans, according to Enser et al. (1998), the PUFA/SFA ratio must be below 0.4. Moreover, the values recorded in treatments with sunflower seed in this study were similar to those observed by Enser et al. (1999), who reported average values of 0.11 in beef. According to Duckett et al. (1993), the PUFA/SFA ratio decreases with increase in the fat content, up to a point where stabilization occurs because of the longer period animals stay in confinement, once there is increased body
fat percentage, and marbling of fat in animals, which is composed of 44% SFA, 45% MUFA, 5% odd-chain fatty acids and only 5% of PUFA.

Silva et al. (2002) reported a ω-6:ω-3 ratio from 1.0 to 6.3. Enser et al. (1998) and Wood et al. (2003) recommended maximum values of 4.0 for a healthy diet. These values are lower than those observed in this study, which ranged from 11.15 to 17.97. As the optimal ω-6 and ω-3 ratio was estimated at 2:1 to 3:1, four times lower than the current intake, it is recommended that humans consume more ω-3 fatty acids from marine sources and plants (Mahan & Scott-Stump, 2005; Martin et al., 2006).

According to all the parameters evaluated in this study, it was not possible to state that the use of organic sources of selenium has advantages over the conventional use of selenium associated with seed oil, since this source did not improve the concentrations of major unsaturated fatty acids. Moreover, it was not possible to affirm the superiority of a lipid source, because no one was better in most of the characteristics analyzed. The sunflower seed stood out by its higher content of CLA, the soybeans had the best ω-6:ω-3 ratio and was similar to other lipid sources.

Conclusions

The most part of the fatty acid profile of longissimus muscle is not affected by the lipid or selenium sources. However, the inclusion of sunflower seed and organic selenium to finishing Nellore steers results in a healthier meat due to the increase of CLA (C18:2 cis-9 trans-11) and selenium concentration in the meat, respectively.

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References


