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Meat characteristics of Nellore steers fed whole cottonseed[1]

Dorival Pereira Borges da Costa[2], Roberto de Oliveira Roça[3], Quézia Pereira Borges da Costa[4], Dante Pazzanese Duarte Lanna[5], Erico da Silva Lima[4], Wander Miguel de Barros[6]

[1] Project financed by FAPESP.
[2] Instituto Federal do Mato Grosso, Campus Campo Novo do Parecis/MT, Brazil.
[3] UNESP/FCA, Campus Botucatu/SP, Brazil.
[4] UNESP/FMVZ, Campus Botucatu/SP, Brazil.
[5] Departamento de Zootecnia, ESALQ/USP, Piracicaba/SP, Brazil.
[6] Instituto Federal do Mato Grosso, Campus Cuiabá – Bela Vista /MT, Brazil.

ABSTRACT - The objective of this study was to evaluate the effect of addition of different levels of cottonseed (0, 14.35, 27.51 and 34.09 kg/100 kg) in the diet of Nellore steers on the meat characteristics. Thirty-six steers (average initial body weight of 333.50 and average age of 20 months) were kept in feedlot stalls for 94 days, each stall with three animals. The experiment was a completely randomized design with four treatments and nine replications. The values of protein, fixed mineral residue, shear force, lipid oxidation, meat color and fat color were similar among the treatments. The increase of the cottonseed levels in the diet reduced the amount of intramuscular fat and increased the moisture content in the meat linearly. Meat aroma and flavor were negatively influenced when cottonseed addition was greater than 27.51 and 34.09 kg/100 kg of the diet, respectively. Cottonseed diet did not modify the concentration of conjugated linoleic acid, saturated fatty acids or total unsaturated fatty acids, but linearly reduced the concentration of monounsaturated fatty acids in the meat.

Key Words: aroma, color, conjugated linoleic acid, flavor, lipid oxidation, shear force

Introduction

In recent years, consumers have been increasingly interested in foods that can provide beneficial substances to their health, besides having desirable nutritional characteristics. Therefore, the food industry and research centers seek foods of this nature (Oliveira et al., 2008).

Products from ruminants are also part of this group because milk as well as meat and the fat tissue contain conjugated linoleic acid, a proven carcinogenic fatty acid (Bauman & Griinari, 2000).

Oilseeds and oils are added to beef cattle diet to increase the content of beneficial fatty acids in the meat to provide healthier meat to consumers; one example is conjugated linoleic acid (Mir et al., 2003; Sackmann et al., 2003; Gibb et al., 2004). Mir et al. (2008) observed that sunflower oil supplementation in diets for steers increased the content of conjugated linoleic acid and trans isomers in meat. However, Hristov et al. (2005) showed that sunflower oil supplementation in finishing diets changed ruminal biohydrogenation of polyunsaturated fatty acids without affecting the concentrations of conjugated linoleic acid in the meat.

Among the oilseeds, whole cottonseed is an excellent option for feedlot because the association of high protein content with high energy content makes the formulation of low cost diets easy (Medeiros et al., 2005; Cranston et al., 2006). Another advantage of cottonseed utilization in beef cattle diets is the production of more unsaturated profile of fat in the meat (Huerta-leidenz et al., 1991; Medeiros et al., 2005). However, one of the limitations of cottonseed is its association with problems in meat flavor.

The objective of this study was to verify the effect of addition of different levels of cottonseed (0, 14.35, 27.51 and 34.09 kg/100 kg) in the diet of Nellore steers on the meat characteristics of Nellore steers finished in feedlot.

Material and Methods

The experiment was carried out on Mateirinha Ranch, located in the municipality of Guiratinga, Mato Grosso State, Brazil, latitude 16º 20' 58'' S and longitude 53º 45' 30'' W, average altitude of 510 m. The region is characterized by hot and wet season generally from October to April, followed by another cold and dry season, from May to September. The climate of the region is classified as AW (wet tropical savanna with dry winter and rainy summer) according to the Köppen climate classification (1938).

Thirty-six Nellore steers, average age of 20 months and average initial body weight of 333.5±14.7 kg were utilized.

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Corresponding author: zoodoc_ufrrj@yahoo.com.br

The animals were identified, vaccinated and subjected to parasite control before the experimental period.

Steers were distributed equally in four treatments which corresponded to the diets with increasing amounts of cottonseed as follows: 0.00, 14.35, 27.51 and 34.09 kg/100 kg of whole cottonseed in relation to dietary dry matter (DM).

The animals were kept in 120 m^2 feedlot stalls for 94 days, including 14 days of adaptation to diets and handling. The feedlot consisted of 12 uncovered stalls with sand floor and each stall had a feed bunker and a drinker; the animals had water *ad libitum*.

The formulation of isoprotein diets was based on the NRC (1996) for non-castrated growing animals and presented the same concentrate:roughage ratio (50:50). The diets were provided three times a day (06h00, 13h00 and 17h00) in a complete diet system, allowing surplus of approximately 5 kg for each 100 kg of provided feed. Sugarcane was used as roughage associated with urea, ground corn grain, whole cottonseed and soybean meal (Table 1).

During the experimental period, the animals were weighed twice a month, after being feed-deprived for 8 hours, to monitor daily weight gain, obtained by the difference between the initial and final body weight divided by the number of days of the period. Once a month, during five consecutive days, the feed supplied and the surplus in the bunker were weighed daily. Samples of surplus were collected to quantify DM intake in each treatment. The diet adjustments were based on the amount of surplus and the daily weight gain.

Table 1 - Proportion of ingredients of experimental diets on a dry matter basis and percentage of dry matter, crude protein and ether extract of treatments

Ingredients (kg/100 kg)	Whole cottonseed in diet (kg/100 kg)			
	0	14.35	27.51	34.09
Sugarcane	50.00	50.00	50.00	50.00
Whole cottonseed	0.00	14.35	27.51	34.09
Ground corn grain	28.13	18.68	10.19	6.01
Soybean meal	19.47	14.57	9.90	7.50
Urea	1.20	1.20	1.20	1.20
Mineral mixture[1]	0.84	0.84	0.84	0.84
NaCl	0.36	0.36	0.36	0.36
Total	100.0	100.0	100.0	100.0
Dry matter[2]	59.35	59.85	60.30	60.53
Crude protein[2]	14.11	14.17	14.16	14.13
Ether extract[2]	2.99	4.98	6.81	7.73
Neutral detergent fiber[3]	35.17	39.63	43.70	45.73
Acid detergent fiber[3]	23.33	26.33	29.06	30.42
Total digestible nutrients[4]	71.76	71.27	70.84	70.63

[1] Composition/kg: P - 136.80 g; Ca - 205.00 g; Mg - 11.00 g; S - 21.23 g; Zn - 10.50 g; Cu - 3.75 g; Mn - 1.50 g; Fe - 4.50 g; Co - 0.20 g; I - 0.30 g e Se - 28.82 mg.
[2] Determined by the method described by Silva & Queiroz (2002).
[3] Determined by the method described by Van Soest et al. (1991).
[4] Values estimated according to the NRC (2001).

The average final body weight of the animals after 94-day feedlot was 446.6±28.0 kg. The steers were transported to Sadia slaughterhouse in the city of Várzea Grande, Mato Grosso State, Brazil, 345 km away from Mateirinha Ranch. The animals were slaughtered by stunning and jugular cutting after a 24-hour feed deprivation. After exsanguination, skinning and evisceration, the carcass were identified, washed, weighed and chilled at 7 ºC for 24 hours. From the left half-carcass of each animal, sirloin steaks were taken from the area of the 12th thoracic vertebrae, 3rd lumbar vertebrae, individually packaged in identified plastic bags and frozen at -18 ºC.

A 2.5 cm transversal sirloin sample, taken from each animal, from the 12th thoracic vertebrae, was utilized to determine shear force. According to the method described by Savell et al. (1998), samples were cooked until reaching internal temperature of 71 ºC, cut into 1.27$^{\varnothing}$cm cylinders, chilled (at 4 ºC for 12 hours) and evaluated in a texture meter, model TA-XT 2i, Stable Micro System (UK) equipped with a set of Warner-Bratzler blades. Eight evaluations were performed on each sample.

The colorimetric system, which indicates color differences corresponding to the human sensitivity, was used to determine meat and fat color. A 2.5 cm transversal sirloin sample of each animal, from the 13th thoracic vertebrae, was thawed for 24 hours until the temperature of 4 ºC and exposed to atmospheric air for 30 minutes; then, a reading of the surface of each sample was done using Chroma Meter. Five readings were done in different points of the sample for meat color and fat color. The evaluated parameters were L*, a* and b*, of the CIELab system, where L* represents luminosity, a* is the red intensity and b*, the yellow intensity.

A 2.5 cm wide transversal sirloin sample from each animal, from the 1st lumbar vertebrae, was used to obtain the centesimal composition and lipid oxidation. The moisture content was obtained according to the 39.1.02 method of the AOAC (2007). The Kjeldahl-micro method was utilized to determine total nitrogen utilized. Crude protein was calculated in function of the total nitrogen content, multiplied by factor 6.25. The ether extract was determined according to the AOAC (2007), item 39.1.05. The same recommended method was used to obtain the amount of fixed mineral residue. The lipid oxidation was evaluated by the formation of substances reactive to 2-tiobarbituric acid (TBA), utilizing the method of Wyncke (1970) for meat samples under 75-day storage.

A 2.5 cm wide transversal sample, from the 2nd lumbar vertebrae, was used to evaluate the fatty acid profile, utilizing the method described by Hara & Radin (1978) to extract lipids. The method proposed by Christie (1982), using a methanol solution of sodium metoxide, was utilized for trans esterification of fatty acids and conjugated linoleic acid.

The transmethyled samples were analyzed in a gas chromatograph model Focus CG- Finnigan, with flame ionization detector, capillary column CP-Sil 88 (Varian), with 100 m of length, 0.25 μm of internal diameter and 0.20 μm of film thickness. Hydrogen was used as carrier gas at a flow of 1.8 mL/min. For initial oven temperature program the wait time was 4 min; for 175 ºC (13 ºC/min), wait time was 27 min, and for 215 ºC (4 ºC/min) wait time was 9 min; and then it increased to 7 ºC/min for 5 min until reaching 230 ºC, totalizing 65 min. The vaporizer temperature was 250 ºC and the detector was set at 300 ºC.

A 1 μL aliquot of esterified extract was injected in the chromatographer and the identification of fatty acids was done by comparing retention times; the percentages of fatty acids were obtained through software Chromquest 4.1 (Thermo Electron, Italy).

Fatty acids were identified by comparing retention times of methyl esters of the samples with fatty acid standards. The fatty acids were quantified by the normalization of methyl ester areas.

A conjugated linoleic acid is a mixture of linoleic acid isomers. In the present study, the conjugated linoleic acid is the sum of $C_{18:2\ trans\ 11\ -\ cis\ 15}$, $C_{18:2\ cis\ 9\ -\ cis\ 12}$, $C_{18:2\ cis\ 9\ -\ trans\ 11}$ and $C_{18:2\ trans\ 10\ -\ cis\ 12}$ fatty acids. Desirable fatty acids are the sum of unsaturated fatty acids and acid $C_{18:0}$. Undesirable fatty acids are the sum of $C_{14:0}$ and $C_{16:0}$ acids (Huerta-Leidenz et al., 1991).

A 2.5 cm wide transversal sirloin sample from the 3rd lumbar vertebrae of each animal was used for the sensory analysis. Ten trained tasters performed the flavor and aroma evaluations. For the flavor analysis, the samples were salted (100 g/L) for 60 minutes at 5 ºC, at a 1:1 ratio. Next, they were wrapped in aluminum foil and heated in a pre-heated electric griddle at 250 ºC for 30 minutes. When the final internal temperature measured in the geometric center was 90 ºC, they were taken from the griddle. Then the samples were cut into similar size cubes and all fat and burned pieces were removed. The samples were offered to tasters in plastic plates, heated in microwave oven for 30 seconds until reaching 50 ºC. For the aroma analysis, the samples were completely immersed in water in a 100 mL beaker, and cooked in water bath for 10 min. After, they were given to tasters in the beakers on a heated plate at 100 ºC.

The multiple comparison test was applied for the strange aroma intensity and strange flavor with the following scale: 1 - extremely less intense than the control; 2 - much less intense than the control; 3 - fairly less intense than the control; 4 - slightly less intense than the control; 5 - equal to control; 6 - slightly more intense than the control; 7 - fairly more intense than the control; 8 - much more intense than the control; and 9 - extremely more intense than the control. The control refers to the samples from the steers that did not receive whole cottonseed addition to the diet; all the other samples from treatments with cottonseed were compared with the control.

The experiment was set in a completely randomized design with 4 treatments and 9 replications. When significant (P>0.05), the effects of the treatments were estimated by regression analysis. For the statistical analysis, PROC MIXED of SAS (Statistical Analysis System, version 8.0) was utilized.

Results and Discussion

There was no difference (P>0.05) among the treatments for the content of protein and fixed mineral residue in meat (Table 2). The increase of cottonseed in the diet reduced (P<0.05) the concentration of intramuscular fat linearly and increased (P<0.05) the moisture content linearly. This occurred because polyunsaturated fats represent the main components of ether extract found in cottonseed and do not undergo ruminal fermentation for the production of volatile fatty acids, inhibiting the growth of ruminal microbiota and affecting the synthesis and flow of microbial protein to the abomasum, which probably slowed down muscle development (Owens et al., 1995). It was concluded that the developmental pace of steers fed greater contents of cottonseed was depressed due to the smaller concentration of intramuscular fat in meat. However, Madruga et al. (2008), when using 0, 20, 30 and 40% of cottonseed in the diet of sheep, did not find any difference among the treatments for meat centesimal composition.

There was no difference (P>0.05) for shear force among the treatments. Similarly, a lipid-rich diet with flaxseed did not interfere in tenderness or succulence of meat from steers (Labrune et al., 2008). However, the addition of frying oil in the diet of steers promoted a more tender meat (Nelson et al., 2008).

There is evidence that a high value of shear force is a characteristic of *Bos taurus indicus* (Shackelford et al., 1994) cattle and that the increasing participation of zebu genes (*Bos taurus indicus*) in the genotype results in harder meat (Sherbeck et al., 1996). However, other factors influence the texture, such as the feedlot time and feed composition. Junqueira (1996), for example, verified shear force value of 4.6 and 4.0 kg, respectively, on the 3rd and 14th day after slaughter in the meat from young ½Marchigian × ½Nellore cattle fed 60% of concentrate and 40% of oat hay for 182 days.

Lipid oxidation is a change that may occur during the processing, distribution and preparation of meat, causing other changes in the food and altering the nutritional quality, color, flavor, aroma and texture (Shahidi & Wanansundara, 1992). The meat with abundant unsaturated fat is more susceptible to oxidation and has been associated with unpleasant flavor (Mottram, 1998).

We observe that the values of substances reactive to 2-thiobarbituric acid were similar (P>0.05) among the treatments. Therefore, it was not confirmed thad lipid oxidation aggravation is related to supplementing cattle with cottonseed. Thus, it is unnecessary to utilize antioxidants in the diet of steers fed cottonseed. Similarly, the addition of frying oil did not interfere in meat shelf life (Nelson et al., 2008).

Color is one of the main attributes of meat that most affects the amount and chemical state of the principal pigment, myoglobin (Felício, 1999), so the higher the concentration, the darker the meat. Consumers discriminate dark meat because they associate it with more mature animals, and consequently, with greater meat toughness (Sainz, 1996). Older animals and the ones that exercise intensively have darker color meat (Felício, 1999). Young bull meat is darker than the meat from male and female calves (Purchas et al., 1990).

Analyzing meat color (Table 2), we can observe that luminosity (L*) ranged from 32.758 to 33.611; the red intensity varied from 13.967 to 14.598; and yellow intensity from -0.226 to 0.071. The average L* was 32.85, which is similar to the average related by Rodrigues & Andrade (2004). There was no change (P>0.05) of luminosity, red intensity and yellow intensity when cottonseed was utilized in the diet. This possible similarity may be related to the similar ages of animals and the same feedlot regimen. Costa et al. (2008), studying the meat color of castrated Nellore calves, slaughtered at 36 and 48 months of age, found similar values of luminosity.

The results (Table 3) for luminosity, red intensity and yellow intensity of fat were similar (P>0.05) among the treatments. Cattle fat color depends on age, gender and breed. Diet is a more extrinsic factor, but it depends on the feed time (Dunne et al., 2009). Color variation of cattle fat is a result of carotenoid accumulation that makes it yellowish (Felício, 1999). Cattle raised on pasture generally have darker yellow fat than animals raised in intensive systems with high levels of concentrate because of carotenoids present in the forage (Dunne et al., 2009). A consumer usually rejects yellow fat and prefers the white one, although there are no problems nutritionally, but the yellow intensity is usually associated with older animals (Felício, 1999).

The appearance of cattle fat is mainly affected by the absorbance of carotene and derivatives of hemoglobin, reflectance, transmittance, fat fluorescence, reflectance and fluorescence of non-lipid components such as connective tissue and cell membranes (Irie, 2001). The similarity of

Table 2 - Meat characteristics of Nellore steers according to cottonseed levels in diet

Item	Cottonseed in diet (kg/100 kg)				Standard deviation	P-value	
	0	14. 35	27.51	34.09		Linear	Quadratic
Moisture (g/100 g)[1]	74.73	75.14	75.18	75.27	0.47	0.013	0.367
Protein (g/100 g)	22.79	22.80	22.81	22.81	0.40	0.897	0.987
Intramuscular fat (g/100 g)[2]	1.42	1.01	0.97	0.90	0.26	0.001	0.010
Fixed mineral residue (g/100 g)	1.02	1.00	1.00	0.99	0.07	0.417	0.980
Shear force (kg)	4.50	4.38	4.93	4.76	0.67	0.161	0.620
SRTBA	0.013	0.014	0.014	0.012	0.01	0.962	0.586
Luminosity	32.611	33.165	32.874	32.758	2.04	0.923	0.592
Red intensity	14.192	14.352	13.967	14.598	1.24	0.773	0.756
Yellow intensity	-0.014	-0.063	-0.226	0.071	1.44	0.977	0.806

SRTBA - substances reactive to 2-tiobarbituric acid (mg of malonaldehyde/ kg of sample).
[1] $\hat{Y} = 0.01479x + 74.80$ ($r^2 = 0.86$).
[2] $\hat{Y} = -0.014x + 1.353 + 0.005$ ($r^2 = 0.842$).

Table 3 - Fat color of Nellore steers according to cottonseed levels in diets

Item	Cottonseed in the diet (kg/100 kg)				Standard deviation	P-value	
	0	14. 35	27.51	34.09		Linear	Quadratic
Luminosity	61.84	62.84	61.86	63.00	2.22	0.530	0.839
Red intensity	7.35	7.40	8.08	8.23	1.56	0.170	0.677
Yellow intensity	8.50	7.95	7.53	8.08	1.13	0.159	0.227

the breeding system and the calf ages may possibly be the reason why there was so difference among the treatments for luminosity, red intensity and yellow intensity of fat. The results of luminosity are similar to the ones obtained by Rodrigues & Andrade (2004) and Costa et al. (2008).

Butyric ($C_{4:0}$), caproic ($C_{6:0}$) and caprylic ($C_{8:0}$) fatty acids were not detected in any treatment. It was possible to verify that oleic ($C_{18:1\ cis\ 9}$) fatty acid had the greatest concentration, followed by palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) fatty acids (Table 4). These results are similar to the ones reported by Silva et al. (2006) and Silva et al. (2009).

The concentration of lauroleic ($C_{12:1}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), undecanoic ($C_{11:0}$), decenoic ($C_{10:1}$) and pentadecanoic ($C_{15:0}$) acids was not altered (P>0.05) by cottonseed diet. Madruga et al. (2008) did not find a difference among the treatments for the content of $C_{10:0}$ and $C_{12:0}$ utilizing 0, 20, 30 and 40% of cottonseed in the diet of sheep. Mir et al. (2008) did not verify a difference in the content of $C_{15:0}$ when adding 15% of sunflower seed oil in the diet of finishing cattle.

With the addition of cottonseed in the diet, the contents of *iso*-tridecanoic ($C_{13:0\ ISO}$), *anteiso*-tridecanoic ($C_{13:0\ ANTEISO}$), *iso*-pentadecanoic ($C_{15:0\ ISO}$), *anteiso*-pentadecanoic ($C_{15:0\ ANTEISO}$) and *iso*-heptadecanoic ($C_{17:0\ ISO}$) fatty acids increased linearly (P<0.05). However, reduction (P<0.05) of myristoleic ($C_{14:1\ cis\ 9}$), palmitoleic ($C_{16:1\ cis\ 9}$) and margaric ($C_{17:0}$) acids is verified. According to Silva et al. (2009), the addition of 15% of sunflower seed in the diet of finishing cattle did not change the concentration of $C_{14:1\ cis\ 9}$ acid, but resulted in a smaller content of $C_{16:1\ cis\ 9}$. Mir et al. (2008) verified decrease in the content of $C_{17:0}$ when sunflower seed was included in the cattle diet.

The lipid-rich diet did not change (P>0.05) the content of myristic ($C_{14:0}$), $C_{16:0}$ and iso-hexadecanoic ($C_{16:0\ ISO}$) fatty acids, but there was a linear increase (P<0.05) of the content of *iso*-tetradecanoic ($C_{14:0\ ISO}$). Felton & Kerley (2004) verified that the meat from animals that were fed diets with great content of lipids had smaller concentrations of $C_{14:0}$ and $C_{16:0}$.

The increase in the amount of cottonseed in the diet decreased (P<0.05) the concentration of heptadecenoic ($C_{17:1}$) acid. Silva et al. (2009) also verified a reduction of the content of $C_{17:1}$ in the meat from Nellore calves that received diets with protected fats.

The increase of cottonseed in the diet (P<0.05) elevated the concentration of $C_{18:0}$ linearly because $C_{18:0}$ is abundant in cottonseed. It is important to point out that $C_{18:0}$, unlike other saturated fatty acids, acts on the reduction of serum cholesterol in humans (Bonanome & Grundy, 1988). This reduction promoted by $C_{18:0}$ can be explained by the decrease of the cholesterol absorption and increase of endogenous cholesterol excretion (Schneider et al., 2000). Preston et al. (1989) observed that the concentration of $C_{18:0}$ in meat fat was also increased with the addition of cottonseed to the cattle diet.

The inclusion of cottonseed in the diet did not influence (P>0.05) the concentration of most isomers of octadecenoic acid ($C_{18:1}$) e.g.: $C_{18:1\ trans\ 6\ -\ trans\ 9}$, $C_{18:1\ trans\ 12}$, $C_{18:1\ cis\ 11}$, $C_{18:1\ cis\ 12}$, $C_{18:1\ cis\ 13}$, $C_{18:1\ trans\ 16}$ and $C_{18:1\ cis\ 15}$. However, for the content of fatty acid $C_{18:1\ trans\ 10\ -\ trans\ 11}$ there was an increase (P<0.05), whereas there was a reduction (P<0.05) for the concentration of oleic acid ($C_{18:1\ cis\ 9}$). The results indicate that cottonseed also keeps a favorable biohydrogenation route to $C_{18:1\ trans\ 10\ –\ trans\ 11}$ accumulation as the main fatty acid $C_{18:1\ trans}$ in the rumen. The content of $C_{18:1}$ acid in buffalo meat was not modified by the inclusion of soybean in the diet but there was an increase of vaccenic acid concentration (Oliveira et al., 2008). Mir et al. (2008) obtained a reduction of $C_{18:1\ cis\ 9}$ and $C_{18:1\ cis\ 11}$ concentration.

The cottonseed-rich diet, that is, rich in unsaturated lipids, did not modify (P>0.05) the content of γ-linolenic ($C_{18:3\ n6}$) and α-linolenic ($C_{18:3\ n3}$). Similarly, in the experiment of Huerta-Leidenz et al. (1991), the concentration of $C_{18:3}$ did not follow the addition of cottonseed in the cattle diet.

Linolenic fatty acid ($C_{18:3}$) is considered essential because it is a synthesis precursor of polyunsaturated fatty acids that have special nutritional properties (Bressan et al., 2004). Among the acids commonly present in animal fat are linoleic acid ($C_{18:2}$) and arachidonic acid ($C_{20:4}$), which are essential compounds for humans (Rice, 1984). Although polyunsaturated fatty acids are mostly not essential, they have an important role to reduce serum cholesterol (Bressan et al., 2004).

This information is confirmed by other authors in the literature, who reported low occurrence of heart diseases, although there was a high intake of fats in Mediterranean countries where there is a diffuse use of olive oil and similar products that provide substantial absorption of fatty acids, especially oleic acid (Wood et al., 1999). As a result of this olive-oil rich diet, the occurrence of serum cholesterol reduction in humans was verified and can be compared to low-fat diets (Wood et al., 1999).

There was no difference (P>0.05) among the treatments for the content of behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$) fatty acids. Madruga et al. (2008) did not find influence of the cottonseed increase to sheep diet on the percentage of $C_{22:0}$. Silva et al. (2009) verified the increase of $C_{22:0}$ concentrations and the reduction of $C_{24:0}$ content in the meat from calves that received protected-fat diet.

The percentage of erucic ($C_{22:1}$), nervonic ($C_{24:1}$), gadoleic ($C_{20:1}$), eicosatrienoic ($C_{20:3}$), arachidonic

Table 4 - Fatty acid profile of the meat from Nellore steers according to cottonseed levels in the diets

Item	Cottonseed in diet (kg/100 kg)				Standard deviation	P-value	
	0	14. 35	27.51	34.09		Linear	Quadratic
$C_{10:0}$	0.087	0.123	0.080	0.130	0.055	0.379	0.860
$C_{11:0}$	0.000	0.000	0.002	0.001	0.003	0.425	0.918
$C_{10:1}$	0.113	0.006	0.024	0.026	0.165	0.292	0.334
$C_{12:0}$	0.083	0. 118	0.111	0.122	0.051	0.156	0.464
$C_{13:0\ ISO}$[1]	0.005	0.008	0.009	0.011	0.004	<0.001	0.762
$C_{13:0\ ANTEISO}$[2]	0.003	0.004	0.007	0.008	0.003	<0.001	0.481
$C_{12:1}$	0.030	0.027	0.027	0.032	0.009	0.865	0.138
$C_{14:0\ ISO}$[3]	0.060	0.072	0.077	0.076	0.018	0.033	0.442
$C_{14:0}$	3.489	3.279	3.221	3.121	0.569	0.184	0.863
$C_{15:0\ ISO}$[4]	0.230	0.267	0.278	0.289	0.061	0.036	0.644
$C_{15:0\ ANTEISO}$[5]	0.236	0.264	0.304	0.297	0.066	0.017	0.772
$C_{14:1\ cis\ 9}$[6]	0.794	0.532	0.565	0.543	0.203	0.007	0.057
$C_{15:0}$	0.479	0.459	0.476	0.487	0.072	0.761	0.454
$C_{16:0\ ISO}$	0.225	0.181	0.254	0.208	0.085	0.748	0.559
$C_{16:0}$	24.229	23.395	23.759	23.440	1.393	0.344	0.500
$C_{17:0\ ISO}$[7]	0.373	0.417	0.506	0.498	0.122	0.007	0.960
$C_{16:1\ cis\ 9}$[8]	3.217	2.477	2.611	2.500	0.527	0.003	0.040
$C_{17:0}$[9]	1.037	0.996	0.918	0.911	0.127	0.014	0.913
$C_{17:1}$[10]	0.712	0.538	0.441	0.500	0.164	<0.001	0.070
$C_{18:0}$[11]	17.532	21.486	21.468	21.249	3.933	0.035	0.130
$C_{18:1\ trans\ 6\ trans\ 9}$	0.052	0.013	0.061	0.047	0.077	0.783	0.370
$C_{18:1\ trans\ 10\ trans\ 11}$[12]	0.549	0.560	0.980	1.028	0.466	0.006	0.389
$C_{18:1\ trans\ 12}$	0.000	0.116	0.000	0.000	0.148	0.651	0.098
$C_{18:1\ cis\ 9}$[13]	33.223	30.265	29.575	29.360	3.317	0.008	0.297
$C_{18:1\ cis\ 11}$	1.285	1.186	1.267	1.327	0.341	0.757	0.424
$C_{18:1\ cis\ 12}$	0.426	0.434	0.447	0.343	0.155	0.431	0.337
$C_{18:1\ cis\ 13}$	0.180	0.083	0.165	0.113	0.122	0.537	0.328
$C_{18:1\ trans\ 16}$	0.063	0.155	0.154	0.120	0.090	0.095	0.118
$C_{18:1\ cis\ 15}$	0.079	0.098	0.118	0.108	0.053	0.148	0.635
$C_{18:2\ trans\ 11-\ cis\ 15}$	0.025	0.040	0.051	0.038	0.023	0.098	0.196
$C_{18:2\ cis\ 9\ –\ cis\ 12}$	4.726	5.560	5.150	6.151	2.435	0.342	0.997
$C_{18:3\ n6}$	0.118	0.152	0.140	0.140	0.052	0.443	0.297
$C_{20:1}$	0.000	0.031	0.066	0.007	0.048	0.972	0.217
$C_{18:3\ n3}$	0.357	0.328	0.319	0.365	0.126	0.915	0.523
$C_{18:2\ cis9\ -\ trans11}$	0.229	0.257	0.286	0.242	0.068	0.347	0.246
$C_{18:2\ t10\ -\ cis12}$	0.003	0.003	0.003	0.000	0.007	0.546	0.562
$C_{22:0}$	0.034	0.037	0.021	0.030	0.027	0.437	0.919
$C_{24:0}$	0.222	0.321	0.300	0.340	0.241	0.370	0.708
$C_{22:1}$	1.278	1.443	1.366	1.342	0.874	0.910	0.727
$C_{20:3}$	0.011	0.010	0.013	0.011	0.010	0.745	0.970
$C_{20:4}$	0.004	0.007	0.004	0.006	0.007	0.951	0.678
$C_{22:2}$	0.020	0.055	0.062	0.030	0.069	0.577	0.215
$C_{20:5}$	0.236	0.263	0.246	0.290	0.170	0.624	0.945
$C_{24:1}$	0.016	0.002	0.003	0.008	0.024	0.432	0.272
$C_{22:5}$	0.001	0.000	0.001	0.000	0.002	0.666	0.798
Not identifiable	3.928	3.932	4.064	4.105	--	-	

[1] $\hat{Y}= 0.000209x + 0.005$ ($r^2 = 0.958$).
[2] $\hat{Y}= 0.000139x + 0.002$ ($r^2 = 0.949$).
[3] $\hat{Y}= 0.0004964x + 0.06175$ ($r^2 = 0.88$).
[4] $\hat{Y}= 0.00416x + 0.3706$ ($r^2 = 0.428$).
[5] $\hat{Y}= 0.002015x + 0.2376$ ($r^2 = 0.92$).
[6] $\hat{Y}= -0.006x + 0.734$ ($r^2 = 0.646$).
[7] $\hat{Y}= 0.004x + 0.370$ ($r^2 = 0.933$).
[8] $\hat{Y} = -0.018x + 3.050$ ($r^2 = 0.633$).
[9] $\hat{Y} = -0.003997x + 1.104$ ($r^2 = 0.96$).
[10] $\hat{Y} = -0.006x + 0.678$ ($r^2 = 0.795$).
[11] $\hat{Y} = 0.1043x + 18.48$ ($r^2 = 0.65$).
[12] $\hat{Y} = 0.015x + 0.477$ ($r^2 = 0.849$).
[13] $\hat{Y} = -0.110x + 32.71$ ($r^2 = 0.876$).

($C_{20:4}$), eicosapentaenoic ($C_{20:5}$), docosadienoic ($C_{22:2}$) and docosapentanoic ($C_{22:5}$) fatty acids was not changed (P>0.05) by the test diet. Similarly, Silva et al. (2009) verified that the concentration of $C_{24:1}$, $C_{20:1}$, $C_{20:3}$ and $C_{22:2}$ acids were not altered by the lipid-rich diet. Oliveira et al. (2008) observed an increase in the percentage of $C_{20:4}$ in the meat from buffalo fed soybean.

There was no difference (P>0.05) among the treatments for the percentage of evaluated isomers of octadecadenoic acid ($C_{18:2}$) e.g.: $C_{18:2\ trans\ 11\ -\ cis\ 15}$, $C_{18:2\ cis\ 9\ -\ cis\ 12}$, $C_{18:2\ cis\ 9\ -\ trans\ 11}$ and $C_{18:2\ trans\ 10\ -\ cis\ 12}$. Thus, the amount of $C_{18:2}$ was not changed by the diet test, although the amount of this fatty acid is greater in cottonseed. This shows that unsaturated fatty acids in cottonseed are not totally protected in ruminal degradation, contradicting the reports of Baldwin & Allison (1983) that suggest that seed oils are protected from ruminal degradation. Nelson et al. (2008) report lower content of palmitoleic and oleic acids with the addition of frying oil in the diet and higher concentration of linoleic and vaccenic acids.

The content of conjugated linoleic acid was not changed (P>0.05) by the cottonseed diet (Table 5). This can be explained by the slow release of triglycerides in the grain, which allowed normal ruminal biohydrogenation by microorganisms. Oliveira et al. (2008), when increasing the lipid content of ration through the addition of whole soybean grain, did not observe the increase of conjugated linoleic acid concentration. However, the addition of 15% of sunflower seed in the diet of finishing sheep increased the percentage of conjugated linoleic acid in the meat (Bolte et al., 2002), suggesting that cottonseed and soybean grain in the rumen may have a different behavior from sunflower seed.

Nevertheless, some authors report that the addition of oil in the diet increases the amount of conjugated linoleic acid in the meat from ruminants. For example, in the experiment of Mir et al. (2004), the concentration of conjugated linoleic acid of cattle meat was quadruplicated after animals received with sunflower. A similar result was observed with the inclusion of soybean oil in the diet that increased the content of conjugated linoleic acid in the meat (Oliveira et al., 2008). The percentage of conjugated linoleic acid of cattle meat was increased with the addition of frying oil to the diet (Nelson et al., 2008). Possibly, in these cases, the presence of unsaturated fatty acids from the vegetable oil induced ruminal microorganisms to perform the biohydrogenation process because the existence of double bonds causes negative effects on the microbial population. During the breaking process of double bonds and consequent hydrogen addition, there is the formation of intermediate compounds, among them the conjugated linoleic acid (Jenkins & McGuire, 2006). Because unsaturated fatty acids are completely and rapidly available in the rumen, it is possible to state that the capacity of microorganisms to completely saturate the volume of linoleic acids may have been exceeded, making intermediate fatty acids from biohydrogenation reactions go to the intestines; that caused the increase of conjugated linoleic acid concentration (Oliveira et al., 2008).

The amount of saturated and undesirable fatty acids was not changed (P>0.05) by the cottonseed diet. Likewise, Huerta-Leidenz et al. (1991) did not find a difference for saturated fatty acid content between the treatments with and without cottonseed. Madruga et al. (2008) did not observe a change in the composition of saturated fatty acids in the meat from sheep cottonseed.

Contrary to the results found by Huerta-Leidenz et al. (1991), there was a reduction (P<0.05) of monounsaturated fatty acids in the meat from steers fed cottonseed. Gillis et al. (2004) reported lower concentration of monounsaturated fatty acids in the meat from calves fed lipid-rich diets. There

Table 5 - Proportions of fatty acids, strange aroma intensity and strange flavor intensity of the meat of Nellore steers according to cottonseed levels in the diets

Item	Cottonseed in diet (kg/100 kg)				Standard deviation	P-value	
	0	14. 35	27.51	34.09		Linear	Quadratic
CLA (g/100 g)	4.98	5.84	5.49	6.43	2.41	0.318	0.960
SFA (g/100 g)	48.32	51.42	51.79	51.22	4.72	0.960	0.314
MUFA (g/100 g)[1]	42.02	37.95	37.81	37.40	3.48	0.003	0.118
PUFA (g/100 g)	5.73	6.67	6.3	7.27	2.66	0.332	0.949
UFA (g/100 g)	47.75	44.62	44.11	44.67	3.79	0.057	0.205
DFA (g/100 g)	65.28	66.11	65.58	65.92	1.89	0.273	0.583
OFA (g/100 g)	27.72	26.67	26.98	26.56	1.75	0.607	0.568
Aroma	5.00	5.01	5.51	5.99	0.45	0.122	<0.001
Flavor	5.00	5.02	5.13	5.99	0.45	0.230	<0.001

CLA - conjugated linoleic acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - total unsaturated fatty acids; DFA - desirable fatty acids (UFA + $C_{18:O}$); OFA - undesirable fatty acids ($C_{14:0}$ + $C_{16:O}$); aroma - strange aroma intensity; flavor - strange flavor intensity.
[1] $\hat{Y} = -0.127x + 41.20$ ($r^2 = 0.786$).

was no alteration (P>0.05) in the content of polyunsaturated fatty acids, and the experimental diet also had no influence (P>0.05) on the amount of total unsaturated fatty acids. The percentage of desirable fatty acids was not modified either (P>0.05). Madruga et al. (2008) did not notice the change in the percentage of monounsaturated, polyunsaturated, total unsaturated and desirable fatty acids in the meat from sheep fed cottonseed. Felton & Kerley (2004) did not observe alterations in the concentration of monounsaturated, polyunsaturated and saturated fatty acids in the meat from animals that did not receive diets with greater amounts of lipids.

Regarding the sensorial analysis (Table 5), it is verified that the cottonseed addition over 34.09 kg for every 100 kg of the diet (P<0.05) resulted in slightly more intense strange flavor. This result is in accordance with the ones found by Pesce (2008), who did not find any alteration in the flavor of meat from cattle fed up to 20% of cottonseed in the diet when studying the cottonseed influence in meat attributes.

Concerning meat flavor, the addition of cottonseed over 27.51 kg/100 kg of the diet of Nellore cattle caused (P<0.05) a slightly more intense strange aroma than the control. Similarly, Pesce (2008) did not verify strange aroma in the meat from cattle supplemented with up to 20% of cottonseed in the diet.

Wood et al. (2003) verified that the muscles with high levels of $C_{18:2}$ oxidate quickly when heated, producing several volatile compounds, including pentanal and hexanal aldehydes, which affected the aromatic quality of the meat. However, the content of $C_{18:2}$ seems not to be the reason for the alteration of the meat flavor and aroma when the diet with cottonseed did not increase the concentration of $C_{18:2}$, even though $C_{18:2}$ corresponds to more than 50% of the fatty acids present in cottonseed.

The influence of fatty acids in meat flavor was studied by Jenschke et al. (2008), who observed that when the percentage of $C_{18:2\ n\text{-}6}$ increased, the unpleasant flavor decreased, while when the concentration of $C_{18:2\ cis\ 9\ -\ trans\ 11}$ and $C_{20:1\ n\text{-}9}$ increased, the unpleasant flavor was more evident. LaBrune et al. (2008) reported that a lipid-rich diet with flaxseed increases the percentage of linoleic acid without changing the flavor.

The quicker oxidation of unsaturated fatty acids, especially those with more than two double bonds, is an important regulator of meat shelf life (Wood et al., 2003). Arachidonic acid, for example, is more susceptible to lipid oxidation during heating and has been associated with unpleasant flavor (Mottram, 1998). However, this association of lipid association with alteration of aroma and flavor does not seem to be confirmed since the content of total unsaturated fatty acids was unaltered.

Conclusions

There is a decreasing linear relationship between the cottonseed level in the diet and the intramuscular fat of meat from Nellore steers. The increase of cottonseed levels in the feed of Nellore calves does not change the concentration of protein, ashes, conjugated linoleic acidor saturated and total unsaturated fatty acids. The utilization of up to 34.09 kg of cottonseed per 100 kg of diet of Nellore steers does not cause lipid oxidation of meat and does not change shear force or meat and fat color. The aroma and flavor of meat are negatively altered by the addition of cottonseed above 27.51 and 34.09 kg/100 kg of the diet, respectively.

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