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Whole cottonseed, vitamin E and finishing period affect the fatty acid profile and sensory traits of meat products from Nellore cattle

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ABSTRACT

This study investigated how different finishing periods and the inclusion of whole cottonseed and vitamin E in diets fed to feedlot cattle affect meat lipid composition and sensory traits of fresh beef and hamburgers. Fifty-four Nellore bulls were fed 3 different diets (C: control; WCS: 30% whole cottonseed; WCSE: 30% whole cottonseed plus vitamin E) during finishing periods of 83, 104, and 111 days. The inclusion of cottonseed did not affect saturated fatty acid levels (SFA), but increased the levels of certain polyunsaturated fatty acids (PUFA) in meat. The SFA levels and n-6/n-3 ratio increased over the length of finishing period. In general, meat products from animals fed the WCS and WCSE diets were more tender and juicier ($P < 0.05$); however, an off-flavor was detected by the panelists ($P < 0.05$). The sensory difference test results showed that the WCS hamburger flavor was not significantly different for the studied lengths of finishing period. Addition of 30% DM cottonseed in diets for cattle did not promote changes likely to affect human health, and it provided a more tender and juiciness meat, however differences in the off flavor were perceived only by panelist.

1. Introduction

The consumption of meat products, especially fresh and processed beef have been increased in recent years due to better income distribution, stimulating the consumption of added-value products (Delgado, 2003). Moreover, beef is rich in B vitamins, minerals, and high biological value proteins. But, meat fat is considered unhealthy by consumers since it has been associated with increasing cholesterol, obesity, and cardiovascular diseases in humans as reviewed by Scollan et al. (2006).

On the other hand, fat and fatty acids (FA) contribute to the sensory quality of meat, since they affect meat tenderness, juiciness, and flavor. Additionally, the intramuscular fat released during the cooking period is involved in stimulating salivation and the perception of juiciness and tenderness (Wood et al., 2003).

Some studies have shown that the diet fed to ruminants changes meat FA profile (Kemp, Mahyuddin, Ely, Fox, & Moody, 1981; Woods & Fearon, 2009). Lipid sources, rich in polyunsaturated FA (PUFA), added to finishing diets of cattle may improve FA profile in beef, increasing the concentrations PUFA and conjugated linoleic acid (CLA) (Oliveira et al., 2011; Shingfield, Bonnet, & Scollan, 2013). According to Bertrand, Sudduth, Condon, Jenkins, and Calhoun (2005) cottonseed contains 55.9% of PUFA, even though a considerable portion of PUFA will be biohydrogenated in the rumen (Harfoot & Hazlewood, 1997). Several PUFA intermediates produced in the rumen are accumulated in the muscle and adipose tissues (Aldai, Renobales, Barron, & Kramer, 2013). It has been suggested that unsaturated FAs in oilseeds are partially protected from biohydrogenation when fed with the intact seed coat (Baldwin & Allison, 1983).

However, some unsaturated FA has been associated with the development of bad odors and taste (off-flavor) in meat that result from oxidation, reducing consumer acceptance (Calkins & Hodgen, 2007; Wood & Enser, 1997). Therefore, one strategy to minimize FA oxidation could be to use antioxidants. Vitamin E has been used in animal feed to inhibit lipid oxidation and increase the shelf life of the final product (Juárez et al., 2012).

Another factor that could alter lipid composition of meat, besides animal nutrition, is the length of finishing period in feedlot (Aldai et al.,

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2011; Warren et al., 2008). Duckett, Wagner, Yates, Dolezal, and May (1993) found linear decrease in PUFA level over the finishing period, while monounsaturated FA (MUFA) increased in the *longissimus* muscle.

There are some studies that investigated the addition of whole cottonseed (Costa et al., 2013; Polviset et al., 2015) and vitamin E in cattle diets (Machado Neto et al., 2014), however there are no studies that associated these ingredients with different lengths of finishing period affecting meat sensory traits and FA profile. To this end, this study evaluated how the addition of 30% DM whole cottonseed and 500 IU vitamin E in diets fed to Nellore bulls confined during 83, 104, and 111 days affected beef FA profile, and beef and hamburger sensory traits.

2. Material and methods

The experiment was conducted in a feedlot of the University of Sao Paulo, located in Pirassununga, SP, Brazil. The experimental procedures were approved by the Animal Ethics Committee of the School of Veterinary and Animal Science, University of Sao Paulo, 500393, and Human Research Ethics Committee of the Brazil, 17115813 and 16386113.

2.1. Animal handling and diets

Fifty-four Nellore bulls reared on pasture with twenty-four months and an initial body weight of 350 kg ± 30 kg were used. These animals were randomized into three blocks according to the initial weight and divided in pens of three animals. Adaptation period was of 21 days. They were fed a diet with a forage:concentrate ratio of 14:86, where forage used was dehydrated sugar cane bagasse and nutritional demands were estimated by the NRC system (1996) with the aim of daily mean weight gain of 1.2 kg/day. Three feeding rations were studied: 1) control, without cottonseed (C); 2) 30% DM of whole cottonseed (WCS); and 3) 30% of whole cottonseed and 500 IU vitamin E/kg DM (WCSE; Table 1). The diets were offered *ad libitum* once a day in the morning. Vitamin E supplementation (Lutavit E 50 from BASF®) was placed in troughs under the concentrate and consisted of 50% alpha-tocopherol acetate. Bulls were slaughtered after 83, 104 and 111 days of feeding.

2.2. Slaughtering, sampling and hamburger processing

The animals were slaughtered in the teaching slaughterhouse of the University of Sao Paulo in Pirassununga, SP, Brazil, supervised by the State Inspection Service. After slaughter, carcasses were cooled at 0–2 °C for 24 h and then the *longissimus lumborum* muscle was removed. From the muscle 2.5 cm thick steaks were obtained between the 12 and 13th ribs, individually vacuum-packed, and frozen immediately at − 18 °C for further analyses.

Additionally, *longissimus thoracis* samples with fat of each animal were removed and used to prepare hamburgers. The meat and fat samples were ground separately in industrial grinder. Then, ground meat was prepared mixing lean (85.4%), fat (12%), salt (2%), garlic paste (0.3%), and emulsifier (0.3%) from which 90 g individual hamburgers were shaped and packed in polyethylene bags, and stored at − 18 °C.

2.3. Fatty acid composition analysis

Lipids were extracted from experimental diets and *longissimus lumborum* muscle using a 2:1 mixture of chloroform and methanol (Folch, Lees, & Stanley, 1957). For diets, lipid extracted was methylated and the methyl esters were formed according to Kramer et al. (1997). For *longissimus lumborum* muscle, after extraction, two lipid aliquots (10 mg) were methylated separately using acidic (methanolic HCl; Supelco Inc., Bellefonte, PA, USA) and base (sodium methoxide; Supelco Inc., Bellefonte, PA, USA) catalysis according to Kramer et al. (1997); Kramer, Hernandez, Cruz-Hernandez, Kraft, and Dugan (2008). For quantitative purposes, internal standard (1 mg of 23:0 methyl ester; Nu-Chek Prep Inc., Elysian, MN, USA) was added before methylation. Fatty acid methyl esters (FAMEs) were analyzed by GC/FID (Agilent Technologies 7890A) using a 100 m SP2560 column and two temperature programs (175 and 150 °C) as described by Kramer et al. (2008). The samples were also subjected to a second GC/FID analysis using a 100 m ionic column SLB-IL111 to determine the CLA isomers and other FAME overlaps (Delmonte et al., 2011).

For identification purposes, specific reference standards and other useful literature detailed in Bravo-Lamas, Barron, Kramer, Etaio, and Aldai (2016) have been used. When confirmation of the chemical structure was required, FAME fractionation with silver-ion solid phase extraction cartridges was performed (Belaunzaran, Bravo-Lamas, Kramer, & Aldai, 2014; Kramer et al., 2008). Chromatographic areas were corrected according to theoretical response factors (Wolff, Bayard, & Fabien, 1995) and the internal standard was used to calculate quantitative data. Contents were expressed in % of total FAMEs quantified. The data for total lipid content of *longissimus lumborum* muscle was obtained by ether extract analysis according to AOAC method (960.39, 2000.

Table 1
Ingredients, chemical composition and fatty acid composition of the experimental diets.

Item	Diet[a]		
	C	WCS	WCSE
Ingredient (% DM)			
Whole cottonseed	–	30.47	30.47
Ground corn	57.22	28.14	28.14
Citrus pulp pellets	18.3	18.3	18.3
Dehydrated sugar cane bagasse	13.42	13.42	13.42
Soybean meal 45%	8.17	8.17	8.17
Mineral mix[b]	0.6	0.6	0.6
Calcareous	0.4	0.4	0.4
Urea	1.37	0.27	0.27
Vitamin E (IU/kg DM)	–	–	500
Chemical composition			
Dry matter (% natural matter)	89.19	91.04	91.04
Crude protein (% DM)	15.19	16.7	16.7
Ether extract (% DM)	3.74	8.32	8.32
Neutral detergent fiber (% DM)	23.46	35.74	35.74
Acid detergent fiber (% DM)	17.1	26.5	26.5
Lignin (% DM)	5.53	13.69	13.69
Ca (% DM)	1.57	1.82	1.82
P (% DM)	1.27	1.47	1.47
Crude energy (cal/kg DM)	4007.0	4398.0	4398.0
Fatty acid composition			
14:0	0.39	0.45	–
16:0	18.64	20.23	–
18:0	2.81	2.74	–
9c-18:1	27.93	24.42	–
18:2 n-6	44.76	47.92	–
18:3 n-3	1.28	1.17	–

[a] C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU vitamin E/kg DM (50% alpha tocopherol acetate).

[b] Estimated amount of mineral ingredients in the mixture assuming dicalcium phosphate, sodium chloride, cobalt sulfate and copper are 18, 40, 21 and 25% P, Na, Co, and Cu, respectively.

2.4. Sensory analysis

Fresh meat was analyzed by descriptive analysis with a trained panel. Hamburgers were analyzed by affective (acceptance) test and a difference from control test, with untrained panelists.

In the descriptive test, twelve trained panelists participated, using a 9-point structured scale for the following attributes: tenderness (1: extremely hard; 9: extremely tender), juiciness (1: extremely dry; 9:

extremely juicy), characteristic flavor and aroma (1: extremely bland; 9: extremely intense), off-flavor and off-aroma, (1: extremely intense to 9: none). The test was carried out in individual booths, under controlled temperature and lighting.

Three steaks from each treatment were randomly distributed into four sessions. The steaks were thawed (4 °C) before analysis for 24 h and cooked in an electric oven (Tedesco®, Brazil) at 180 °C (AMSA, 1995) until the core internal temperature of 71 °C.

The data were collected automatically by FIZZ (software for sensory analysis/Biosystem).

The acceptance test of hamburger was performed by one hundred untrained laboratory panelists who evaluated tenderness, juiciness, flavor and aroma using a hedonic nine-point scale (Meilgaard, Civille, & Can, 1999), as 1: disliked very much and 9: liked very much, and off-flavor and off-aroma attributes using a five-point scale, 1: absent and 5: very strong. The hamburgers were grilled on electric grill preheated to 180 °C for four minutes on each side until the internal temperature reached 71 °C (AMSA, 1995).

For the descriptive and acceptance tests, the samples were encoded with three-digit number and provided one at a time (monadic presentation) to the panelists (Ferreira, 2000). The presentation order of the samples was balanced between the panelists to minimize the effect of presentation order on the evaluation.

The difference from the control test for hamburgers Four sessions were performed, according to finishing period, with 23 panelists each session. The hamburgers were cooked in an electric oven (Tedesco®, Brazil) at 180 °C to internal temperature of 71 °C (AMSA, 1995), cut in pieces and served as the follows. In the first three sessions, the control sample (C) was compared to the other treatments (the control sample itself - C, WCS and WCSE) within each finishing period (83, 104 and 111 days in feedlot). In the fourth session, the control sample corresponded to the hamburger from animals fed with WCS for 83 days (the shorter finishing period) which was compared to itself (WCS for 83 days) and hamburgers processed with meat from animals fed WCS for 104 and 111 days. The aim of this last session was to determine if the panelists would find difference between samples processed with the meat from animals fed WCS for different times.

The samples (control + 3 coded samples) were simultaneously provided for each panelist in all sessions. Subsequently, the panelist was asked to compare the three coded samples to the control sample and assign a respective score to grade the flavor differences between the samples and control as follows zero: no difference, and 9: extremely different.

2.5. Statistical analysis

The FA composition data was checked for residual variance homogeneity considering a linear model including diets, length of finishing and interaction as fixed model, using the IBM SPSS Statistics 20. Means were compared by Tukey test ($P < 0.05$).

The sensory parameters obtained in the descriptive analysis were analyzed using a random block design, considering a mixed linear model including the fixed effect of diet and length of finishing, and random block effect (panelist). Statistical analyses were performed by the SAS® PROC MIXED software (version 9.2), using the restricted maximum likelihood method for obtaining solutions of fixed and random effects.

The acceptance test data were analyzed by SAS® PROC MIXED considering a mixed linear model including the fixed effect of diet and length of finishing, and random block effect (panelist). Means were compared by Tukey test ($P < 0.05$).

The means estimated for difference from the control test were compared using the Dunnett multiple comparison test at $P < 0.05$ significance level.

Table 2
Effect of finishing diets on the fatty acid composition (% of total fatty acids) of *longissimus lumborum* muscle from Nellore bulls.

Fatty acid	Diet[1]			SE	*P*
	C	WCS	WCSE		
SFA	39.83	40.57	41.08	0.482	0.5689
12:0	0.082	0.080	0.083	0.003	0.9290
14:0	2.759	2.865	3.097	0.091	0.3122
16:0	22.54	22.63	22.90	0.278	0.8455
18:0	12.53	13.47	13.47	0.239	0.1581
BCFA	1.520[a]	1.180[b]	1.220[b]	0.036	0.0006
ai-17:0	0.453[a]	0.335[b]	0.342[b]	0.010	< 0.0001
i-17:0	0.342[a]	0.261[b]	0.283[b]	0.008	0.0002
cis-MUFA	33.30[a]	30.00[b]	27.70[b]	0.437	< 0.0001
9c-16:1	2.340[a]	1.910[b]	1.770[b]	0.058	0.0008
9c-18:1	26.90[a]	23.70[b]	21.80[b]	0.377	< 0.0001
trans-MUFA	5.150[b]	6.200[b]	7.780[a]	0.254	0.0004
10*t*-18:1	2.770[b]	2.610[b]	4.480[a]	0.211	0.0011
11*t*-18:1	0.970[b]	1.710[a]	1.390[ab]	0.106	0.0259
PUFA	11.64	13.82	13.90	0.541	0.1371
18:2 n-6	5.670[b]	8.490[a]	8.580[a]	0.275	< 0.0001
20:4 n-6	2.517	2.131	2.113	0.123	0.3106
18:3 n-3	0.335[b]	0.401[ab]	0.426[a]	0.019	0.0449
20:5 n-3	0.499	0.441	0.474	0.034	0.7485
22:5 n-3	1.140	0.936	0.937	0.056	0.2096
22:6 n-3	0.112	0.126	0.111	0.007	0.5868
CLA	0.543	0.573	0.526	0.016	0.5204
9*c*,11*t*-	0.362	0.399	0.323	0.017	0.2242
7*t*,9*c*-	0.069	0.072	0.077	0.003	0.5148
10*t*,12*c*-	0.019[b]	0.032[ab]	0.049[a]	0.002	< 0.0001
nc-dienes (18:2)	0.587[b]	0.826[a]	0.847[a]	0.012	< 0.0001
9*c*,13*t*-/8*t*,12*c*-	0.113[b]	0.192[a]	0.168[a]	0.006	< 0.0001
11*t*,15*c*-	0.072[b]	0.112[a]	0.117[a]	0.003	< 0.0001
10*t*,15*c*-	0.069	0.063	0.076	0.002	0.1179
n-3 PUFA	2.246	2.038	2.090	0.106	0.7083
n-6 PUFA	9.096[b]	11.55[a]	11.60[a]	0.404	0.0215
n-6/n-3	4.177[b]	5.904[a]	5.901[a]	0.107	< 0.0001
P/S	0.294	0.334	0.342	0.017	0.4685

Averages followed by the same letter are not significantly different by Tukey test at 5%. SFA, saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; nc, non-conjugated; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acids; P/S, PUFA/SFA.

SFA (10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 19:0; 20:0; 21:0; 22:0; 24:0); BCFA (*i*-14:0; *i*-150; *ai*-15:0; *i*-16:0; *i*-17:0; *ai*-17:0; *i*-18:0; *ai*-19:0); *cis*-MUFA (7*c*-14:1; 9*c*-14:1; 9*c*-15:1; 7*c*, 11*c*, 12*c*, 13*c*-16:1; 9*c*, 11*c*-17:1; 6*c*, 8*c*, 9*c*, 11*c*, 12*c*, 13*c*, 14*c*, 15*c*, 16*c*-18:1; 13*c*-19:1; 5*c*, 7*c*, 8*c*, 9*c*, 11*c*-20:1; 13*c*-22:1; 15*c*-24:1); *trans*-MUFA (9*t*-15:1; 10*t*; 6*t*, 7*t*, 8*t*, 9*t*, 11*t*, 12*t*, 3*t*, 14*t*-16:1; 4*t*, 5*t*, 6*t*, 8*t*, 9*t*, 10*t*, 11*t*, 12*t*, 15*t*, 16*t*-18:1; 8*t*, 9*t*-20:1); PUFA (18:2 n-6; 18:3 n-6; 18:3 n-3; 20:2 n-6; 20:3 n-9; 20:3 n-6; 20:3 n-3; 20:4 n-6; 20:4 n-3; 22:2 n-3; 20:5 n-3; 22:3 n-3; 22:4 n-6; 22:4 n-3; 21:5 n-3; 22:5 n-6; 22:5 n-3; 22:6 n-3); CLA (7*t*,9*c*; 8*c*,10*t*; 9*c*,11*t*; 9*t*,11*c*; 10*t*,12*c*; 11*t*,13*c*).

[1] C: without whole cottonseed; WCS: diet with 30% of whole cottonseed; WCSE: diet with 30% of whole cottonseed plus 500 IU vitamin E/kg DM (50% alpha tocopherol acetate).

3. Results and discussion

3.1. Fatty acid profile

For the FA studied, there was no interaction between diet and length of finishing ($P > 0.05$) and, therefore, each factor was studied separately. Diet did not affect total and individual SFAs although differences were observed for branched-chain FAs (BCFA), *cis* and *trans*-MUFA, non-conjugated dienes (nc-diene) and some individual PUFAs (Table 2).

BCFA and *cis*-MUFA levels were higher in meat from cattle fed C diet compared to WCS and WCSE, which were similar. This fact was evident in the major individual FAs within each group, *i.e.*, iso- and *anteiso*-17:0 in BCFAs and 9*c*-16:1 and 9*c*-18:1 in the *cis*-MUFA group. Andrade et al. (2014) reported higher level of 9*c*-18:1 in meat from Angus x Nellore bulls fed without supplementation with protected lipids. Huerta-Leidenz et al. (1991) also reported that MUFA decreased in the meat of cattle fed cottonseed. The MUFA decrease can be due to high oleic acid

content in control diet, compared with cottonseed diets (Table 1), consequently, animals fed C diet presented higher oleic acid content in meat. Furthermore, MUFA content could be associated by the fact that the cottonseed can inhibit the activity of delta-9 desaturase enzyme responsible for the endogenous unsaturation of SFA to MUFA, due to the high amount of FA cyclopropenoid (sterculic and malvalic) present in cottonseed (Yang, Larsen, Smith, & Tume, 1999). Yang et al. (1999) reported that delta-9 desaturase activity decreased by 50% in Angus-cross cattle fed rumen protected cottonseed oil.

Total *trans*-MUFA and 10*t*-18:1 contents were higher in the WCSE diet (7.8% and 4.5%, respectively) compared to WCS diet (6.2% and 2.6%, respectively), while vaccenic acid (11*t*-18:1) content was higher in the WCS diet. Kay, Roche, Kolver, Thomson, and Baumgard (2005), Pottier et al. (2006) and Juárez et al. (2010) reported that high-level PUFA diets supplemented with vitamin E reduced the total *trans*-MUFA content and prevented the '11*t*-18:1 to 10*t*-18:1 shift' in plasma, milk, and backfat. Even though the mechanisms of these findings have not been clarified, present results are different from those generally reported that vitamin E would reduce oxidative stress of ruminal bacteria, resulting in a lower production of *trans* FAs, especially 10*t*-18:1 (Juárez et al., 2010; Pottier et al., 2006).

Essential FAs (18:2 n-6, 18:3 n-3) were significantly higher in muscle from animals that were fed the WCS and WCSE diets, compared to C diet, reflecting the higher content of these FAs in diets containing cottonseed (Huerta-Leidenz et al., 1991), and possibly the result of the seed coat protection (Baldwin & Allison, 1983). Shingfield et al. (2013) reported that despite the ruminal process, part of dietary PUFA bypasses, that is, it passes intact through the rumen, and it is absorbed and deposited in the animal body fat.

Rumenic acid (9*c*,11*t*-18:2) was the main CLA isomer in all muscle samples, while no differences were observed in the total amount of CLA and the two major isomers (9*c*,11*t*- and 7*t*,9*c*-18:2) among diets. On the other hand, 10*t*, 12*c*-18:2 content increased in muscle from animal fed the WCS and WCSE diets for which anti-carcinogenic, anti-obesity and anti-inflammatory properties for human health have been reported (Benjamin & Spener, 2009). Pires et al. (2008) also reported higher 10*t*, 12*c*-18:2 contents in steers fed diets with protected lipids. The accumulation of major nc-dienes (*9c*, *13t/8t*, *12c*-18:2 *11t*, *15c*-18:2) was higher in the WCS and WCSE diets compared to C diet.

In terms of total n-3 and n-6 contents, higher n-6 content and, therefore, a higher n-6/n-3 ratio were observed in the intramuscular fat of animals fed WCS and WCSE diets possibly associated to the diets lipid composition. Overall, the n-6/n-3 and P/S ratios obtained in the present study are not the ones recommended in the daily intake of humans (4.0 or below for n-6/n-3, and over 0.45 for P/S; DHSS, 1994; Wood et al., 2003).

Overall, the length of finishing also affected the FA profile of meat (Table 3). Total SFA increased while PUFA decreased with increasing finishing period, leading to an undesirable P/S ratio. The n-6 PUFAs were not affected by the finishing periods, however most of n-3 PUFAs decreased when finishing period increased, resulting a highest n-6/n-3 ratio in meat from animals slaughtered at 111 days of finishing. Likewise, Aldai et al. (2011) compared Asturianas de los Valles beef cattle finished either on pasture or in feedlot for 30 and 60 days and reported similar results.

The MUFA, CLA and nc-dienes were not affected by the length of finishing. However, the second most abundant CLA isomer, 7*t*,9*c*-18:2, presented higher concentration at 104 days of finishing. This CLA isomer is related to high levels of 10*t*-18:1 (Aldai, Dugan, Kramer, Mir, & McAllister, 2008) and the highest levels of both isomers occurred at 104 days in the feedlot.

Furthermore, another interesting observation was the higher muscle content of 20:5n-3 (21.4%) and 22:5n-3 (47.2%) compared to their precursor 18:3n-3, which was only present at 18.8% of the total n-3 PUFA content. The high content of long-chain n-3 PUFA has been reported in some cattle breeds (Kraft, Kramer, Schoene, Chambers, & Jahreis, 2006; Ruiz et al., 2005), including Zebu-type cattle (Bressan et al., 2011), evidencing the potential to elongate the 18:3 n-3 regardless of diets provided to the animals.

Table 3
Effect of finishing period on the fatty acid composition (% of total fatty acids) of *longissimus lumborum* muscle from Nellore bulls.

Fatty acid	Length of finishing (days)			SE	*P*
	83	104	111		
SFA	38.47^{b}	41.08^{a,b}	41.93^{a}	0.482	0.0146
12:0	0.080	0.085	0.080	0.003	0.6822
14:0	2.610	3.126	2.985	0.091	0.0691
16:0	21.56^{b}	23.49^{a}	23.01^{a,b}	0.278	0.0124
18:0	12.60^{b}	12.69^{b}	14.17^{a}	0.239	0.0100
BCFA	1.383	1.238	1.294	0.036	0.2572
ai-17:0	0.388	0.354	0.389	0.010	0.2932
i-17:0	0.319	0.278	0.289	0.008	0.0710
cis-MUFA	29.46	30.51	31.00	0.437	0.3692
9c-16:1	1.923	2.109	1.987	0.058	0.4375
9c-18:1	23.36	24.13	24.90	0.377	0.2771
trans-MUFA	5.999	6.854	6.280	0.254	0.3794
10*t*-18:1	2.928	3.798	3.135	0.211	0.2252
11*t*-18:1	1.411	1.246	1.405	0.106	0.7769
PUFA	15.12^{a}	12.32^{a,b}	11.92^{b}	0.541	0.0289
18:2 n-6	8.359	7.315	7.078	0.275	0.1178
20:4 n-6	2.681	2.079	2.001	0.123	0.0503
18:3 n-3	0.517^{a}	0.327^{b}	0.318^{b}	0.019	< 0.0001
20:5 n-3	0.626^{a}	0.404^{b}	0.383^{b}	0.034	0.0040
22:5 n-3	1.252^{a}	0.893^{b}	0.868^{b}	0.056	0.0077
22:6 n-3	0.142^{a}	0.112^{a,b}	0.095^{b}	0.007	0.0211
CLA	0.568	0.546	0.527	0.016	0.6138
9*c*,11*t* -	0.392	0.342	0.350	0.017	0.4634
7*t*,9*c* -	0.064 ^{b}	0.085 ^{a}	0.069 ^{b}	0.003	0.0057
10*t*,12*c* -	0.031	0.040	0.030	0.002	0.0600
nc-dienes (18:2)	0.742	0.745	0.774	0.012	0.5386
9*c*,13*t*-/8*t*,12*c*-	0.149	0.156	0.168	0.006	0.4140
11*t*,15*c* -	0.108	0.093	0.100	0.003	0.1323
10*t*,15*c*-	0.071	0.069	0.067	0.002	0.6994
n-3 PUFA	2.716^{a}	1.865^{b}	1.793^{b}	0.106	0.0011
n-6 PUFA	12.11	10.24	9.911	0.404	0.0679
n-6/n-3	4.576^{b}	4.649^{b}	5.757^{a}	0.107	< 0.0001
P/S	0.392^{a}	0.298^{a,b}	0.281^{b}	0.017	0.0213

SFA, saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; nc, non-conjugated; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acids; P/S, PUFA/SFA.
Averages followed by the same letter are not significantly different by Tukey test at 5%.
SFA (10:0; 11:0; 12:0; 13:0; 14:0; 15:0; 16:0; 17:0; 18:0; 19:0; 20:0; 21:0; 22:0; 24:0); BCFA (*i*-14:0; *i*-150; *ai*-15:0; *i*-16:0; *i*-17:0; *ai*-17:0; *i*-18:0; *ai*-19:0); *cis*-MUFA (7*c*-14:1; 9*c*-14:1; 9*c*-15:1; 7*c*, 11*c*, 12*c*, 13*c*-16:1; 9*c*, 11*c*-17:1; 6*c*, 8*c*, 9*c*, 11*c*, 12*c*, 13*c*, 14*c*, 15*c*, 16*c*-18:1; 13*c*-19:1; 5*c*, 7*c*, 8*c*, 9*c*, 11*c*-20:1; 13*c*-22:1; 15*c*-24:1); *trans*-MUFA (9*t*-15:1; 10*t*; 6*t*, 7*t*, 8*t*, 9*t*, 11*t*, 12*t*, 3*t*, 14*t*-16:1; 4*t*, 5*t*, 6*t*, 8*t*, 9*t*, 10*t*, 11*t*, 12*t*, 15*t*, 16*t*-18:1; 8*t*, 9*t*-20:1); PUFA (18:2 n-6; 18:3 n-6; 18:3 n-3; 20:2 n-6; 20:3 n-9; 20:3 n-6; 20:3 n-3; 20:4 n-6; 20:4 n-3; 22:2 n-3; 20:5 n-3; 22:3 n-3; 22:4 n-6; 22:4 n-3; 21:5 n-3; 22:5 n-6; 22:5 n-3; 22:6 n-3); CLA (7*t*,9*c*; 8*c*,10*t*; 9*c*,11*t*; 9*t*,11*c*; 10*t*,12*c*; 11*t*,13*c*).

3.2. Sensory analysis

The descriptive test of fresh meat showed an interaction effect between diet and finishing period only for tenderness ($P < 0.01$; Fig. 1). Beef obtained from treatments containing whole cottonseed (WCS and WCSE) were more tender compared to control group (C), regardless of finishing period. However, beef from the C and WCSE diets improved the tenderness over the finishing days, while WCS not presented difference with increasing finishing period. According to Miller (2014), the improving tenderness probably resulted from the increase in muscle fat. In this study, animals from WCS and WCSE diets presented 6.08% and 5.56% of ether extract in beef, while group C showed 4.70%.

Meat juiciness, characteristic flavor, off-flavor and characteristic aroma parameters were significantly ($P < 0.05$) different between diets (Table 4). Meat from bulls fed the WCS and WCSE diets was the juiciest and had the most intense characteristic flavor, in addition to the

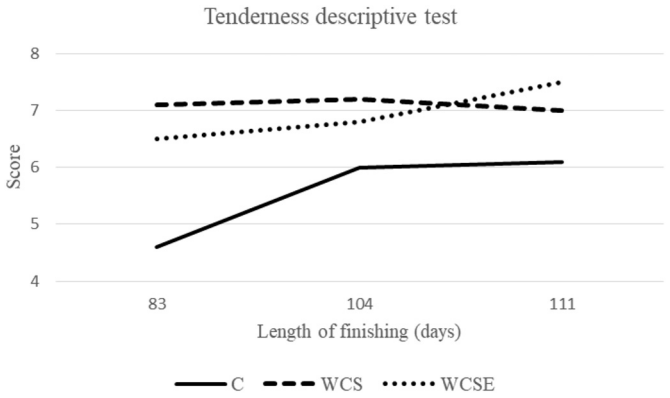

Fig. 1. Interaction for tenderness of *longissimus lumborum* muscle from Nellore bulls fed experimental diets over different finishing periods, evaluated by the descriptive test ($P = 0.0020$).
C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU of vitamin E/kg DM (50% alpha tocopherol acetate). Sensory scores from descriptive test (trained panel): [1](9: extremely tender and 1: extremely tough).

Table 4
Sensory attributes of the *longissimus lumborum* muscle from Nellore bulls fed experimental diets, evaluated by the sensory descriptive test.

Attribute	Diet[1]			*P*
	C	WCS	WCSE	
Juiciness[2]	$5.3^{b} \pm 0.198$	$5.7^{a} \pm 0.200$	$5.7^{a} \pm 0.200$	0.0300
Characteristic flavor[2]	$5.0^{b} \pm 0.244$	$6.1^{a} \pm 0.246$	$5.9^{a} \pm 0.247$	< 0.0010
Off flavor[3]	$7.4^{a} \pm 0.316$	$6.5^{b} \pm 0.319$	$6.8^{b} \pm 0.319$	0.0045
Characteristic aroma[2]	$5.6^{b} \pm 0.235$	$6.3^{a} \pm 0.237$	$5.9^{ab} \pm 0.237$	0.0053
Off aroma[3]	7.6 ± 0.277	7.8 ± 0.280	7.3 ± 0.280	0.1429

Averages followed by the same letter are not significantly different from each other, by Tukey test at 5%.
[1] C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU of vitamin E/kg DM (50% alpha tocopherol acetate).
[2] Sensory scores from descriptive test (trained panel): 9: extremely juicy, extremely intense; 1: extremely dry, extremely bland).
[3] Sensory scores from descriptive test (trained panel): (9: none; 1: intense).

most intense off-flavor as well. Meat from animals fed WCS diet had the most intense aroma compared to C diet, while the WCSE diet did not differ from the others. However, the off-aroma was not significantly ($P > 0.05$) different among diets.

Costa et al. (2013) added 0, 14.35, 27.51, and 34.09% of whole cottonseed in the diet of Nellore and reported a negative effect on aroma and flavor when cottonseed addition was higher than 27.51%. Research has shown that changing meat flavor and aroma of diets containing whole cottonseed is possibly associated with the polyphenolic compound, gossypol, present in this ingredient (Aneja, Dass, Prakash, & Chandra, 2004; Lordelo, Davis, Calhoun, Dowd, & Dale, 2005; Trischitta & Faggio, 2008).

The acceptance test results showed an interaction between diet and length of finishing for tenderness ($P = 0.0206$), juiciness ($P = 0.0049$), and flavor attributes ($P = 0.0178$) of hamburgers. The hamburgers from bulls fed WCSE and slaughtered at 104 days of finishing had lower acceptability for tenderness by consumers compared to C and WCS diets for the same finishing period. However, the scores varied little and were 7.0, 7.1 and 6.4 for C, WCS, and WCSE, respectively (Fig. 2a). The juiciness highest scores were given to hamburgers from animals fed WCSE for the 111-day finishing period and the lowest, to C group for the same period (Fig. 2b). The flavor scores assigned to hamburgers from bulls fed WCS, slaughtered at 83 days, were higher than C group

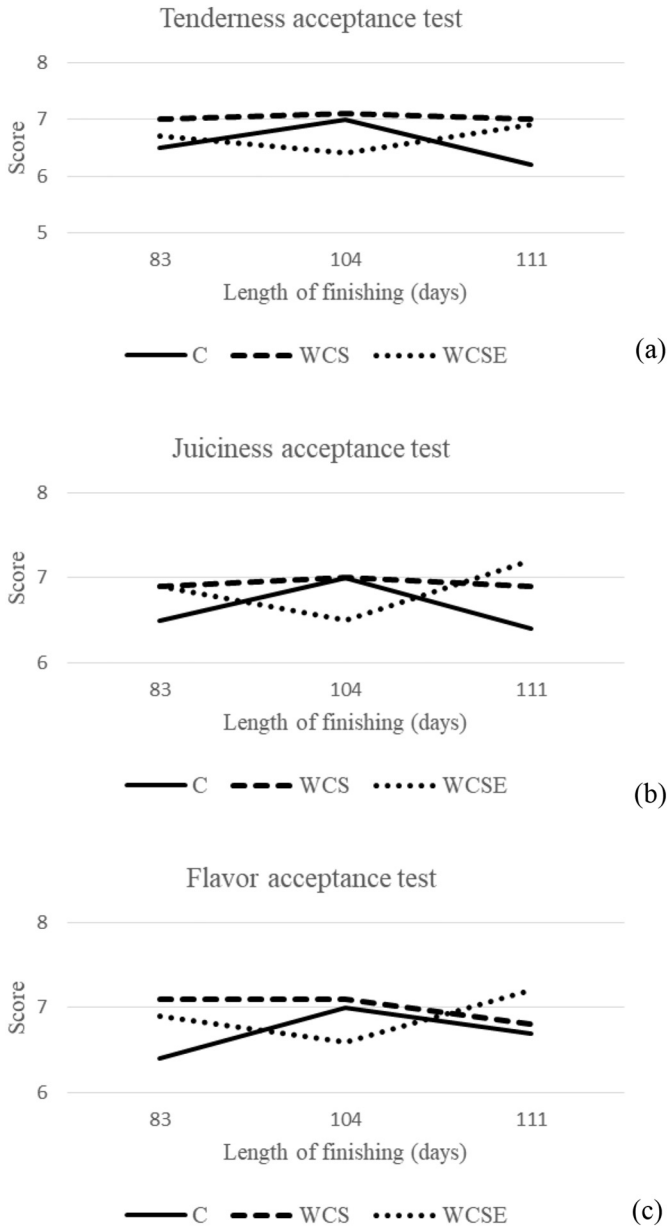

Fig. 2. Interaction for tenderness, juiciness, and flavor of hamburgers from Nellore bulls fed experimental diets over different finishing periods, evaluated by the acceptance test. C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU of vitamin E/kg DM (50% alpha tocopherol acetate).Sensory scores from affective test: (9: like extremely; 1: dislike extremely).

for the same finishing period. However, the hamburgers from animals fed WCSE and finished at 111 days were more acceptable than those slaughtered at 83 days (Fig. 2c).

In general, the WCS and WCSE diets provided tender and juicy fresh meat and hamburgers. Cônsolo et al. (2014) fed Nellore with 0, 8, 16 and 24% DM whole raw soybean and reported that up to 24% DM soybean, the meat was more tender for consumers.

Meat tenderness, measured by sensory analysis, may be related with backfat thickness (BF), since animals fed WCS and WCSE presented higher BF (4.12 and 3.29 mm, respectively) compared to treatment C (2.00 mm; unpublished data). The BF provides protection for the carcass against the low temperatures observed in cold storage, preventing the shortening of muscle fibers caused by abrupt temperature drop on the muscle surface, thereby reducing meat toughness (Smith, Dutson, Hostetler, & Carpenter, 1976). This fat increase is related to the deformation theory, based on the weakening of the perimysium

Table 5
Sensory attributes of hamburgers from Nellore bulls fed experimental diets, evaluated by the acceptance test.

Attribute	Diet[1]			SE	P
	C	WCS	WCSE		
Off flavor[2]	1.8[ab]	2.0[a]	1.8[b]	0.117	0.0212
Aroma[3]	6.7	6.8	6.9	0.164	0.3076
Off aroma[2]	1.7	1.9	1.7	0.111	0.0730

Averages followed by the same letter in the row are not significantly different from each other, by Tukey test at 5%.
[1] C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU of vitamin E/kg DM (50% alpha tocopherol acetate).
[2] Sensory scores from affective test: (1: none; 5: very strong).
[3] Sensory scores from affective test: (9: like extremely; 1: dislike extremely).

Table 6
Results from the difference from the control test, regarding the flavor of hamburgers from Nellore cattle, fed experimental diets over different finishing periods.

Length of finishing (days)	Diet[1]		
	C (standard)	WCS	WCSE
83	3.192	3.615	3.769
104	2.696[b]	3.804[a]	3.482[a]
111	2.380[b]	4.040[a]	3.720[a]

Averages followed by the same letter in the row are not significantly different from each other, by Dunnett test at 5%.
[1] C: without whole cottonseed, WCS: diet with 30% of whole cottonseed and WCSE: diet with 30% of whole cottonseed plus 500 IU of vitamin E/kg DM (50% alpha tocopherol acetate).

connective tissue that causes tissue deformation, consequently, decreasing meat cold shortening effect.

Furthermore, tenderness characteristic can be associated with energy density. Diets containing oilseed, WCS and WCSE had higher energy density. Miller, Cross, Crouse, and Jenkins (1987) and Cranwell, Unruh, Brethour, and Simms (1996) observed that high energy density diets increased the solubility of collagen, contributing to meat tenderness.

For hamburgers evaluated by acceptance test, there was difference for off-flavor ($P < 0.05$) among diets, hamburgers from bulls fed the WCS diet had the lowest acceptability, while no difference was observed ($P > 0.05$) for off-aroma and characteristic aroma (Table 5).

When comparing acceptance and descriptive tests, the aroma was different only for fresh meat. The trained panel detected a more intense aroma in the meat from animals fed WCS in only one score compared to the C group that received neither intense nor bland score, while there was no difference in this attribute by acceptance test for hamburgers.

The characteristic flavor was more intense for meat from the WCS and WCSE diets, regardless of finishing period and vitamin E addition (Table 4). This attribute was classified as slightly intense, differing from C diet, which scored neither intense nor bland, demonstrating that diets with high lipid content (8.32%) and rich in oilseeds result in tasty meat products. The red meat flavor is highly dependent on the diet; high-energy diets rich in grains yield meat with more intense flavor than low-energy diets rich in forage (Bowling et al., 1978; Melton, 1990).

Consumers that performed the acceptance test detected a slight off-flavor assigned to WCS hamburger, however hamburgers from WCSE had lower score, indicating no off-flavor. Hamburgers from C diet did not present difference among WCS and WCSE diets (Table 5). On the other hand, the descriptive test results indicated that both WCS and WCSE diets yielded meat with more intense off-flavor, corresponding to the slightly bland score (Table 4). Therefore, the inclusion of vitamin E in the WCSE diet not efficiently minimized off-flavor, while meat from cattle fed the C diet had moderately bland flavor. The difference between results from sensory analysis can be explained by the use of trained panel and the addition of ingredients to make the hamburgers, which could mask meat flavor.

Furthermore, the off-flavor difference between C and WCS or WCSE diets could be linked to the fatty acids composition (Richards & Morrison, 1969; Love & Pearson, 1971). In general, the thermally induced oxidation of PUFA produces volatile compounds that can contribute to the desirable or undesirable meat flavor, depending on the type, amounts and proportions in the meat (Elmore, Mottram, Enser, & Wood, 1999). For example, the PUFA 18:2 n-6 content was higher in meat from WCS and WCSE when compared to C group (8.49, 8.58 and 5.67%, respectively). Calkins and Hodgen (2007) concluded that the oxidation of linoleic and arachidonic FA originate hydroxyperoxide-9 and hydroxyperoxide-11, respectively, during cooking, which can produce 2,4-decadienal, 2-nonenal, hexanal, and other important compounds that determine the characteristic meat flavor and aroma. However, the high concentrations of the 2,4-decadienal and hexanal compounds resulting from the oxidation of linoleic acid can also produce undesirable flavor and aroma in cooked meat.

For the difference test, there was no difference ($P > 0.05$) among hamburgers from bulls fed the experimental diets and slaughtered at 83 days, indicating that the use of cottonseed with or without vitamin E supplementation did not change meat flavor at 83 days of finishing. Nevertheless, the same was not observed for hamburgers from animals slaughtered at 104 and 111 days of finishing. For these finishing periods, the consumer detected a difference ($P < 0.05$) in the flavor of hamburgers from WCS and WCSE diets compared to C diet (Table 6). Regarding to cattle fed WCS for different length of finishing, hamburgers from animals slaughtered at 104 and 111 days of finishing did not differ from the 83 days (Table 7), suggesting that regardless of feeding period, the diet with cottonseed affected the meat product similarly.

The results of the difference and descriptive tests showed that vitamin E was not effective to improve meat flavor when animals are fed cottonseed. The lack of response of the evaluated traits to the use of vitamin E may be explained by the low vitamin E concentration used. Juárez et al. (2012) evaluated the meat from cattle fed with 10% DM ground flaxseed and vitamin E (451 and 1051 IU) and reported no difference in tenderness, juiciness, characteristic flavor, and off-flavor attributes.

Regarding the length of finishing, to our knowledge, the literature review showed that there are no studies simultaneously investigating how the use of whole cottonseed in cattle finishing diets and the feeding period affect meat sensory attributes. We expected that the off-flavor detected in meat products would be mild or even imperceptible to consumers for the shorter length of finishing. Costa et al. (2013) fed Nellore cattle diets containing different levels of whole cottonseed during 94 days in feedlot and reported off-flavor in the meat from animals fed a diet with 34.09% cottonseed. Moreover, Medeiros et al. (2005) used only 9.5% DM whole cottonseed for about 180 days of feedlot and concluded that the use of 9.5% did not affect the meat flavor, even for extended periods. Likewise, Gibb et al. (2004) investigated the inclusion of 10.8 and 14% DM sunflower seeds and 1000 IU/animal/day vitamin E in the diet of British cattle at 172 days

Table 7
Results from the difference from the control test for the flavor of hamburgers from Nellore cattle fed the 30% DM whole cottonseed diet over different finishing periods.

Length of finishing (days)	Diet
	WCS
83 (standard)	3.056
104	3.352
111	3.463

WCS: diet with 30% of whole cottonseed.

and reported similar results.

It is noteworthy that although some differences were observed in the sensory attributes in this study, these differences were less than one score and probably would be not easily detected by the consumers. Additionally, different sensory analysis methods were performed. The use of trained and non-trained panels to evaluate the meat products including hamburger, a meat product processed with the addition of other ingredients may have resulted in small discrepancies between the results of different methodologies.

It is important to highlight that in addition to whole cottonseed added to the beef cattle diet and the finishing period, other factors like the variety of cottonseed used (gossypol concentration), the forage: concentrate ratio and type of forage and concentrate added to the diet can alter the lipid composition of meat products could alter meat sensory attributes.

4. Conclusion

The inclusion of 30% DM whole cottonseed in diets for beef cattle does not promote changes likely to affect human health. However, the lipid composition is affected by finishing period. Animals finished a shorter period, like commonly used in brazilian feedlot provide better lipid composition. The whole cottonseed provides more tender and juicier fresh beef and hamburgers 111 days finished animals. On the other hand, this ingredient gave an off-flavor for the fresh beef. However, use of whole cottonseed in diets is not unfeasible, because for hamburgers the off-flavor was not perceptible. Therefore, the perception of the off-flavor can decrease overall acceptability of meat. The inclusion of vitamin E affected negatively the lipid composition of the meat due to 10*t*-18:1 increases, furthermore, vitamin E did not affect meat sensory attributes, especially to prevent the off-flavor associated to whole cottonseed fed animals.

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