Sensory characteristics and volatile compounds of beef in feedlots with cottonseed

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Abstract

The aim of this study was to evaluate the sensory characteristics of meat from cattle fed a cottonseed diet. A total of 100 beef cattle, with a mean initial live weight of 386.19 ± 4.48 kg and a mean slaughter weight of 527.82 ± 17.96 kg, aged between 13 and 48 months, were maintained in the feedlot for 88 days. This study was conducted with two treatments: a control treatment and feeding cattle whole cottonseed at a concentration of 15.9%. The animals were slaughtered for determination of sensory and volatile compounds in the beef. There was a difference in aroma, with better grades attributed to beef not fed cottonseed. The use of cottonseed promoted the occurrence of a strange taste in beef. There was a difference in the profile of volatile compounds between the groups. In general, feeding cattle cottonseed did not modify the qualitative sensorial aspects of beef; however, there was a greater chance of the development of some strange flavors of high intensity. The volatile compound profile of beef from cattle fed cottonseed was different than that of beef from control group.

Keywords: beef cattle; nutrition; byproduct; meat quality.

Practical applications: Sensory traits may be affected when cottonseed is used to feed cattle in feedlots.

1 INTRODUCTION

Fattening cattle in feedlot systems is a method used worldwide to achieve better performance and carcass characteristics in a short time. Despite these advantages, it is very important that the feed used in this system provides sufficient nutritional benefits, is low in cost, contributes to reducing food– feed competition, and decreases the environmental impact of livestock (Esteves et al., 2017; Lima et al., 2016; Polizel Neto et al., 2022; Salami et al., 2019). In addition to these aspects, byproducts from cotton harvest have been used to feed cattle in feedlots in Brazil and worldwide in different ways, such as whole cottonseed, hulls, gin trash, and meal (Rogers et al., 2002). Whole cottonseed is useful as an energy source in the diet and is used for the feed of cattle and cows (Lobato et al., 2014; Müller et al., 2021).

Cottonseed is a byproduct of the textile industry that is used in animal production due to its economic profitability and ability to demonstrate nutritional performance similar to that of conventional feeds as a source of protein, lipids, and physically effective neutral detergent fibers (Cranston et al., 2006; Schneid et al., 2022). The use of this byproduct has increased annually in Brazil, and although some farms have used whole cottonseed in feedlots for fattening cattle, others have avoided the use of this byproduct, fearing the appearance of a "strange flavor," such as "liver" or other flavors, in beef. To answer this question, several authors have studied beef from cattle fed cottonseed at different levels or compared it to other feeds; however, the authors were not able to determine the influence of using this

byproduct on the sensory traits of meat from fattening animals (Costa et al., 2013; Ferrinho et al., 2018; Polizel Neto et al., 2022).

The effects of modifying the fatty acid composition on the lipid profile and the total fat content in beef were the main results reported for feeding cattle with cottonseed. This occurs because this byproduct is rich in lipids and is a source of polyunsaturated fatty acids (Huerta-Leidenz et al., 1991; Müller et al., 2021; Salami et al., 2019). Thus, this ingredient can be easily oxidized (Zia et al., 2022), and when exposed to environmental conditions, it can produce substances such as iron. Aldehydes and ketones can transfer this flavor to meat by themselves (Gianelli et al., 2012) or by changing the meat composition when the profile of fatty acids is altered, and aromatic compounds associated with an unpleasant flavor from the Maillard reaction or lipid oxidation are produced during cooking (Kosowska et al., 2017; Legako, 2020; Mottram, 1998).

To determine the cause of this difference, an analysis of sensory traits is needed to determine whether there is any change in beef flavor when cottonseed is used to feed cattle. This is complementary to analytical techniques, making it possible to determine, which compounds are related to a sensory trait. In addition to evaluating sensorial traits, gas chromatography combined with mass spectrometry can aid in understanding the differences in juiciness and palatability in meat through the identification of flavor compounds present in the meat (Lee et al., 2011; Stetzer et al., 2008; Watanabe et al., 2008). Previously, studies have associated changes in beef flavor with an increase in linoleic acid in the meat to

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cattle fed in feedlots using different feedstuffs (Neethling et al., 2016). This difference could be attributed to the large number of derivative compounds produced from this fatty acid when the meat is heated and cooked (Elmore et al., 2004; Ferrinho et al., 2018; Polizel Neto et al., 2022).

Therefore, the objective of this study was to evaluate the effects on sensory traits and the composition of volatile compounds in the meat of commercial cattle fed in a feedlot using whole cottonseed in the diet.

2 MATERIALS AND METHODS

2.1 Experimental design, diets, and sampling

The field experiment was conducted in a feedlot with 100 intact Nellore males randomly distributed into two groups according to the experimental diet with the addition or absence of 15.9% whole cottonseed. These cattle had an initial weight of 386.19 \pm 4.48 kg and were slaughtered at 527.82 \pm 17.96 kg between 13 and 48 months of age.

The experimental design was completely randomized into two treatments: control treatment (CT), animals in which there was no cottonseed in the diet, and cottonseed treatment (CS), animal feed contained cottonseed at a level of 15.9% in the diet. Each treatment group had 50 replications, where each animal was considered a replicate. The animals in the CT and CS groups were held in a feedlot for 88 days, and at the end of the experiment, they were slaughtered after a 12-h solid fast and under humanitarian conditions.

After carcass cooling for 24 h at \pm 1°C, samples were taken from the *longissimus thoracis* muscle between the 12th and 13th ribs (sirloin cut), packed with film paper (polyethylene), and identified and frozen at -18°C for transportation and subsequent laboratory analysis. This study was approved by the Committee on Ethics in the Use of Animals (CEUA) of the UFLA, protocol number 040/12, and by the Committee of Ethics in Research in Human Beings (COEP), protocol number 337.475.

2.2 Sensorial analysis

The sensorial analysis was carried out after defrosting the samples under refrigeration at 4°C for 24 h followed by cooking until 72°C (the internal temperature of the samples) according to Fahmy et al. (1992).

For sensorial analysis, a sensory taster panel (100 untrained tasters) was used, where the samples were coded and presented to the panelists in a paired manner: CT–CS (samples from treatment CT and CS) and CS–CT (samples from treatment CS and CT). The sensorial parameters evaluated were color, flavor, aroma, tenderness, and overall impression. The samples were evaluated on a hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (Realini et al., 2013); the data were subjected to statistical analysis. Furthermore, each taster assessed the absence or presence of abnormal taste; in a positive case, the taster's degree of intensity was low or high.

2.3 Volatile compound analyses

The extraction of volatile compounds (VCs) was performed from *longissimus thoracis* muscle samples according to the methodology of Donadel et al. (2013) and Marçal et al. (2022). Seven samples weighing 100 ± 0.1 g were randomly chosen from each treatment and were wrapped in aluminum foil and cooked on an electric plate (MegaGrill; Britânia, Curitiba, PR, Brazil) at 150°C for approximately 20 min until they reached an internal temperature of 72°C. Afterward, the samples were cooled at room temperature and crushed, and 7 g of each sample was placed in a 20-mL vial and subjected to headspace solid-phase microextraction (HS-SPME) for the isolation and identification of VCs. A divinylbenzene/carboxen/polydimethylsiloxane, 24ga (DVB/CAR/PDMS, 10 mm Supelco, Bellefonte, PA, USA) adsorption fiber was used to capture the compounds via an automatic sampler, and the fiber was exposed to the headspace of the sample for 45 min with the vial heated at 60°C in an extractor coupled to the equipment. Before extraction, each vial containing the samples was exposed to the same extraction temperature, and the fiber was exposed to the headspace for 10 min (equilibrium time). Afterward, the fiber was automatically inserted into the chromatograph injector for thermal desorption of the analytical compounds.

The VCs were separated and identified on a gas chromatograph coupled to a mass spectrometer (Shimadzu GC/MS-QP 2010 Plus; Agilent Technologies, Inc., Palo Alto, CA, USA). Thermal desorption during fiber analysis was performed with a GC injector at 250°C in split-less mode, and the fiber was kept exposed inside the injector for 10 minutes to eliminate the memory effect. The VCs were separated by a fused silica capillary column (5% diphenyl and 95% polysiloxane; model SLBTM-5MS Supelco; 30 m \times 0.25 mm \times 0.25 µm) (Supelco, Inc., Bellefonte, PA, USA). The temperature program of the column started at 35°C, was maintained for 2 minutes, and then increased to 80°C at a rate of 2°C min-1. From this temperature on, the temperature increased to 150°C with a heating ramp of 4°C min-1 and then to 230°C at a rate of 8°C min-1, after which the mixture remained in the isotherm for 5 minutes. The drag gas used was He at constant pressure and an initial flow rate of 1 mL min-1. The analyzer was operated in sweeping mode, monitoring masses from 35 to 350 m/z.

The compounds were evaluated by integration of the chromatographic peaks, followed by identification of their mass spectra with those of the GC/MS spectral library (Whiley 8 and FFNSC 1.2 Libraries), as well as by the Kovats index of the literature by the experimental time retention of the compounds and the series of alkanes used with a standard (C8 to C24) and their molecular weight according to the methodology used by Adams (2007).

2.4 Statistical analysis

For the statistical analysis of the data, the Wilcoxon test (α) $= 0.05$) was used to evaluate the sensory parameters since the samples were analyzed in a paired fashion. For the analysis of the occurrence of off flavors in meat, the χ^2 test or Fischer's exact

test (α = 0.05) was used. For the VCs in the samples, analysis of variance was performed by means of F (α = 0.05).

The data sets were analyzed, and principal component analysis (PCA) was performed. The criterion for choosing the number of principal components was one of the interpretable factors that determined that number, which together account for more than 70% of the variance. All analyses were performed using the Statistical Analysis System (SAS) statistical software program, Student version 9.1.21.

3 RESULTS AND DISCUSSION

The sensory analysis revealed that the tasters considered most of the parameters analyzed, with average scores between 5.30 and 6.74 according to the hedonic scale, e.g., most scores were indifferent (5) or like moderately (7) (Table 1). Similar results, with scores ranging from 6.7 to 7.2, were reported by Eiras et al. (2017), who analyzed parameters of acceptability of meat from young bulls fed cottonseed hulls; additionally, scores ranging from 6.4 to 7.1 were reported for meat from Nellore bulls that were fed whole cottonseed and whole cottonseed plus vitamin E by Ferrinho et al. (2018). In general, these results showed that the score of the sensory traits of beef could not be associated with the specific use of cottonseed in the feed of animals. Several authors have reported that the source of feed, which is rich in fat or lipids when it is used for fattening cattle in feedlots, tends to have a negative influence on the sensorial parameters of beef due to an increase in linoleic acid in the meat (Ferrinho et al., 2018; Polizel Neto et al., 2022). Thus, worse beef grades are more strongly associated with changes in meat quality in the production system than with the use of specific feedstuffs.

The worst grades were observed for color and tenderness parameters, independent of treatment (Table 1). These aspects are very important for consumer acceptance and are related to the production system, as they are associated with sensory aspects by consumers and impact their willingness to buy beef (Neethling et al., 2016). The main aspect to be considered in the present study was that the animals used in this study were Nellore cattle, and the beef from these animals was not aged. In the literature, animals such as *Bos indicus indicus* have been characterized as having tough meat due to the high activity of calpastatin (Scheffler, 2022), and only a period of 10 days of aging is associated with an improvement in tenderness (Bressan

et al., 2011). Therefore, these parameters can vary according to livestock practices or technological processes after slaughter, and the adoption of practices that improve sensorial parameters is recommended to enhance beef quality, which is desirable for all consumers.

The flavor parameter in the sensory analysis of beef from the animal fed cottonseed had worse scores according to the tasters (*p* = 0.0333). Similar results for beef flavor were reported by Costa et al. (2013) when more than 27.51% cottonseed was used to feed cattle in feedlots. Ferrinho et al. (2018) analyzed the sensorial traits of beef from Nellore bulls fed cottonseed or cottonseed in combination with vitamin E at a level of 30%. These authors reported greater flavor intensity in beef samples than in those from control diets when animals were not fed cottonseed. The main factor is associated with this change in lipid composition because an increase in the C18:2w6 (linoleic acid) content could be responsible for the oxidation of compounds associated with characteristic meat flavors or undesirable flavors (Ferrinho et al., 2018). In contrast, in beef in the present study, there were no effects on ether extract or fatty acid composition (Esteves et al., 2017). This could be associated with the lower level of cottonseed feed provided to the animals in the present study, which is not sufficient to change the fatty acid profile of the meat.

For the other parameters analyzed, such as color, taste, tenderness, and overall impression, the tasters did not find any difference $(P > 0.05)$ between meat from animals fed cottonseed and not (Table 1). There was no influence of cottonseed supplementation on color parameters indicated by the tasters; moreover, other authors have reported no negative influence of cottonseed or other byproducts used for feeding cattle on the sensorial attributes of beef (Eiras et al., 2017; Gomes et al., 2016; Polizel Neto et al., 2022). Despite the physicochemical analysis of the raw beef revealed a change in the objective meat color and pH (pH 24 h), there was an increase in the yellowness index (higher value of b^*) and a decrease in pH, similar to the results reported by Esteves et al. (2017). Similarly, Stelzleni et al. (2013) investigated the influence of objective color parameters of beef from finished heifers fed cottonseed pellets (which yielded a lower brightness index), and no differences in meat quality or sensory traits were reported. Thus, although there were changes in objective parameters measured in beef from cattle fed cottonseed, after the meat was cooked, these changes could not be detected by tasters.

*Wilcoxon test (α = 0.05); ID: interquartile deviation; SD: standard deviation; 1 based on a hedonic nine-point scale (1 = dislike extremely to 9 = like extremely).

There was no difference in the occurrence of an off flavor in beef samples from cattle fed cottonseed ($p = 0.1410$) or the control diet, and 82% of the tasters did not note an off flavor (Table 2). Using 15.9% cottonseed supplementation in the feed of cattle in our study did not directly affect beef sensorial traits or introduce an off flavor, showing that this byproduct can be included up to 15.9% in cattle feed. Gomes et al. (2016) reported similar results in beef cattle, reaching a level of 11.11% with the use of trained and untrained panelists to evaluate sensorial traits. Thus, these results were unexpected because some off flavors or strange flavors in beef have been reported when the level of cottonseed is greater than 30% in the diet of cattle (Costa et al., 2013; Ferrinho et al., 2018) or up to 40% for sheep (Vieira et al., 2010).

However, when some kind of off flavor was observed by tasters in beef samples from animals fed cottonseed, there was a five-fold greater chance of the flavor being characterized as high intensity by 68.2% of the tasters ($p < 0.039$, *OR* = 5.37; 95%CI 1.237–23.207). However, in beef from the CT, 71.4% of the tasters classified the samples as having a low-intensity off flavor (Table 2). In general, this increase in flavor intensity was associated with a change in the fatty acid profile (Ferrinho et al., 2018) and modification of the profile of VCs related to flavor (Vieira et al., 2010). This aspect should be considered when a feed component rich in lipids is used to feed cattle, as the profile of lipids can influence the meat composition and, consequently, the flavor of the meat.

The occurrence of off flavors modified the taste perception of some of the sensory traits evaluated. Among the samples where tasters recognized the occurrence of off flavors, they gave lower scores for color ($p = 0.0090$) and flavor ($p = 0.0271$) in the CT treatment group and for color ($p = 0.0120$), taste ($p = 0.0120$) $= 0.0021$), and overall impression ($p = 0.0030$) in the beef from the animals fed cottonseed (Table 3). Meat flavor is one of the major attributes associated with quality and beef consumer preference (Legako, 2020). Like our study, Muchenje et al. (2010) reported a relationship between perceptions of the occurrence of off flavors and aroma scores for beef. Therefore, the occurrence of off flavors could affect a consumer's sensorial perception, acceptability, and willingness to purchase beef once strange flavors create an undesirable aroma.

After the analysis of VCs, a total of 71 compounds were identified in the samples from the top series (Table 4). The principal groups of compounds were aldehydes, ketones, esters, alkanes, and alcohols, and they are associated with the formation of cooked meat flavors and odors (Kosowska et al., 2017). These compounds were generated from chemical reactions during the heating process through the Maillard reaction and lipid and protein degradation according to the bromatological composition and lipid profile of the meat (Donadel et al., 2013; Mottram, 1998).

Samples of beef from animal fed cottonseed had the highest means ($p < 0.05$) for heptanal (C903) and 12-methyltridecanal (C1576) in the aldehyde group; decan-3-one (C1186) in the ketone group; 5-isopropenyl-1-methyl-1-cyclohexene (C1027), 8-isopropyl-1,3-dimethyl-tricyclo [4.4.0 (2,7)] dec-3 ene (C1376), gamma-gurjunene (C1473), and 1-chloro-decane (C1633) in the hydrocarbon group; methyl (E)-2-hexenoate

	Diets				Overall			
Occurrence of strange taste	Control	Cottonseed (15.9%)					p-value*	OR (95%)
	(n)	(%)	(n)	(%)	(n)	(%)		
Absence	86	86	78	78	164	82	0.1410	
Present	14	14	22	22	36	18		
Level of strange taste								
High intensity	4	28.6	15	68.2	19	52.8	0.0391	5.357
Low intensity	10	71.4		31.8	17	47.2		

Table 2. Evaluation of the occurrence of off flavors in the meat and their intensity levels with the use of cottonseed (15.9%) in the feed of feedlot cattle.

 $\gamma \chi^2$ test (α = 0.05); OR: odds ratio; CS: cottonseed (15%); CT: control treatment without cottonseed.

*Wilcoxon test (α = 0.05); MD: median; AE: average; ID: interquartile deviation; SD: standard deviation; FST: found strange taste; CS: 15.9%, diet with cottonseed; TD: tenderness; OI: overall impression. 1 Based on a hedonic nine-point scale $(1 =$ extremely dislike to $9 =$ extremely like).

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*Continues.

*F-test (0.05); SEM: Standard error mean.

(C1284), methyl decanoate (C1313), heptan-2-yl butyrate (C1391), 3-pentanol, and 3-methylcarbamate (C1352) in the ester group; and 1-octanol, 2-butyl (C1534), and 2-isopropyl-5-methylcyclohexanol (C1173) in the alcohol group.

However, in control samples, there were greater values for (E)-2-decenal (C1262), dodecanal (C1410), and tetradecanal (C1614) in the aldehyde group; 3,7-decadiene (C989), 3,7-dimethyldodecane (C1320), and 2-methyltetradecane (C1483) in the hydrocarbon group; and ethyl decanoate (C1394), (E)-5-hydroxy-2-isopropenyl-5-methyl-3-hexenyl isobutyrate (C1487), and 2-methyl-4,6-dinitrophenyl acetate (C1716) in the ester group (Table 4).

In the present study, hexanal (C804), an aldehyde, was the main volatile compound found, and there was no difference in the level of this compound between the samples ($p = 0.0871$). 2,4-Decadienal and 2-nonenal originate from the oxidation of several fatty acids, such as linoleic (C18:2n6C) and arachidonic (C20:4n6C) fatty acids, and are associated with the characteristics of meat flavor (Ferrinho et al., 2018; Watanabe et al., 2008). Stetzer et al. (2008) reported that the off flavor ("liver flavor") of meat was positively correlated with pentanal, hexanal, 3-hydroxy-2-butanone, and hexanoic acid levels, compounds that can contribute to pungent, grassy or greasy, buttery, and sweat

odors, respectively. Thus, although treatment with cottonseed causes a direct occurrence of off flavors, the hexanal level was almost two times higher in meat samples from the cottonseed treatment group than in those from the control group and can contribute to the major perception of off flavors in the meat from the cottonseed group.

In relation to the compounds observed in the ketone group, there was only a difference for the decan-3-one (C1186) content, which had high values in meat from animals fed cottonseed; this compound is related to fruity and mold flavors. In addition to these compounds, the following esters and alcohols were found, namely, methyl (E)-2-hexenoate, methyl decanoate, heptan-2-yl butyrate, 3-pentanol, 3-methyl, and carbamate and 1-octanol, 2-butyl, and 2-isopropyl-5-methylcyclohexanol, with the respective pungent aromatic, fat, wax, citrus, oil and woody, cut grass, wine, fatty, and fruity flavors, according to Van Ba et al. (2012), for beef from cattle fed cottonseed.

According to the profile of VCs in beef samples from cattle fed cottonseed, there were higher concentrations of 2-isopropyl-5-methylcyclohexanol and 1-octanol and 2-butyl (Table 4). Similarly, Elmore et al. (2004) reported similar results in beef from cattle fed high-grain diets. In general, these compounds are associated with roasted, sweet, fruity, and fat odors in meat,

and some of them are related to the deterioration of meat flavor according to Resconi et al. (2013) and the lipid oxidation process (Gianelli et al., 2012). An increase in alcohol compounds in beef was associated with a stronger liver flavor (Kosowska et al., 2017).

Even though there were significant differences in the levels of some VCs according to treatment, PCA revealed that other compounds contributed to the differences between the samples. Of the 71 compounds identified in the samples, 43 were significantly different according to the PCA plot (Figure 1), which contributed to the formation of two distinct groups between the beef samples from the control and cottonseed groups (Figure 2). According to PCA, the results did not follow the same trend as the statistical results shown in Table 4 for the profiles of the compounds in each group of substances in the beef samples. Thus, considering the variable analyzed (the use of cottonseed to feed cattle), no specific associations could be established between the percentages of some compounds and treatments.

The compounds 2,5-dimethyl-3-furanthiol (C967), hexyl acetate (C1015), 5-isopropenyl-1-methyl-1-cyclohexene (C1027), 2-isopropyl-5-methyl-cyclohexanol (C1173), (Z)-4 hepten-2-yl butyrate (C1199), heptan-2-yl pentanoate (C1291), 3-methyl-3-decen-1-ol (C1308), 3-pentanol-3-methyl-carbamate (C1352), heptan-2-yl butyrate (C1391), and hexadecane (C1399) were correlated with principal component (PC) 2, and the other compounds (C892, C954, C983, C989, C1000, C1030, c1060, C1074, C1077, C1105, C1186, C1207, C1219, C1264, C1000, C1074, C1077, C1105, C1100, C1207, C1215, C1204, 5
C1274, C1320, C1376, C1407, C1410, C1413, C1437, C1473, C1478, C1483, C1534, C1539, C1576, C1614, C1633, C1679,

Eigenvalues of the correlation matrix									
	Eigenvalue			Difference Proportion Cumulative					
1	19.8480551	11.9225978	0.4616	0.4616					
$\mathbf{2}$	7.9254572	2.9169774	0.1843	0.6459					
3	5.0084799	1.6649393	0.1165	0.7624					

Figure 1. Principal component analysis (PCA) of volatile compounds in beef samples.

C1716, C1962, and C2122) were correlated with PC 1. Despite the difference in the correlation of compounds with each PC, with a greater correlation with PC 2, Figure 2 shows that the beef samples from the cattle fed cottonseed were less dispersed than those from the CT.

However, there was a set of compounds relatively frequently found in beef samples according to the treatments studied. We can distinguish three main clusters (Figure 3). One group was formed mainly by samples from the CT, with animals not fed cottonseed (yellow); the second group (red), and the third group (blue) were formed exclusively by beef samples from cattle fed cottonseed and control cattle, respectively.

The results in Figure 3 are similar to those shown in Figure 2; a greater similarity was observed for most beef samples from animals fed cottonseed (5 out of 7 samples) due to the profile of VCs that influence beef from cattle fed cottonseed in the diet.

Figure 2. PCA of CS (cottonseed) and CT (control) samples based on the profiles of volatile compounds.

Figure 3. Dendrogram of the agglomerative hierarchical clustering (AHC) of volatile compounds in beef samples showing the three main clusters (yellow, red, and blue) for the CS (cottonseed) and CT (control).

In summary, in the present study, any differences in sensory traits noted by the tasters were not associated directly with a specific compound found in each treatment.

However, most authors have established an association between the formation of VCs and oxidation processes on fatty acids in beef. In the present study, this association was not detected; there was no difference in the fatty acid profiles between beef samples (Esteves et al., 2017). Therefore, these differences in VCs are not related only to the lipid profile of beef, considering the influence of the characteristics and composition of the raw material (whole cottonseed) used to feed animals. Other studies with new approaches that focus on differences in the composition, kind of processing, and storage of whole cottonseed should be explored to establish associations that could exist between strange flavors and the quality of this byproduct; however, these associations were not considered in the present study.

4 CONCLUSION

In general, feeding cottonseed up to an inclusion level of 15.9% did not modify the qualitative sensory aspects of beef in feedlots; however, there was a greater chance of the development of some strange flavors with greater intensity with the use of this byproduct.

There was a difference in VCs between cattle fed cottonseeds and control cattle, particularly for aldehydes, ketones, hydrocarbons, esters, and alcohols; moreover, the VCs tended to have similar profiles with little variation between samples.

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