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Effects of feeding legume-grass pasture and different concentrate levels on fatty acid profile, volatile compounds, and off-flavor of the *M. longissimus* thoracis

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ABSTRACT

Pasture-finished beef is becoming more popular among consumers due to concerns related to fatty acid content and sustainable practices. The effects of finishing crossbred steers on legume-grass pasture comprised of oats, ryegrass, and clover (PAST), legume-grass pasture plus whole corn grain (WCG) supplementation (SUPP), and only with WCG (GRAIN) on fatty acids profile, volatile compounds, sensory, and texture attributes were studied. Pasture diets (PAST and SUPP) led to lower n-6/n-3 ratio ($P < 0.001$), and highest deposition of C18:2 *cis*-9 *trans*-11 ($P < 0.001$) in the lean. Beef from steers fed GRAIN had the highest values of volatile compounds associated with lipid oxidation. Off-flavor intensity was significantly greater on beef from steers fed GRAIN when compared to PAST. Overall, muscles from steers finished on PAST and SUPP showed similar attributes but differ when compared to GRAIN. The presence of forage is essential to improve fatty acid profile, decrease volatile compounds associated with lipid oxidation, and minimize off-flavor.

1. Introduction

Grazing pastures and freshly cut herbage are predominantly the most important feed sources for cattle in the world (Fanchone, Archimede, Baumont, & Boval, 2010; Freitas et al., 2014; Oliveira & Millen, 2014; Schlegel, Wyss, Arrigo, & Hess, 2016). The utilization of pastures and fresh forage with high nutritional quality such as legume-grass mixtures can improve animal performance and maintain the meat quality standard of steers finishing on pasture (Chail et al., 2016; Dierking, Kallenbach, & Grün, 2010).

Pasture finishing leads to greater deposition of *n*-3, CLA fatty acids, and lower n-6/n-3 ratio in the lean when compared with grain-finishing (Aldai et al., 2011; Duckett, Neel, Lewis, Fontenot, & Clapham, 2013; Patino, Medeiros, Pereira, Swanson, & McManus, 2015). From a nutritional standpoint, this change may be beneficial to consumers due to increased levels of desirable fatty acids (Ferguson, 2010; Scollan et al., 2014). The incorporation of *n*-3 PUFA in the muscle is facilitated by higher concentration of linolenic acid which is often found in roughages. Although higher levels of PUFA are associated with higher lipid oxidation (De Mello et al., 2012), roughage is a natural source of antioxidants (Lindqvist, Nadeau, & Jensen, 2011), which lowers lipid oxidation rates that are usually observed in grass-finished beef. Therefore, pasture finishing improves color and lipid stability of beef and also alters concentration of volatile compounds formed during cooking due to changes in fatty acid profile (Duckett et al., 2013; Humada, Sañudo, & Serrano, 2014; Kerth, Braden, Cox, Kerth, & Rankins, 2007; Luciano et al., 2013; Ponnampalam et al., 2017; Tansawat, Maughan, Ward, Martini, & Cornforth, 2013).

When comparing meat from steers finished on grain, pasture, and pasture with supplementation, previous work suggested that concentrate levels and forage ratios are factors that may affect meat attributes, however, some research also reported minimal or no effects on meat quality based on different levels and ratios (Duckett et al., 2013; Kerth et al., 2007; Patino et al., 2015; Ponnampalam et al., 2017). Reasons of this inconsistency in results may be related to the presence of preserved roughage in some dietary treatments tested by those authors. Roughage conservation methods such as haying and silage fabrication reduce the initial concentration of antioxidants and PUFA. However, Lindqvist et al. (2011) and Stefanello et al. (2018) demonstrated that conserved forage is still a significant source of antioxidants,

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whereas Boufaïed et al. (2003) and Eriksson and Pickova (2007) showed that this type of feedstuff has significant levels of linolenic acid.

Currently, there is few research that studied the effects of finishing ruminants with levels of 100% concentrate and legume-grass pasture including ryegrass, oat, red and white clover. Luciano et al. (2012) showed expressive difference in oxidative stability between grass and concentrate fed, however, comparison based on supplementation level was not performed. Fruet et al. (2016) observed that there were differences in total lipids, fatty acid profile, and lipid oxidation in muscle from ewes finished on grass and only concentrate. Both studies used ovine as experimental units, which metabolize glucose and lipids from feedstuff differently than bovine (Smith & Prior, 1986). Therefore, in order to accurately understand the effects of feeding grain and forage on beef quality attributes, studying distinct diets based only on grain or roughage may provide a better overview of those effects since most of grain-fed beef is finished with diets containing different levels of roughage. The objective of this study was to evaluate texture profile, fatty acid composition, volatile compounds profile, sensory attributes, and instrumental color of beef finished on legume-grass pasture, supplemented with whole corn grain, and only with whole corn grain.

2. Material and methods

2.1. Animals, dietary treatments, and sample collection

Eighteen crossbred steers (Hereford, Angus, and Nelore) [18 to 20 months of age, initial body weight (BW) 333 ± 27.87 kg], previously reared on pasture, were randomly assigned to one of three finishing diet treatments based on legume-grass pastures comprised of black oats (*Avena strigosa*), ryegrass (*Lolium multiflorum*), white clover (*Trifolium repens*) and red clover (*Trifolium pretense*) (PAST), PAST with whole corn grain (WCG) supplementation of 1.4% of BW (SUPP), and WCG only (GRAIN). The pasture was divided in 12 paddocks whereas six were used to individually house steers assigned to PAST (0.6 ha each) and six for steers assigned to SUPP (0.4 ha each). A continuous grazing system with variable stocking rate was adopted by following the methodology described by Mott and Lucas (1952). In order to keep desired forage mass between 1200 and 1500 kg DM/ha during 91 experiment days, forage mass was evaluated based on the visual estimation technique described by Campbell and Arnold (1973) at intervals of 14 days. Steers allocated to treatment GRAIN were confined in a feedlot-covered facility in individual pens of 13.5 m^2 (n = 6). Steers assigned to treatment GRAIN were individually fed with 2.8% of BW of concentrate and the diet consisted of 85% of WCG and 15% of protein-vitamin-mineral pellet supplement. The removal of all roughage sources from the GRAIN diet was possible due to the higher particle size of WCG and the addition of 150 mg of ionophore (inside of the pellet supplement) in order to avoid acidosis. All animal handling and care procedures were approved by the IFF Ethics Committee on the Use of Animals (protocol 003/2015). Composition of finishing diets is shown in Table 1. Average daily gain (kg) of steers fed GRAIN, SUPP, and PAST was 1.81, 1.51, and 1.33, respectively (SEM = 0.06 and $P < 0.001$).

Steers were backgrounded on natural pasture consisting of grasses including *Paspalum notatum*, *P. dilatatum* and *Coelorachis selloana*, *Stipa setigera, S. hyalina*, *Piptochaetium bicolor* and *P. stipoides* as well as legumes including *Trifolium polymorphum* and *Adesmia bicolor* (Freitas et al., 2014). Prior entering the experiment, steers were acclimatized to facilities and respective diets during a period of 21 days. Steers assigned to SUPP and GRAIN received a gradual supply of concentrate until levels of WCG achieved 1.4 and 2.8% of BW, respectively. After the adaptation period, steers were fed for 91 days and slaughtered at the Farroupilha Federal Institute abattoir. Carcasses were chilled for 24 h at 4 °C and left rib sections from the 5th to 13th rib were fabricated. The outer fat cover of the bone-in section between the 9th and 11th rib was removed for subcutaneous fat color analysis. Subsequently, the *M. longissimus thoracis* (LT) was excised from the rib and 2.54 cm steaks were fabricated, individually vacuum packaged, and stored at −80 °C until analysis could be performed.

Table 1
Chemical composition of dietary treatments offered to steers finished on three different feeding regimes.

Nutrients	Finishing system		
	GRAIN[a]	SUPP[b]	PAST[c]
Crude protein[d]	14.9	15.9	20.9
Ether extract	2.3	2.5	2.5
Neutral detergent fiber corrected for ash	16.8	30.9	49.5
Ash	3.6	7.2	12.5
Non-fiber carbohydrate	62.2	43.5	14.4
Metabolizable energy[e]	2.89	2.75	2.50
α-tocopherol[f]	2.16	2.76	4.18
Carotenoids	0.62	1.89	3.18
16:0[g]	15.06	16.49	19.31
18:0	3.86	3.83	4.34
18:1n9	29.29	17.96	7.29
18:2n6	43.82	17.96	7.29
18:3n3	2.20	27.22	52.27

[a] 85% whole corn grain +15% protein-vitamin-mineral pellet supplement.
[b] Pasture and concentrate allowance (50:50 ratio).
[c] Pasture only.
[d] Crude protein, ether extract, neutral detergent fiber corrected for ash, ash, and non-fiber carbohydrate, all values expressed on a dry matter basis (%).
[e] All values expressed in Mcal/kg; ME = TDN (g/kg DM) × 4.4 × 0.82 (*NRC*, 2000).
[f] α-tocopherol and carotenoids expressed in mg/100 g.
[g] All fatty acids expressed as % of FAME.

2.2. Chemical composition and fatty acids profile

Thirty grams of one LT steak was lyophilised (Terroni, LS3000B, Brazil) under optimal conditions (Carpentier, Dens, Houwe, Swennen, & Panis, 2007) for chemical composition analysis. Moisture, crude protein, and ash were quantified according to AOAC (1995). The remaining portion of the steak was used to analyse total lipid values (Hara & Radin, 1978) and transesterification of fatty acid profile (Christie, 1989). Fatty acid methyl esters (FAME, %) were quantified using a gas chromatograph (GC) (Agilent, 45,813–01, CA, USA) equipped with a flame ionisation detector (FID). Separations were accomplished using a fused silica capillary column (0.25 mm × 60 m, Supelco SP™-2362, PA, USA). Oven temperature was programmed from 100 °C to 240 °C, whereas injector and detector temperature were 250 °C and 280 °C, respectively. The carrier gas was nitrogen at a flow rate of 0.6 mL per min. Individual fatty acids were identified by comparison of retention times with known standards (Supelco Mix 37 components FAME; trans-11-vaccenic acid methyl ester; conjugated linoleic acid methyl ester; cis −7.10,13,16,19 - Docosapentaenoic methyl ester). Fatty acids were quantified by incorporating an internal standard, methyl tricosanoic (C23:0) acid, into each sample during methylation.

2.3. Volatile compounds

Volatile compounds were evaluated following the methodology described by Donadel et al. (2013) with some modifications. Thirty grams of a 2.54 cm of LT steak was autoclaved at 121 °C for 10 min. Subsequently, samples were homogenized using a hand blender and aliquots of 5 g were placed into 20 mL glass vials, capped with a polytetrafluoroethylene rubber septum, and placed in a 60 °C water bath and allowed to equilibrate for 15 min. Volatiles were extracted by headspace solid phase micro extraction (HS-SPME) using a 75 μm × 10 mm carboxen/polidimetilsiloxane fiber (Supelco, PA, USA) that was exposed into the headspace of the vial for 50 min. A Shimadzu QP2010 *Plus* GC-Mass Spectrometer (MS) (Shimadzu Co., Japan) was used to detect and separate volatile compounds. The MS was equipped