

RESEARCH ARTICLE

Pasture type effects over beef quality: A comparison

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ABSTRACT

The beef is one of the main sources of nutrients for a balanced diet. In this context, several authors have reported that grass-fed beef presents low levels of intramuscular fat and high levels of polyunsaturated fatty acids. Some studies have noted that forage fatty acid content is variable between species and their varieties, and that can affect fatty acid profile in beef. The aim of this study was to evaluate beef quality from three different types of pastures in southern Chile. A group of 45 Holstein Friesian steers were finished in the spring months with three different types of pastures from temperate rainy climate: Improved pasture (P), *Lolium perenne* L.-based pasture (LT), and *Lolium multiflorum* Lam.-based pasture (LM). The steers were separated into groups of 15 for treatment. Nonsignificant differences were found in carcass measurements and P treatment showed a higher intramuscular content than LM treatment (2.26% vs. 1.42%) and greater presence of SFA: 42.4% vs. 40.3% reported in LM treatment. While LM presented a higher n-6 fatty acids: 4.42% related to P treatment: 3.13%. Regardless of the type of pasture, it can be confirmed that meat from pasture systems presents low intramuscular fat and a better ratio of n-6/n-3.

Key words: Beef quality, fatty acids, intake, pastures, steers.

INTRODUCTION

The beef is one of the main sources of nutrients for a balanced diet. In this context, several authors have reported that grass-fed beef presents low levels of intramuscular fat and high levels of polyunsaturated fatty acids (FA). Among of factors that affect the nutritional content of beef, the diet of the animal is one of the most important variables that affects intramuscular fat (IMF) content and its FA profile (Aldai et al., 2012). The amount of IMF is an important meat quality attribute, and it is positively associated with flavor and juiciness (Bessa et al., 2015). In addition, IMF is a relevant source of fat in terms of FA composition and its effect on human health (Scollan et al., 2014).

Beef produced from grass-fed systems has less IMF (2%-3%), a better ratio n-6/n-3 FA and a higher concentration of conjugated linoleic acid (CLA), as compared to beef from grain-fed systems (Morales et al., 2012; Duckett et al., 2013). The n-6/n-3 ratio and CLA play important roles in the prevention of cardiovascular disease, type 2 diabetes, rheumatoid arthritis, asthma, and several cancers, among other beneficial properties (Simopoulos, 2016).

Feeding high-forage diets is frequently associated with a decrease in the concentration of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Also, it is related to an increase in the concentration of n-3 FA in beef (Duckett et al., 2013). These differences are also affected by other factors, such as slaughter weight and fat percentage of the carcass (Scollan et al., 2014). Furthermore, changes in nutritional values of grass-fed beef are related to the high n-3 FA content of herbages and pastures (Krusinski et al., 2022). Higher n-3 FA concentrations in pastures can lead to a more unsaturated FA profile in beef and milk, due to the incomplete biohydrogenation process of FA in the rumen (Mwangi et al., 2022). Nevertheless, FA content in

pastures is variable among species, cultivars, harvest time, and growing season, so several factors can affect the FA profile of beef originating from grass-fed animals (Krusinski et al., 2022).

It is possible to find several differences in FA profiles in pasture species, although few studies are comparing the effect of different pastures from temperate rainy climates on IMF and FA profiles in beef.

We hypothesized that in steers grazing botanically diverse sward, the intramuscular fat (IMF) content could have higher quality than that of steers that grazed monospecies. Thus, the aim of the present study was to evaluate IMF and FA profiles and other characteristics in beef produced using three different types of pastures, widely employed in temperate rainy climates from southern Chile for grass-finished steers.

MATERIALS AND METHODS

Animals and treatments

Forty-five Holstein-Friesian steers of similar age (24 ± 3 mo) were finished during 80 d in the spring months (October to December) in the Animal Production unit of the Instituto de Investigaciones Agropecuarias (INIA), INIA Remehue, Osorno ($40^{\circ}31'13.1''$ S; $73^{\circ}03'58.6''$ W; 73 m a.s.l.; annual rainfall 1300 mm), Chile.

The average initial weight of the steers was 334 ± 25.9 kg. The steers were blocked by weight and assigned to one of three treatment groups. All steers were allocated in replicated paddock (three paddocks per treatment, five animals per paddock) and this distribution of animals was maintained throughout the experiment. The grazing treatments ($n = 15$ steers) were improved pasture primarily comprising *Lolium perenne* L. ('Nui'), *Bromus valdivianus* Phil. (P), pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* L. ('Bounty') (LT), and pasture of *Lolium multiflorum* ('Abermagic AR1') (LM).

The pastures of LT and LM were established in the autumn (6 mo prior to the beginning of the experiment). The P consisted of improved grassland with an approximate yield of $10 \text{ t DM ha}^{-1} \text{ yr}^{-1}$. Steers grazed in nine paddocks, i.e., three paddocks per treatment. Each paddock had a 1.2 ha surface, and grazing strips were adjusted with electric fencing, according to target daily gain.

Grazing management consisted of allowing variable grazing stripes to cattle based on the weekly DM offer using electric fences. The pre-grazing sward height was recorded daily using a rising plate meter (F200, Farmworks, Feilding, New Zealand) in 60 random locations within the area to be grazed the next day. Post-grazing sward heights were recorded daily in 20 locations randomly chosen within each treatment area grazed the previous day. Mean herbage DM intake (DMI) per animal was estimated daily according to the difference between pre- and post-grazing herbage mass to ground level, divided into the number of steers per treatment and replicate. For the estimation of herbage mass, a plate meter was weekly calibrated.

The differences generated by body weight in animals were corrected using metabolic weight as a standard unit for comparison of the DMI and fatty acids (FA) (Anderson et al., 1988) and mechanism to correct the differences associated with the decrease in the contribution of FA in mature vegetative stages of the grassland. The FA intake was quantified with the observed live weights of each steer at the same time as the sampling of the vegetation cover.

The botanical, chemical and FA compositions of the pastures were estimated with samples from simulated grazing activity. The samples were collected in plastic bags from each pasture paddock. Three samplings from each paddock were collected once a month during the experiment, with a total of nine samples obtained upon completion. All the samples were transported refrigerated to the laboratory and were dried for 48 h at 60°C for chemical analyses. The chemical content of the feed samples was analyzed at INIA Remehue Laboratory in Osorno, Chile. The variables measured were DM, crude protein, ether extract, neutral detergent fiber, and soluble carbohydrates (AOAC, 2005) whereas metabolizable energy (ME) was estimated by Garrido and Mann (1981).

Slaughter and sample procedure

The steers were slaughtered at a commercial meat plant when all steers obtained a minimum live weight of 460 kg, at an average age of 26.5 ± 3 mo. The average live weight of the steers before slaughter was 496.3 ± 33.69 kg. The animals were slaughtered following standard procedures at a commercial meat plant licensed for export. Briefly, animals were stunned with a captive bolt gun, followed by exsanguination an electrical stimulation was applied for 30 s. Afterward, carcass was suspended from the Achilles tendon and eviscerated.

Subsequently, the carcasses were dressed and chilled and entered the chillers (0 °C) after the killing. The cold and warm carcass weights and the pH values were measured 24 h postmortem at the abattoir. Three pH measurements were taken per carcass using a pH penetration electrode (Hanna FC232) of a portable pH-meter (Hanna 99163; Hanna Instruments, Woonsocket, Rhode Island, USA). A section of the *Longissimus thoracis et lumborum* muscle was removed from the ninth thoracic vertebrae until the last lumbar vertebrae of each carcass. This section was cut into three equal parts that were vacuum packaged and aged for 21 d at 4 ± 2 °C, and then stored frozen at -18 ± 2 °C until analysis.

Instrumental color and texture

The raw steaks were allowed to bloom for 30 min at room temperature before the analysis. Color measurements were made using the CIELAB color space, using L* (lightness: 0 = black to 100 = white), a* (redness/greenness: Positive values, red; negative values, green), and b* (yellowness/blueness: Positive values, yellow; negative values, blue), with a chroma meter (CR-400; Minolta Inc., Osaka, Japan) with illuminant D65 and a 2° viewing angle. The readings were taken from three randomly selected locations of the upper surface of each steak to obtain a representative reading of the surface color. The instrumental color of the external fat surface was also measured at three different points.

After measuring the color, two steaks of 2.54 cm thick were used to determine shear force. The samples were cooked in a preheated oven (EKA, KF 620; Famava, Santiago, Chile) at 170 °C until an internal temperature of 71 °C was reached. After the cooking procedure, the steaks were wrapped in film and stored for 24 h at 4 ± 2 °C. Subsequently, at least six cores of 13 mm diameter were extracted from each steak for the Warner-Bratzler shear force analysis, which followed the USDA methodology. The test was performed using a Warner-Bratzler shear blade in a texture analyzer (TA-XT2i, Stable Micro Systems, Godalming, UK).

Sensory analysis

A 12-member trained panel participated in the sensory analysis. The training and testing sessions were conducted at the sensory laboratory of INIA Remehue. The assessors were selected from a group of 30 people without previous experience in sensory evaluation; the panelists were trained following the standards of American Society for Testing and Materials (ASTM) and International Organization for Standardization (ISO). The sensory laboratory was designed according to ISO standards with separate booths, and the samples were evaluated in a sequence established to avoid the effect of sample order presentation, and the first-order or carry-over effects.

The panelists evaluated the beef color intensity, fat color intensity and level of marbling in three raw steaks per session, and beef flavor, tenderness, and juiciness in the cooked samples. References of each sensory attribute were described, and have been published previously (Morales et al., 2013). The assessors evaluated the 45 cooked samples in duplicate, and three samples were analyzed in each session, completing a total of 30 sessions.

Immediately after the raw steaks were evaluated, they were covered with aluminum foil and cooked in a preheated oven (EKA, KF 620; Famava, Santiago, Chile) at 170 °C, until an internal temperature of 71 °C was reached, which was measured with individual thermocouples inserted into the geometric center of each steak. The cooked steaks were diced into pieces of 20 mm × 20 mm × 25 mm (length × width × height), then placed in coded trays and served to the assessors, at temperatures ranging from 30 to 35 °C. The descriptors were quantified using a hybrid scale that ranged from 0 (absence) to 10 (maximum intensity).

Chemical composition

Moisture, protein, and ash content were measured according to AOAC (2005) procedures. After removing all external fat from the steaks, the intramuscular fat was measured by extraction, using the Soxhlet equipment with petroleum ether (AOAC, 2005).

Fatty acid composition

The samples of pasture were analyzed according to the combined extraction method of direct transesterification and thin-layer chromatography, as described by Alves et al. (2008). Beef samples were extracted from 1 g freeze-dried muscle, using a mixture of chloroform-methanol (1:1, v/v), according to Kramer et al. (2008). Lipid aliquots (~ 10 mg) from each steak were methylated separately using acidic (methanolic HCl) and basic (sodium methoxide) reagents to ensure complete methylation of all lipids and to avoid isomerization

of conjugated linoleic acids (CLAs), respectively (Kramer et al., 2008). For quantitative purposes, 1 mL internal standard (1 mg mL⁻¹ 23:0 methyl ester, n-23-M from Nu-Chek Prep Inc., Elysian, Minnesota, USA) was added before methylation. The contents of fatty acid methyl esters (FAMES) were expressed as percentages (%) of the total FAME quantified.

The FAMES were analyzed using gas chromatographer (GC) equipped with a flame ionization detector (GC-2010 Plus; Shimadzu, Kyoto, Japan). A 100 m SP-2560 column (Supelco, Bellefonte, Pennsylvania, USA) was operated at two complementary GC temperature programs that plateaued at 175 and 150 °C (Kramer et al., 2008). In addition, a 100 m SLB-IL111 ionic liquid column (Supelco) was used to confirm the identification of several biohydrogenation intermediates like CLA isomers and others. For both columns, hydrogen was used as a carrier gas with a constant flow rate of 1 mL min⁻¹, and the injector and detector temperatures were set at 250 °C.

For peak identification purposes, two reference standards, individual FAMES (21:0, 23:0, 26:0), and a CLA mixture (9c, 11t-/8t, 10c-/11c, 13t-/10t, 12c-/8c, 10c-/9c, 11c-/10c, 12c-/11c, 13c-/11t, 13t-/10t, 12t-/9t, 11t-/8t, 10t-18:2) were used, all of which were obtained from Nu-Chek Prep Inc. Isomerized mixtures of linoleic (18:2 n-6) and linolenic (18:3 n-3) acids were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), and branched-chain FA (BCFA) were identified using a bacterial FAME mixture from Matreya (Pleasant Gap, Pennsylvania, USA). Several of the *trans*-18:1 and CLA isomers together with other non-conjugated dienes, not included in the standard mixtures, were identified by their retention times and elution orders, as reported in the literature, and were confirmed using FAME fractions obtained from Ag+-SPE cartridges (Belaunzaran et al., 2017).

Statistical analysis

Dry matter and FA intake, carcass measurements, meat quality and FA data were analyzed by ANOVA using the general linear model (GLM), including the type of finishing pasture (P, LT and LM) as a fixed effect, and the replicate (three paddocks by treatment) was included as a random term in the model. The paddock was the experimental unit and steers were the sampling unit.

Sensory analysis data were analyzed by ANOVA including treatment, panelists, as fixed effects, and sessions and replicate as random terms. The least-square means were separated using Tukey's studentized range ($P \leq 0.05$). All statistical analyses were calculated using the JMP 12.1.0 software (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Botanical, chemical and FA composition from the three different pastures is shown in Table 1. The LT treatment showed a higher quantity of ash and a lower content of 18:2n-6 (linoleic acid, LA) than other treatments ($P < 0.05$).

These results differ from those obtained by other authors (Elgersma, 2015), who provided evidence of higher levels of LA in legumes (white clover) compared to perennial ryegrass and annual ryegrass. Lower lipolysis activity in vegetal tissue has been attributed to the clover species, due to being a source of various types of saponin compounds present in white clover (Karri et al., 2019). Nevertheless, the amount of white clover in LT treatment represented only 13.9% of its total composition. In addition, Morales et al. (2015) obtained similar values of LA, and higher values of linolenic acid (ALA), as compared to the results of the present study (ALA; about 70% of total FA in pasture), when using improved grassland similar to P treatment.

The comparison of daily voluntary consumption was similar between treatments ($P = 0.28$), with a higher value in LT (112 g DM kg^{-0.75}) by 9.8% than LM (102 g DM kg^{-0.75}), and by 5.6% to treatment improved pasture treatment (106 g DM kg^{-0.75}). The intake 16:0 was lower in the LT (-13.6%) and LM (-22.1%) in contrast to the pasture (P) treatment ($P = 0.001$; Table 2). The higher LNA intake of grazing steers was P treatment ($P = 0.006$) with an increase of the order of 20%-30% compared to the LT and LM treatments. The naturalized grassland treatment generated higher intakes (mg kg^{-0.75}) in total FA ($P = 0.007$), total saturated FA (SFA) ($P = 0.004$), monounsaturated FA (MUFA) ($P = 0.0013$) and polyunsaturated FA (PUFA) ($P = 0.008$), favorable scenario for rumen biohydrogenation (Bessa et al., 2015). The relationship between pasture type and DM intake constitutes the basis for designing grazing practices to adipogenesis with a better healthy FA index, integrating stearoyl-Coa desaturase (SCD) activity and insulin sensitivity regulation.

Table 1. Average botanical and chemical and fatty acids composition of the diets (n = 3 per treatment). P: Grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus*; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFA: total fatty acids. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

	P	LT	LM	RMSE	P value
Botanic composition, %					
<i>Lolium perenne</i>	45.3 ± 29.2	65.9 ± 6.4		-	-
<i>Lolium multiflorum</i>			77.8 ± 18.5	-	-
<i>Trifolium repens</i>	1.3 ± 2.2	13.9 ± 3.6		-	-
<i>Holcus lanatus</i>	10.1 ± 8.1			-	-
<i>Festuca arundinacea</i>	0.8 ± 2.3			-	-
<i>Plantago lanceolata</i>	0.4 ± 1.3			-	-
<i>Bromus valdivianus</i>	27.4 ± 26.2			-	-
<i>Dactylis glomerata</i>	2.3 ± 5.1			-	-
Weeds	5.4 ± 5.9	11.0 ± 4.8	16.3 ± 15.7	-	-
Dead tissue	1.0 ± 0.9	1.6 ± 1.6	NC	-	-
Others	5.8 ± 3.6	7.6 ± 3.1	5.9 ± 4.3	-	-
Chemical composition					
Dry mater, %	20.2 ^a	17.4 ^b	19.9 ^{ab}	2.264	0.041
Crude protein, %	23.5	23.4	22.0	3.678	0.582
Metabolizable energy, Mcal kg ⁻¹	2.77	2.80	2.83	0.568	0.453
Neutral detergent fiber, %	44.6	41.2	42.6	6.603	0.139
Ash, %	9.12 ^b	10.19 ^a	8.80 ^b	0.708	0.001
Ether extract, %	3.12	2.81	2.76	0.728	0.524
Soluble carbohydrates, %	12.6	13.2	15.1	2.728	0.129
Fatty acids, %					
16:0	13.1	12.8	13.3	1.438	0.805
18:2n-6	11.1	9.29	10.3	1.903	0.216
18:3n-3	63.8	64.3	63.3	3.713	0.864
SFA	19.1	20.2	19.9	1.998	0.577
MUFA	4.51	4.44	4.73	0.650	0.629
PUFA	75.4	74.3	74.3	2.519	0.588
Fatty acids, mg 100 g ⁻¹ fat tissue					
16:0	15.4	13.8	15.8	2.845	0.352
18:2n-6	12.8 ^a	10.0 ^b	12.1 ^a	2.119	0.046
18:3n-3	78.7	69.1	75.3	19.063	0.617
SFA	22.5	21.8	23.7	4.590	0.710
MUFA	5.50	4.83	5.70	1.631	0.533
PUFA	92.34	79.9	88.1	20.405	0.491
TFA	120.4	106.5	117.5	25.361	0.535

Table 2. Daily fatty acids average intake by steer to treatment pasture (n = 3 per treatment). P: Grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil.; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error; FA: fatty acid; SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

Fatty acid	P	LT	LM	RMSE	P value
	mg kg ^{-0.75}				
16:0	15.40 ^a	13.30 ^b	12.00 ^b	0.593	0.001
18:2n-6	11.10	10.30	10.10	0.482	0.200
18:3n-3	77.10 ^a	63.30 ^b	58.50 ^b	2.740	0.006
Total FA	119.80 ^a	99.70 ^b	91.70 ^b	4.369	0.007
Total SFA	24.20 ^a	19.90 ^b	17.60 ^b	0.865	0.004
Total MUFA	5.33 ^a	4.73 ^{ab}	4.13 ^b	0.215	0.013
PUFA	89.10 ^a	74.20 ^b	69.20 ^b	3.243	0.008
∑ n-6	11.40	10.50	10.40	0.491	0.193
∑ n-3	77.60 ^a	63.70 ^b	58.80 ^b	2.756	0.005

The FA profile in beef is associated with dietary and animal factors (Bessa et al., 2015). The amount and type of forage have a role in increasing the biohydrogenation of FA in the rumen, highlighting that higher consumption of MUFA and PUFA stimulates their escape with a greater deposit in tissue fat (Vahmani et al., 2015). The biohydrogenation for LA and ALA FA is related to the amount in the forage, therefore the differences generated by type of forage and season of the year have an important role in intake, biohydrogenation and fat deposition (Bessa et al., 2015).

Final live weight (FW) did not differ among treatments (Table 3); however, LT showed higher carcass yield (CY) than the other two treatments ($P < 0.05$). Also, the observed CY values were higher than those obtained by McNamee et al. (2014), who reported 49.1% CY in grass-fed Holstein Friesian (HF) steers of 578.2 kg FW. In general, the three groups presented CY values according to grass-fed HF steers, ranging between 450-550 kg FW (Catrileo et al., 2014; Morales et al., 2015).

There were no differences among treatments for beef color and pH ($P > 0.05$; Table 3). The subcutaneous fat b^* values for all treatments were higher than Morales et al. (2015), who reported 15.23 and 14.64 (b^* values, respectively) using improved grassland. Grass-fed beef often contains yellow fat due to carotenoid content in green forages (Daley et al., 2010). The main carotene in plants (β -carotene) has pro-vitamin A activity, and vitamin A has several benefits for human health, such as preventing slow growth and infertility, among others (Pogorzelska-Nowicka et al., 2018). Therefore, fat yellowness could be used as a quality attribute to differentiate grass-finished beef from other production systems.

The LT showed lower Warner-Bratzler shear force values than LM. However, the results were consistent with those reported by Morales et al. (2015), who obtained < 30 N shear force values (2.85 kgf) in grass-fed HF steers. In addition, the values were below the range of the consumer's acceptability, according to Miller et al. (2001), who established shear force values between 42-48 N to satisfy 86% of consumers.

Regarding beef composition, P and LT presented a higher percentage ($P < 0.05$) of crude protein than LM, whereas P showed higher intramuscular fat than LM ($P < 0.05$; Table 3). These results could be related to higher FA consumption of P treatment (Table 2). Similarly, P was a natural pasture established about 10 yr before the study, and it was composed of various forage species (Table 1), whereas LM was established for this study and only one predominant forage species (*L. multiflorum*), and LT was composed of *L. perenne* and *T. repens*. Despite three pastures showing similar nutrition quality (Table 1), animals from P treatment could select pasture species of higher palatability and quality among all the species available on the pasture. On the other hand, the steers grazing improved pasture (P) had higher initial availability of DM, according to values obtained using a forage measuring plate ($P < 0.0001$) than steers of LM (2.776 vs. 2.069 kg DM ha⁻¹; data not shown). The P also reported better grazing efficiency per paddock than LM at 28% and 23%, respectively. Therefore, steers of P treatment could select and consume the most nutritional parts of the plant due to a better initial height of P pasture than LM pasture.

Table 3. Initial and slaughter weight, carcass measurements and meat quality attributes of the *Longissimus thoracis et lumborum* muscle of steers from each treatment (n = 15 per treatment). P: Grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil.; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

	P	LT	LM	RMSE	P value
Initial weight, kg	329.7	329.7	332.3	27.893	0.913
Slaughter weight, kg	504.1	480.3	504.5	31.020	0.242
Cold carcass weight, kg	248.4	243.2	250.7	16.514	0.683
Carcass yield, %	51.9 ^b	53.3 ^a	52.4 ^{ab}	1.399	0.033
pH	5.68	5.62	5.61	0.077	0.427
<i>Longissimus thoracis et lumborum</i>					
L*	39.7	40.1	39.8	2.104	0.921
a*	23.9	25.4	24.2	1.746	0.182
b*	12.3	13.3	12.8	1.407	0.160
Subcutaneous fat					
L*	63.7	65.5	64.8	2.390	0.757
a*	13.6	13.2	13.5	2.267	0.828
b*	17.2	19.6	17.1	2.335	0.189
Shear force, N	20.3 ^{ab}	17.6 ^b	23.0 ^a	3.125	0.002
Moisture, %	75.3 ^b	75.5 ^b	76.5 ^a	0.817	0.003
Protein, %	21.5 ^a	21.4 ^a	20.7 ^b	0.732	0.007
Ash, %	1.05	1.03	1.03	0.040	0.135
Intramuscular fat, %	2.26 ^a	1.72 ^{ab}	1.42 ^b	0.631	0.003
Cholesterol, mg 100 g ⁻¹ fresh sample	43.7 ^a	40.2 ^{ab}	35.5 ^b	8.000	0.040

The IMF values were similar (< 3.0%) to those reported by Morales et al. (2012) at 2.7%, in a study involving pasture-finished steers. Additionally, the IMF values obtained from all treatments can be considered lean beef in several countries (2%-5% IMF), meeting the needs of healthy food consumers due to its low content of fat (Realini et al., 2016).

Cholesterol showed higher concentrations in P than in LM ($P < 0.05$; Table 3). This is consistent with authors reporting that cholesterol could be positively correlated with IMF content (Bragagnolo, 2009). Cholesterol content in P ranged between 43 to 84 mg 100 g⁻¹, these values are lower than shown by Duckett et al. (2013) for grass-fed animals.

The trained sensory assessors did not find any difference ($P > 0.05$) among the treatments for all the sensory descriptors evaluated. For all treatments, tenderness was the better-evaluated sensory descriptor (Table 4). The MB scores obtained are in accordance with those presented by Morales et al. (2013) for HF steers finished on pasture. Even though many authors have associated higher IMF with more juiciness, higher tenderness, and better flavor (Troy et al., 2016), in this study, no differences were detected between P (higher IMF) and the other treatments. Similar results were obtained by Pordomingo et al. (2012) in beef with low amounts of IMF (< 3%). The panelists may require higher amounts of fat for them to be able to detect differences.

Percentages of total FAME for SFA, straight BCFA, and dimethylacetal are shown in Table 5. Treatment P presented higher values of SFA, myristic acid (14:0) than LM ($P < 0.05$); this can be related to higher consumption of SFA (Table 2) and IMF content (Table 3). The SFA and MUFA increased with higher IMF (Khan et al., 2015), mainly due to triacylglycerols (TG). The TG are rich in SFA and MUFA and showed a wide range of variation (0.2% to more than 5%), whereas phospholipids (PPL) are rich in PUFA and presented low variability (between 0.2% and 1% of muscle), and they are relatively independent of IMF content (Sales et al., 2020). Additionally, the content of 14:0 was very similar to Duckett et al. (2013) study on grass-fed Angus steers with 2.1% IMF. It should be mentioned that 14:0

and lauric acid (12:0) raise cholesterol levels in humans (Pighin et al., 2016). These results are supported by the data of the present study, where P treatment obtained higher cholesterol content than LM ($P < 0.05$; Table 3). Stearic acid and BCFA values did not show differences among treatments; BCFA concentrations were like those obtained by Aldai et al. (2012). Usually, BCFA is expressed better in grass-fed animals, due to higher fiber content, which leads to a more intensive cellulolytic rumen-bacteria activity (Kramer et al., 2008).

Table 4. Sensory attributes of beef from each treatment (n = 15 loins per treatment). P: Grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error.

Sensory attribute	P	LT	LM	RMSE	P value
Red color intensity	4.49	4.48	3.93	0.842	0.350
Fat color intensity	4.49	4.53	4.56	0.743	0.942
Marbling	3.40	2.79	2.89	0.782	0.086
Juiciness	3.86	4.39	3.99	4.079	0.392
Tenderness	6.33	6.03	5.73	1.338	0.594
Flavor	5.64	5.75	5.24	0.849	0.286

Table 5. Saturated straight and branched-chain fatty acid composition and content in the *Longissimus thoracis et lumborum* muscle of steers (n = 15 per treatment). FAME: Fatty acid methyl esters; P: grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error; TFA: total fatty acids; SFA: saturated fatty acids; BCFA: branched-chain saturated fatty acid; AME: alk-1-enyl methyl ethers; DMA: dimethyl acetal. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

Fatty acid	% total FAME					mg 100 g ⁻¹ meat				
	P	LT	LM	RMSE	P value	P	LT	LM	RMSE	P value
TFA						2290.2	1954.9	1836.1	657.219	0.074
SFA	42.4 ^a	41.8 ^{ab}	40.3 ^b	1.901	0.037	974.2	823.6	745.0	293.690	0.071
12:0	0.100	0.105	0.111	0.0239	0.248	2.29	2.03	2.09	0.861	0.338
13:0	0.074	0.083	0.088	0.0204	0.529	1.71	1.55	1.67	0.652	0.741
14:0	2.47 ^a	2.19 ^b	2.02 ^b	0.336	0.004	57.4 ^a	43.1 ^b	37.6 ^b	18.397	0.014
15:0	0.306	0.295	0.281	0.0367	0.251	6.99	5.89	5.24	2.289	0.085
16:0	24.6	23.9	23.2	1.194	0.055	565.6	468.6	430.7	168.930	0.052
17:0	0.775	0.777	0.740	0.081	0.517	17.7	15.6	13.8	6.073	0.141
18:0	12.5	12.9	12.3	1.375	0.318	287.9	254.9	224.6	90.128	0.144
19:0	0.110	0.101	0.100	0.0195	0.435	2.57	2.01	1.87	0.902	0.066
20:0	0.067	0.068	0.064	0.0112	0.501	1.50	1.33	1.16	0.401	0.249
22:0	0.031 ^c	0.039 ^b	0.045 ^a	0.0088	0.0001	0.685	0.731	0.797	0.1833	0.142
24:0	0.016 ^b	0.031 ^a	0.036 ^a	0.0087	0.004	0.355 ^b	0.583 ^a	0.611 ^a	0.1763	0.005
BCFA	1.29	1.35	1.32	0.195	0.697	29.5	27.3	24.8	11.318	0.409
AME and DMA	3.16	3.71	4.20	1.273	0.112	67.7 ^{ab}	65.6 ^b	70.5 ^a	9.490	0.040

Regarding MUFA (Table 6), treatment P presented higher concentrations of 9c-14:1, 9c-16:1 and 11c-16:1 than LM ($P < 0.05$). This can be explained by the differences found in 14:0 and SFA (Table 5), because 9c-14:1, 9c-16:1 and 11c-16:1 depend on the SFA synthesis, and 9c-14:1 is formed from the biosynthesis of 14:0 using delta-9-desaturase enzyme, whereas 11c-16:1 comes from 16:0 desaturation. Also, oleic acid (9c-18:1; most predominant

MUFA in beef) did not present differences among treatments ($P > 0.05$) getting similar values to those reported by other authors in grass-fed cattle (Morales et al., 2012). There were observed differences in some *cis*-18:1 isomers: 13c-18:1, 14c-18:1 and 15c-18:1.

Table 6. Monounsaturated and polyunsaturated fatty acid composition and content in the *Longissimus thoracis et lumborum* muscle of steers ($n = 15$ per treatment). FAME: Fatty acid methyl esters; P: grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

Fatty acid	% total FAME					mg 100 g ⁻¹ meat				
	P	LT	LM	RMSE	P value	P	LT	LM	RMSE	P value
MUFA	46.1	45.1	45.2	3.030	0.396	1065.7	892.4	840.9	345.636	0.066
<i>cis</i> -MUFA	42.1	41.3	41.2	2.960	0.432	974.1	817.5	766.7	315.688	0.062
9c-14:1	0.796 ^a	0.671 ^b	0.524 ^c	0.2011	0.0006	18.6 ^a	12.9 ^b	10.3 ^b	6.308	0.001
<i>cis</i> -16:1	4.50 ^a	4.05 ^b	4.11 ^b	0.673	0.003	103.6 ^a	78.9 ^b	76.3 ^b	32.633	0.007
7c-16:1	0.187 ^b	0.204 ^a	0.213 ^a	0.0230	0.003	4.23	4.00	3.87	1.318	0.543
9c-16:1	4.12 ^a	3.68 ^b	3.76 ^b	0.653	0.003	95.1 ^a	71.8 ^b	69.8 ^b	30.300	0.006
11c-16:1	0.157 ^a	0.132 ^{ab}	0.109 ^b	0.0367	0.008	3.66 ^a	2.54 ^b	2.09 ^b	1.240	0.001
9c-17:1	0.62	0.62	0.65	0.0600	0.572	14.2	12.3	12.0	4.588	0.138
9c-18:1	34.1	34.0	33.9	2.451	0.937	790.1	675.2	630.0	262.117	0.096
11c-18:1/15t-18-1	1.38	1.35	1.49	0.157	0.107	31.5 ^a	25.9 ^b	27.2 ^{ab}	9.095	0.017
12c-18:1	0.072	0.076	0.067	0.0124	0.330	1.67	1.51	1.24	0.585	0.243
13c-18:1	0.357 ^a	0.318 ^{ab}	0.280 ^b	0.0870	0.018	8.36 ^a	6.25 ^b	5.54 ^b	3.228	0.001
14c-18:1	0.040 ^{ab}	0.045 ^a	0.031 ^b	0.0146	0.009	0.935 ^a	0.907 ^a	0.599 ^b	0.4839	0.016
15c-18:1	0.106 ^a	0.091 ^{ab}	0.073 ^b	0.0193	0.020	2.47 ^a	1.85 ^{ab}	1.36 ^b	0.866	0.033
11c-20:1	0.133	0.120	0.130	0.0301	0.507	3.12	2.24	2.47	1.082	0.055
<i>trans</i> -MUFA	3.99	3.74	3.94	0.554	0.579	91.6	74.9	74.2	32.083	0.160
PUFA	6.20	7.36	8.06	2.185	0.064	133.7	132.6	137.2	22.960	0.627
Σ n-6	3.31 ^b	4.04 ^{ab}	4.42 ^a	1.245	0.041	71.3	72.6	74.9	12.739	0.393
18:2n-6	2.09 ^b	2.48 ^{ab}	2.71 ^a	0.707	0.044	45.4	45.3	46.3	8.895	0.867
20:3n-6	0.221	0.276	0.292	0.0927	0.082	4.71	4.89	4.93	0.775	0.367
20:4n-6	0.89 ^b	1.16 ^{ab}	1.28 ^a	0.461	0.049	18.8	20.3	21.2	4.451	0.055
22:4n-6	0.061	0.070	0.082	0.0610	0.612	1.36	1.25	1.57	1.254	0.746
Σ n-3	2.75	3.17	3.47	0.945	0.124	59.3	57.4	59.4	10.791	0.751
18:3n-3	1.14	1.30	1.35	0.302	0.159	25.2	24.3	23.5	5.841	0.619
20:5n-3	0.658	0.778	0.849	0.277	0.159	13.9	13.7	14.4	2.789	0.765
22:5n-3	0.796	0.912	1.072	0.338	0.092	16.9 ^{ab}	16.1 ^b	18.0 ^a	3.224	0.030
22:6n-3	0.128	0.148	0.174	0.064	0.177	2.64	2.64	2.93	0.739	0.664

The LM had higher percentages of 18:2 n-6, 20:4 n-6 and n-6 fatty acids than P treatment and LM also showed the lowest IMF content. In this respect, 18:2 n-6 content of phospholipid has a major influence on total muscle FA composition. But as body fat increases, the proportion of phospholipids in total lipids decreases and this is accompanied by an increase in the proportion of MUFA and a decrease in the proportion of 18:2 n-6 in total lipids (Bessa et al., 2015).

Regarding PUFA, the diet did not influence the development of this FA group in terms of muscle ($P > 0.05$), principally attributed to the PUFA of the pastures used in the present study (Table 1). Additionally, the PUFA content presented in beef (Table 6) was below the reported levels by several authors in grass-fed cattle (Aldai et al., 2012). Likewise, PUFAs 18:2 n-6 (LA) and 18:3 n-3 (ALA) did not show differences among treatments ($P > 0.05$). The LA amount for every treatment was lower, while the ALA content (Table 6) was similar to results in the study reported by Duckett et al. (2013) for grass-fed steers. The n-6/n-3 ratios (1.21, 1.28, 1.27 for P, LT, LM respectively) obtained did not differ between

treatments ($P > 0.05$). Similarly, Duckett et al. (2013) did not report differences in experiments with forage species, e.g., lucerne, pearl millet and mixed pastures.

Biohydrogenated intermediates are shown in the Table 7. There were no differences in *trans*-18:1 isomers, except for 12t-18:1 (%) and 13t/14t-18:1 (mg 100 g⁻¹ meat), which showed higher values in treatment P than in LM ($P < 0.05$). Similarly, P treatment showed a higher concentration of 9c.15c-18:2, 9c.13t/8t.12c- and 8t.13c-18:2 than LM. Glasser et al. (2008) related the amount of these isomers with LA and ALA intakes, estimating that 25% of dietary intakes of both PUFAs are recovered in duodenum digesta as *trans*-18:1.

Table 7. Biohydrogenated intermediates fatty acid composition and content in the *Longissimus thoracis et lumborum* muscle of steers ($n = 15$ per treatment). FAME: Fatty acid methyl esters; P: grazing improved pasture primarily composed by *Lolium perenne* ('Nui') and *Bromus valdivianus* Phil; LT: grazing pasture of *L. perenne* ('One 50 AR1') and *Trifolium repens* ('Bounty'); LM: grazing pasture of *L. multiflorum* ('Abermagic AR1'); RMSE: root mean square error; CLA: conjugated linoleic acid. Different letters in rows indicate differences in Tukey's test ($P \leq 0.05$).

Fatty acid	% total FAME					mg 100 g ⁻¹ meat				
	P	LT	LM	RMSE	P value	P	LT	LM	RMSE	P value
<i>trans</i> -18:1	3.75	3.48	3.67	0.527	0.530	86.1	69.9	69.4	30.440	0.152
6-8t-18:1	0.087	0.087	0.091	0.0286	0.924	2.01	1.77	1.69	0.853	0.643
9t-18:1	0.160	0.149	0.164	0.0525	0.776	3.68	3.13	3.03	1.710	0.460
10t-18:1	0.131	0.108	0.118	0.0414	0.205	3.00	2.29	2.28	1.436	0.160
11t-18:1	1.06	1.01	1.09	0.337	0.744	24.3	20.3	21.2	11.042	0.303
12t-18:1	0.150 ^a	0.129 ^{ab}	0.116 ^b	0.0305	0.040	3.52	2.61	2.21	1.310	0.053
13t/14t-18:1	0.483	0.416	0.407	0.0892	0.055	11.21 ^a	8.16 ^{ab}	7.42 ^b	3.401	0.038
16t-18:1	0.214	0.175	0.159	0.0408	0.136	4.99	3.64	2.92	1.798	0.088
9c.11t-18:2	0.337	0.319	0.399	0.1402	0.163	7.73	6.48	7.92	4.298	0.241
7t-9c-18:2	0.025	0.027	0.024	0.0077	0.611	0.544	0.491	0.422	0.085	0.133
Other-dienes and trienes	0.262	0.263	0.260	0.0715	0.746	5.14	6.09	5.21	2.102	0.213
9c.13t/8t.12c-	0.287 ^a	0.240 ^b	0.236 ^b	0.0359	0.028	6.69 ^a	4.89 ^{ab}	4.38 ^b	2.309	0.029
8t.13c-18:2	0.166 ^a	0.131 ^b	0.133 ^b	0.0183	0.028	3.86 ^a	2.65 ^b	2.46 ^b	1.211	0.020
9c.12t/16c-	0.045	0.047	0.042	0.0108	0.361	1.013	0.959	0.743	0.401	0.136
11t.15c-18:2	0.288	0.245	0.242	0.0730	0.171	6.63	5.05	4.64	2.688	0.089
9c.15c-18:2	0.116	0.118	0.111	0.0230	0.670	2.70 ^a	2.30 ^{ab}	2.10 ^b	0.969	0.032
9c.11t.15c-18:3	0.139	0.140	0.159	0.0322	0.314	3.06	2.56	2.85	0.625	0.150

Regarding ruminant *trans* FA (R-TFA), such as *trans*-vaccenic acid (TVA; 11t-18:1), 10t-18:1, and ruminic acid (RA; 9c,11t-18:2), TVA presented higher concentrations at eight times more than 10t-18:1 in every treatment (Table 7) – a normal result for grass-fed cattle (Bessa et al., 2015). It should be noted that not all R-TFA have benefits for human health; for instance, 10t-18:1 has been associated with an increase in the risk of atherosclerosis (Michas et al., 2014). On the other hand, TVA and RA have been related to prevention of several types of cancers and cardiovascular diseases (Wang et al., 2012). Likewise, RA was the most predominant CLA in all treatments, comprising more than 65% of total CLA isomers. Nevertheless, the RA% of total FAME values, reported in Table 7, were lower than the values reported by other authors in grass-fed beef studies (Morales et al., 2012). Aldai et al. (2012) showed similar RA percentages in bulls fed with ryegrass and clover. Moreover, the concentration of CLA in beef is generally less than 1% of the total FAME (Aldai et al., 2012; Morales et al., 2012).

It is important to note that although few differences in FA profile among treatments were observed, they can be useful to determinate the influence of the type of forage in the beef quality. The FA profile could be a discriminate tool to ensure the animal feeding or geographical origin as was demonstrated by Vasilev et al. (2020), who used FA profile to discriminate lamb meat from different grazing areas. In this context, new studies that included pastures from temperature rainy climates should be conducted to corroborate if differences found among treatments are consistent.

CONCLUSIONS

At the same level of DM intake ($\text{kg}^{0.75}$), there are differences in the consumption of some fatty acids (FA), where improved pasture showed higher intake values of FA of biomedical interest. The three treatments of the present study did not show differences in carcass characteristics. However, beef from improved pasture showed higher intramuscular fat, this indicates that it is possible to modulate the amount of intramuscular fat in grass-fed animals. Regardless of the type of pasture, it can be confirmed that meat from pasture systems presents low intramuscular fat and a better ratio of n-6/n-3.

Author contribution

Conceptualization: I.S. Resources: I.S. Formal analysis: I.S. Data curation: I.S., R.A. Investigation: I.S. Methodology: I.S., R.M. Data curation: R.A.R. Writing – original draft: R.A.R., N.A., R.A. Writing – review & editing: R.A.R., N.A., R.A. Supervision: R.M. Funding acquisition: R.M.

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