SCIENTIFIC NOTE

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Beef production from dairy bulls under two different production systems and its effect on the fatty acid profile and beef quality

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Bulls in dairy production are usually slaughtered at an early age to avoid production problems and unnecessary costs. However, the animals could be a source of additional income and better quality meat. The objective of this work was to determine the characteristic and quantity of fatty acids of the *Longissimus thoracis* muscle of dairy bulls finished at pasture and in an intensive feeding system. Sixteen 14-mo old Holstein Friesian dairy bulls with initial live weights (LW) of 340 ± 20 kg were randomly assigned to bulls with daily pasture silage and kale (*Brassica oleracea* L.) supplements and 1.0% concentrate at pasture (T1) and bulls with pasture silage supplements and 2% concentrate in confinement (T2). No differences ($P > 0.05$) were found between treatments for dressing percentage, rib eye area, and fat cover when animals were slaughtered at 21-mo, with LW 550 kg. However, kidney fat for T1 and T2 of 4.44 and 2.61 kg an^{-1}, respectively, were different ($P < 0.05$) as was pH, where T2 had a higher value (5.72) than T1 (5.46). Significant differences ($P < 0.05$) between treatments were found for all polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA) *c*-9, *t*-11, C18:2 *n*-6 *trans*, C18:3 *n*-3, C22:5 *n*-3, C22:6 *n*-3, with higher levels for T1. Beef from the forage-fed bulls (T1) had an *n*-6: *n*-3 ratio below 4.0.

Key words: Beef quality, *Brassica oleracea*, CLA, fatty acids, Holstein Friesian bulls.

INTRODUCTION

Lean beef from beef cattle finished at pasture has a high nutrient density and is an excellent source of protein, vitamins, minerals, and essential fatty acids. Beef is a source of high quality protein. Unlike some plant sources of protein, beef supplies every essential amino acid for optimal performance. Beef is a valuable source of many minerals, including Fe, Zn, Se, and Cu (Pereira and Vicente, 2013). Moreover, it has low fat content (less than 5% relative to muscle) and low cholesterol content (Johnson, 2009).

Meat and milk from ruminants also represents the major dietary sources of conjugated linoleic acids (CLAs). The rumenic acid C18:2 *cis*-9 *trans*-11 (referred to as CLA) accounts for 75-90% of total CLAs in meat. Conjugated linoleic acids are associated with a number of health-promoting biological activities, including anti-carcinogenic and anti-atherogenic activity, the ability to reduce the catabolic effects of immune stimulation, enhance growth and reduce body fat (Banni et al., 2002).

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Beef from cattle finished on pasture have higher CLA content than beef from cattle finished in feedlots (French et al., 2000; Schmid et al., 2006). There is now more interest in the quality of beef than factors such as growth rate and carcass composition, which had been the main focus of producers. A recent study evaluated the nutritional quality (intramuscular fat, cholesterol content, and fatty acid composition) of beef produced in Chile under different production systems classified according to the type of finishing diet (Morales et al., 2012). Information is available about consumer sensory preferences for Chilean beef from different finishing systems. In general, consumers prefer beef produced on pastures and reject beef with high visual marbling (Morales et al., 2013).

Traditionally, male calves in Chilean dairies have been sacrificed or sold at an early age to avoid difficulties in finishing and unnecessary costs. Dairy breeds are large and late-maturing animals and carcass quality increases with increasing LW (Nogalski et al., 2014), so that dairy bulls could be an alternative for beef production because they have a higher weight gain capacity and can be slaughtered sooner (Vaz et al., 2001). Dairy bulls are more efficient and produce more lean meat than beef steers (Catrileo and Rojas, 2012). Nevertheless, to date there is no information on the quality and quantity of fat in Chilean dairy bulls. The objective of this work was to determine characteristic and quantity of fatty acids of the *Longissimus thoracis* of dairy bulls finished at pasture and in an intensive feeding system.

MATERIALS AND METHODS

Beef selection and sample preparation

The study was carried out in La Araucanía Region, south Chile. Sixteen Holstein Friesian dairy bulls with an average age of 14-mo were studied at the Instituto de Investigaciones Agropecuarias, INIA Carillanca, Temuco (38º41' S, 72º25' W; 200 m a.s.l.) The study was approved by INIA ethics/welfare committee confirming compliance with all requirements of the country for this type of animal experiments.

From 4 to 14-mo age the animals were managed by grazing. At the beginning of winter and after 10-mo on pasture they were randomly divided into two treatments with eight bulls each. Forage diet treatment (FD): bulls with an average initial LW of 338.6 ± 20 kg were fed by continuous grazing on 2 ha of mixed ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.), with additional free pasture silage of mixed ryegrass and clover, access to kale (*Brassica oleracea* L.) and concentrates at 1.0% of their LW. Kale was limited to 30% DM intake using electric fence. Concentrate diet treatment (CD): bulls with an average initial 341.3 ± 17 kg LW were kept together in a pen in a barn with the same silage pasture *ad libitum* and a daily amount of concentrate at 2% LW. The concentrate was the same for both groups and was composed of 65% triticale (×*Triticosecale* spp.), 33% lupine (*Lupinus luteus* L.), 1% mineral salt and 1% sodium bicarbonate. The diet for both treatments were formulated approximately isoproteic for 14% crude protein and isoenergetic for 2.6 Mcal ME kg^{-1} to obtain maximum daily weight gains over 1.0 kg (AFRC, 1995).

The chemical content of feed samples was analyzed at the INIA Remehue Animal Nutrition and Environment Laboratory. Dry matter was measured by the method described by AOAC (1990). Crude protein was determined according to AOAC (1990) and metabolizable energy, ammoniacal N ($N\text{-}NH_3$), pH and neutral detergent fiber according to Sadzawka et al. (2007). The chemical composition of feed is presented in Table 1.

There was a 14 d period before the experiment to accustom the animals to the two feeding system and management conditions. After this period animals were weighed at the beginning of the study at 14-mo age and every 14 d in the morning without fasting. The average initial LW at 14-mo was 340 kg and the average final live weight at slaughter was 552 ± 40 kg for the forage diet group and 549 ± 20 kg LW for the concentrate diet group, with 170 d and 147 d in the study, respectively. The animals were slaughtered according to industrial practice in Chile at a commercial slaughterhouse 20 km from INIA center. After slaughter, carcasses were identified and cooled for 24 h at 2 °C. The pH_{24} (pH 24 h *post mortem*) level was measured three times with a pH penetration electrode (Hanna FC232) of a portable pH-meter (Hanna 99163, Hanna Instruments, Woonsocket, Rhode Island, USA). In the quartering, 200 g of *Longissimus thoracis* was removed from the 8th to the 9th vertebra and transported to the meat science laboratory of the INIA Remehue. Muscle samples were vacuum packed and stored at 4 ± 2 ºC until analysis.

Table 1. Chemical composition of feeds used (DM basis).

Feed	DM	CP	ME	NDF	$N\text{-}NH_3$	pH
	—— % ——		Mcal kg^{-1}	—— % ——		
Pasture[1]	31.5	11.2	2.36	60.2	-	-
Kale (*Brassica olearacea* L.)	12.0	19.8	3.09	18.7	-	-
Pasture silage[2]	34.2	20.1	1.83	47.9	10.5	4.2
Triticale (×*Triticosecale* spp.)	88.1	9.9	3.29	13.8	-	-
Lupine (*Lupinus luteus* L.)	88.1	30.8	3.35	26.7	-	-

DM: Dry matter; CP: crude protein; ME: metabolizable energy; NDF: neutral detergent fiber; $N\text{-}NH_3$: ammoniacal nitrogen.
[1]Mixed pasture of ryegrass (*Lolium perenne*) and clover (*Trifolium repens*).
[2]Silage of mixed pasture of ryegrass and clover.

Color and fat analysis

Steaks were maintained for 30 min at room temperature prior to analysis. Instrumental color measurements were recorded for *L** (lightness; 0: black, 100: white), *a** (redness/greenness; positive values: red, negative values: green), and *b** (yellowness/blueness; positive values: yellow, negative values: blue) using a Minolta chromameter (CR-400, Minolta, Osaka, Japan) with illuminat D_{65} and 2º viewing angle. Readings were taken from three locations of the upper surface of randomly selected steaks to be representative of surface color. After color analysis, all external fat from steaks was removed and samples were ground and intramuscular fat (IMF) was measured by the Soxhlet extraction 920.39 method (AOAC, 1990). The rest of samples were vacuum-packed and stored at -18 ± 2 ºC until fatty acid analysis.

Fatty acid composition

Fat was extracted according to Bligh and Dyer (1959) and Lumley and Colwell (1991). Briefly, 10 g fresh sample was thawed and fat extracted with methanol, chloroform, and water (40:25:16 mL). Samples were then homogenized for 30 min and passed through filter paper in a glass funnel. Water was added until biphasic separation was observed. The fat was concentrated in the chloroform layer. The chloroform phase was collected and removed by evaporation and 2.0 mL of n-hexane was added to the extract, which was then stored at -18 ± 2 °C until analysis. Approximately 0.2 g fat was obtained with this extraction.

Transmethylation was carried out according to the method described by Ichihara et al. (1996). One hundred μL of KOH in 2-n methanol was added and the mixture (sample + 2.0 mL) was agitated for 3 min at room temperature. After phase separation, the supernatant was collected and analyzed by gas chromatography. The fatty acid profile was determined in a gas chromatograph (GC-2010 plus Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). A capillary column SP-2560 (Sigma-Aldrich, Bellefonte, Pennsylvania, USA) of

100 m × 0.25 mm × 0.25 μm film was used. Helium was used as the carrier gas at 1.0 mL min^{-1} with an inlet pressure of 15 psi, using the split injection method (100:1). The injector temperature was fixed at 250 ºC and the detector temperature at 260 ºC. The injected sample volume was 1.0 μL and the oven temperature was programmed to increase from 140 ºC (held for 5 min) to 240 ºC (held for 15 min) at 4 ºC min^{-1}. Fatty acids were identified by comparing the retention times of the chromatograph peaks to those of the methyl esters from a mixture prepared with a 37-component FAME mix standard (Standard: 47885-U, Sigma Aldrich, St. Louis, Missouri, USA), C18:1 *t*-11 methyl ester standard (Standard: 46905-U, Sigma Aldrich) and *c*-9, *t*-11 octadecadienoic conjugated methyl acid (Standard: 10-1823-7, Larodan AB, Malmo, Sweden).

Experiment design and statistical analysis

A completely randomized design was used with eight replicates (animals) per treatment. The results were submitted to an F-ANOVA test at 5% significance, with the XLSTAT 2012 software package.

RESULTS AND DISCUSSION

Table 1 presents the chemical composition of the feed used in the study. As can be observed, the values are in consistent with the traditional nutrient content of local feeds for ruminants (Anrique et al., 2008).

Animal performance (Table 2) indicate that bulls with CD treatment reached a final weight of 549 kg, while those on the forage diet reached a final weight of 552 kg 1-mo later at 21-mo of age and with 1.56 and 1.44 kg an^{-1} d^{-1}, respectively with no differences within treatments ($P > 0.05$). No significant differences were found for dressing percentage, rib eye area, and fat cover ($P > 0.05$). However, kidney fat of 4.44 and 2.61 kg an^{-1}, respectively, were significantly different for both treatments, as well as the pH_{24} (pH 24 h *post mortem*), where the CD treatment presented a higher value (5.72) than the FD (5.46) ($P < 0.05$). Higher levels of kidney fat have been observed in animals finished with concentrates as a consequence of higher levels of lipo enzyme activity (Smith and Crouse, 1984). Regarding pH, grass-fed beef tend to show higher pH_{24} than animals grain-fed beef (French et al., 2001; Del Campo et al., 2008), which could be related to higher levels of muscle glycogen content in animals fed with high-energy diets (Del Campo et al., 2008). However, the pH_{24} value of the animals in the CD treatment is lower than those observed in the study of Catrileo and Rojas (2012) under similar conditions. This could be associated with the confined being more active despite the balanced diet they received.

Table 2. Dairy bulls performance on finishing diets based on forage (FD) or concentrate diet (CD).

	FD	CD
Days on study	170	147
Initial weight, kg	338.6	341.3
Final weight, kg	552.0	549.0
Daily live weight gain, kg animal^{-1}	1.44	1.56
Carcass weight, kg animal^{-1}	295.8	293.2
Dressing percentage, %	53.62	53.37
Rib eye area, cm^2	75.74	76.74
Fat cover, mm	1.19	1.44
Kidney fat, kg animal^{-1}	2.61b	4.44a
pH 24 h *post mortem*	5.46b	5.72a

Letter within a row indicates difference ($P < 0.05$).
CD: concentrate based diet; FD: forage based diet.

The percentage of intramuscular fat (IMF) was significantly higher ($P < 0.05$) for the CD treatment (Table 3). However, levels are lower than those reported in the same muscle by Morales et al. (2012) (2.1% and 2.3% lower), which could be associated with higher rates of daily weight gain of bulls on a concentrate diet. In this respect, Padre et al. (2006) observed a higher lipid content in steers than in bulls. The lower IMF content in bull beef is due to the presence of testosterone, which is associated with the greater capacity for muscle growth in bulls (Field, 1971).

Colour (L^* parameter) differences were found that could be related to the higher pH values for the CD treatment. This in turn could be because bulls in the feedlot (barn), in spite of a diet higher in carbohydrate, were more active (due to unfamiliar surroundings) than the bulls on the forage diet in the field, which caused a higher incidence of dark cutting. On the other hand, several studies indicate that grass-fed beef is darker than beef from grain-finished animals (Realini et al., 2004), because pasture-fed animals have more muscle myoglobin since they are more active than concentrate-fed animals that are kept indoors (Priolo et al., 2001).

***Longissimus thoracis* intramuscular fatty acid composition**

Intramuscular fat from the FD treatment had higher levels ($P < 0.05$) of C14:0, C15:0, C18:0, C20:0, and C22:0 and all saturated fatty acids (SFA), in meat than CD with no differences between treatments in C16:0 and C17:0 fatty acids (Table 4). Total monounsaturated fatty acid (MUFA), oleic acid (C18:1 *c*-9), C16:1 and C17:1 levels were higher in IMF from the CD treatment than from the FD (Table 4). No differences ($P > 0.05$) were found for C14:1, C18:1 n-7, and C18:1 *t*-11.

The percentage of all polyunsaturated fatty acids, PUFA, C18:2 *n*-6 *trans*, C18:3 *n*-3, C22:5 *n*-3, C22:6 *n*-3,

Table 3. Intramuscular fat (IMF) content (%), pH, and color of *Longissimus thoracis* muscle of dairy bulls on two feeding systems.

Parameters	CD	FD	RMSE
IMF	1.94a	0.90b	0.903
L^*	39.90a	35.93b	3.506
a^*	19.97	21.28	2.533
b^*	10.30	10.34	2.288

Different letter within a row indicates significant difference ($P < 0.05$).
CD: concentrate based diet; FD: forage based diet; RMSE: root mean squared error. IMF: intramuscular fat ; L^*, (lightness; 0: black, 100: white); a^* (redness/greenness; positive values: red, negative values: green); b^* (yellowness/blueness; positive values: yellow, negative values: blue).

Table 4. Means for fatty acid composition (%) of *Longissimus thoracis* of dairy bulls on two feeding systems.

Fatty acid	CD	FD	RMSE
C 14:0	2.78b	4.65a	1.301
C 14:1	0.48	0.38	0.169
C 15:0	0.32b	0.70a	0.174
C 16:0	26.93	25.73	1.685
C 16:1	2.84a	1.34b	0.442
C 17:0	1.04	0.93	0.144
C 17:1	0.76a	0.36b	0.175
C 18:0	14.83b	18.53a	1.654
C 18:1 *n*-11 *trans*	2.58	2.09	1.175
C 18:1 *n*-9 *cis*	35.00a	26.38b	4.365
C 18:1 *n*-7	0.81	0.66	0.447
C 18:2 *n*-6 *cis*	6.96	9.23	2.314
C 18:2 *n*-6 *trans*	0.06b	0.22a	0.033
C 20	0.05b	0.13a	0.065
C 18:3 *n*-3	1.35b	3.25a	0.645
C 18:2 *n*-6 CLA 9 *cis* 11 *trans*	0.30b	0.58a	0.131
C 22:0	0.04b	0.19a	0.051
C 20:3 *n*-6	0.37	0.50	0.202
C 20:3 *n*-3	0.10	0.25	0.245
C 20:4 *n*-6	1.62	2.40	0.953
C 22:2	0.03	0.12	0.089
C 20:5 *n*-3	0.06b	0.19a	0.104
C 22:5 *n*-3	0.61b	1.25a	0.444
C 22:6 *n*-3	0.04	0.02	0.052
SFA	45.99b	50.85a	2.877
MUFA	42.46a	31.20b	4.657
PUFA	11.49b	18.00a	4.598
n-3	2.16b	4.96a	1.269
n-6	9.30	12.93	3.425
P:S	0.25	0.36	0.105
n-6:*n*-3	4.54a	2.64b	0.638

Different letter within a row indicates significant difference ($P < 0.05$).
CD: concentrated based diet; FD: forage based diet; RMSE: root mean squared error; CLA: conjugated linoleic acid; SFA: saturated fatty acids: C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0; MUFA: monounsaturated fatty acids: C16:1 + C17:1 + C18:1 *n*-9 + C18:1 *n*-7 + C18:1 *n*-11; PUFA: polyunsaturated fatty acids: C18:2 *n*-6 + C18:2 *n*-6 *trans* + C18:3 *n*-3 + C20:3 *n*-6 + C20:3 *n*-3 + C20:4 *n*-6 + C20:5 *n*-3; C22:5 *n*-3 + C22:6 *n*-3 + CLA c-9 t-11; PS: polyunsaturated:saturated fatty acid ratio; *n*-6:*n*-3: fatty acid ratio.

were significantly higher ($P < 0.05$) for the FD treatment than the CD. The percentages of all PUFAs were higher than those reported in Chilean studies with steers (Morales et al., 2012) and other studies using different animal feed sources (Schor et al., 2008), although similar to those for Holstein Friesian bulls according to Padre et al. (2006).

Although seafood is the major dietary source of *n*-3 fatty acids, red meat also constitutes a significant source for some populations with limited consumption of seafood (Sinclair et al., 1994). In the present study, the pasture-fed beef had higher 18:3 *n*-3 and all *n*-3 fatty acid values.

C18:1 *t*-11 (*trans*-vaccenic acid) levels were similar between the two treatments and similar to levels found in others studies with steers fed on pasture plus supplements and feedlot. Some studies suggest a linear increase in CLA *c*-9, *t*-11 synthesis as the C18:1 *t*-11 content of the diet increases in human subjects (Salminen et al., 1998; Turpeinen et al., 2002), while the C18:1 *t*-11 to CLA *c*-9, *t*-11 conversion rate is estimated at 5% to 12% in rodents and 19% to 30% in humans (Turpeinen et al., 2002).

Conjugated linoleic acid *c*-9, *t*-11 was higher ($P < 0.05$) for the FD treatment. CLA *c*-9, *t*-11 is produced as a result of biohydrogenation in the rumen, where unsaturated fatty acids (mainly C18:2 *n*-6 and C18:3 *n*-3) from the diet are isomerized and then partially saturated (De La Torre et al., 2006). CLA *c*-9, *t*-11 is also synthesized by endogenous conversion of C18:1 *t*-11 (*trans*-vaccenic acid) by the enzyme Δ-9-desaturase in adipose tissue and the mammary gland (Bauman et al., 2011). Biohydrogenation is affected by the level of concentrates in the animal diet (Scollan et al., 2014) and the CLA *c*-9, *t*-11 concentration in the adipose tissue is higher when animals are fed on pasture than on stored forage or grain (French et al., 2000). Other studies have also shown that steers finished on pasture have higher CLA *c*-9, *t*-11 than those fed on grain-based diets (Realini et al., 2004; Del Campo et al., 2008; De la Fuente et al., 2009; Morales et al., 2012).

There were no differences between the treatments in the P:S ratio of IMF ($P > 0.05$). A value of 0.4 or higher is recommended for the P:S ratio (British Department of Health, 1994). However, De la Fuente et al. (2009) indicated that the P:S ratio has limited significance because not all saturated fatty acids increase cholesterol. Moreover, the positive effect of monounsaturated fatty acids such as C18:1 *c*-9 (Lee et al., 1998) for human health is not considered when this ratio is used. C18:1 *c*-9 increases human HDL (high-density lipoprotein)-cholesterol and decreases LDL (low-density lipoprotein)-cholesterol concentrations (Katan et al., 1994), and there is a positive relationship between LDL-cholesterol levels and human cardiovascular diseases. In contrast, HDL-cholesterol reduces the risk of cardiovascular diseases (Kwiterovich, 1997).

Considerable attention has been paid to the relative proportion of *n*-6 and *n*-3 fatty acids, as diets with high *n*-6: *n*-3 ratios have been highlighted as risk factors for certain cancers and coronary heart diseases (Hibbeln et al., 2006). A value of 4.0 or less for a diet is recommended for the *n*-6: *n*-3 ratio (British Department of Health, 1994).

Beef from the FD treatment had *n*-6:*n*-3 ratios below 4.0 (Table 4), which is consistent of other studies (Schor et al., 2008; De la Fuente et al., 2009; Klee et al., 2011; Morales et al., 2012). These authors also found that the *n*-6: *n*-3 ratio increases as the grain or concentrate content in the diet increases.

CONCLUSIONS

Under the conditions of the present study dairy bulls fed the forage diet had higher levels of all polyunsaturated fatty acids, including conjugated linoleic acid, than the bulls fed the concentrate diet. There were no differences in final live weight, dressing percentage, daily weight gain, rib eye area and fat cover, although finishing required one month less with the concentrate-fed bulls.

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