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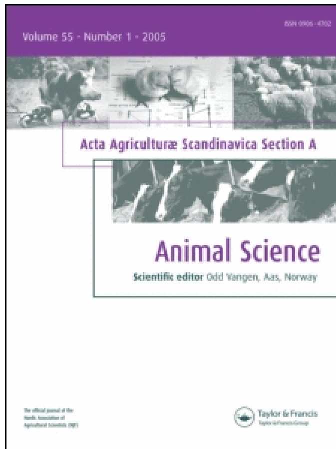

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ORIGINAL ARTICLE

Influence of hempseed cake and soybean meal on lipid fractions in bovine *M. longissimus dorsi*

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Abstract
Sixteen Swedish Red steers were fed a hempseed cake (HC) or soybean meal (SM) protein supplement. Lipid extract from fresh and cooked *M. longissimus dorsi* were analysed. Diet comparison showed that HC had increased monounsaturated fatty acid proportion, primarily 18:1 *cis*-9 in the meat ($P<0.05$). Additionally, HC steers had higher proportions of 18:1 *trans*-11 and 18:2 *c*-9, *t*-11 ($P<0.05$). Furthermore, HC steers had decreased n-6/n-3 fatty acid ratio compared to SM steers ($P<0.05$). Cooking increased polyunsaturated fatty acid (PUFA) and n-6 level, particularly 18:2n-6 and 20:4n-6 ($P<0.05$). Cooking decreased the proportion of 17:0 and 18:0 ($P<0.05$). In polar lipid, HC steers had lower saturated fatty acid ($P<0.05$) and higher PUFA levels ($P<0.05$). Warner-Bratzler shear force and lipid content were unaffected by dietary treatment. Cooking decreased the triacylglycerol level of both groups ($P<0.01$). We conclude that HC is a viable alternate supplement for beef diets which improves the meat FA profile.

Keywords: *Beef, fatty acids, hempseed cake, omega-3, phospholipids, soybean meal, triacylglycerols.*

Introduction

Consumer concerns are increasing regarding the nutritional aspects of their diet, particularly health aspects associated with the lipid content, spurring interest in the lipid profile of consumed products. High dietary saturated fatty acid (SFA) intake has been implicated in the rise of human diseases associated with modern living (Wood et al., 2003). Increased prevalence of diseases in developed countries, including various cancers and cardiovascular disease, has been linked to dietary SFA content (Bruckner, 2000). More recently, cardiovascular health has focused on the ratio and total intake of n-6 and n-3 polyunsaturated fatty acid (PUFA). Wijendran and Hayes (2004) suggest the available mass of 18:2n-6 governs the hyperlipemic effects of SFA, *trans*-monounsaturated fatty acids (MUFA) and cholesterol. Increased use of cereal grains and oilseeds for animal production has resulted in a shift in the fatty acid (FA) profile of agriculture products in industrialised countries. Dramatic increases in human n-6 consumption while static or declining n-3 intake has been linked to increased vegetable oil consumption and use of cereals for animal feeds during the last 40 years (Ailhaud et al., 2006).

Efforts to correct the n-6/n-3 imbalance have included the promotion of increased fish consumption (Williams & Burge, 2006). However, fish consumption is low in many western countries, placing increased reliance on terrestrial livestock for long chain n-3 PUFA (Givens et al., 2006). Givens et al. (2006) illustrate how global meat consumption has increased in the last half century, showing increased relevance of terrestrial meat sources as vectors for altering the lipid intake of humans. In Australia, 43% of dietary long chain n-3 PUFA originates from terrestrial animals, compared to 48% from marine sources (Howe et al., 2006). Ruminant meat makes a significant contribution, accounting for 28% of the total long chain n-3 PUFA consumed in Australia (Howe et al., 2006).

Although beef tissues tend to be high in SFA, averaging 45% of total FA due to extensive

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biohydrogenation within the rumen, they offer a possible alternative source of beneficial FA (Rhee, 2000). 18:2 *c*-9, *t*-11 Conjugated linoleic acid (CLA), unique to ruminant products, is known for its anticarcinogenic and atherogenesis properties (Lock & Bauman, 2004). Additionally, grass-fed beef is a source of beneficial n-3 FA including 20:5n-3 and 22:6n-3 (Howe et al., 2006; Fredriksson Eriksson & Pickova, 2007). Due to the low efficiency of conversion of 18:3n-3 to 20:5n-3 and subsequently 22:6n-3 in man, animal nutrition may hold the key in increasing the n-3 PUFA content of our food supply (Givens et al., 2006). Therefore, it is of importance to understand the influence of animal products originating from sources high in 18:3n-3. The impact of animal products on human nutrition with focus on increasing the n-3 FA dietary contents has been highlighted by De Smet et al. (2004). Grass and green-feed lead to higher proportions of n-3 PUFA in animal products compared to concentrates, which are often produced with cereals and oils rich in 18:2n-6 (Wood et al., 2003; De Henauw et al., 2007; Wood et al., 2008).

Nutritional strategies to increase the n-3 PUFA content of beef tissue, while lowering the n-6/n-3 ratio, have focused on inclusion of fresh forage and lipid sources high in n-3 FA content (Scollan et al., 2006; Fredriksson Eriksson & Pickova, 2007). Inclusion of oilseeds, either as processed or whole, offers varying degrees of rumen bypass efficiency. Linseed is known to have the highest content of 18:3n-3 (Givens et al., 2006), with 73% of the oil as PUFA and 57% of that as n-3 FAs (Flax Council of Canada, 2008). Similarly, hemp oil consists of 84% PUFA, of which 22% is 18:3n-3 (Callaway, 2004). Although there is limited information regarding the feeding value of hempseed in ruminant diets, trials have been conducted using both meal and whole seed. Mustafa et al. (1999) found hemp meal, which contained 32% crude protein and 5.2% ether extract, to be an excellent source of rumen undegraded protein, with digestion characteristics similar to heat-treated canola meal. Inclusion of hemp meal, up to 20% dietary dry matter (DM), had no effect on feed intake for sheep (Mustafa et al., 1999). Alternatively, feeding full-fat oil seed offers greater protection of lipids from biohydrogenation than if fed as a meal (Aldrich et al., 1997). A feedlot diet (Gibb et al., 2005) containing 14% as-fed whole hempseed had no adverse effects on daily intake or gain compared to a soybean control diet. FA profile comparison of *Musculus pars costalis diaphramatic* indicated a higher proportion of 18:3n-3 in hemp-supplemented animals compared to soybean-supplemented animals (Gibb et al., 2005). Whole hempseed provided a good source of bypass protein as well as increasing the content of n-3 PUFA within the tissue. The objectives of this study were to compare the lipid and vitamin E content of fresh and cooked *Musculus longissimus dorsi* (*LD*) muscle from steers supplemented with either cold-pressed hempseed cake (HC) or soybean meal (SM) in addition to the silage:barley (45:55 DM) basal diet. A secondary objective was to determine the influence of diet on meat quality evaluated by shear force.

Materials and methods

Animals

Swedish Red breed steers ($n = 16$) were born spring 2004. Beginning of the indoor winter housing finishing period, 1st November 2005, animals were placed in an insulated barn; with slated floors in the pens. Steers were randomly allocated into four pens per treatment, with two animals per pen. Initial average weight for the HC steers was 397.3 kg (±20.8 kg) and SM steers was 395.9 kg (±38.6 kg). Steers were bunk fed a 45:55 DM grass silage: rolled barley basal diet, with a protein supplement top-dressed and hand-mixed daily. Supplement for each animal from 1st November to 21st December included 0.2 kg as-fed HC or 0.1 kg SM +0.1 kg rolled barley. From December 21st 2005 until the end of the trial 1.4 kg as-fed HC or 0.7 kg rolled barley +0.7 kg SM. Diets were adjusted in December to correct for changing of silage source, and protein content. Animals were shipped and slaughtered based on pen average live weight, endpoint target weights being 620 kg. HC steers were fed 175 d (±18 d), while SM animals were fed a total of 179 d (±16 d). Average live weights were 614 kg (±20.8 kg) and 636.5 kg (±51.4 kg), with corresponding hot carcass weights (−2% shrink) of 321.5 (±11.2 kg) and 327.8 (±32.6 kg) for HC and SM steers respectively, average age 24 months. Further description of diet and feeding conditions are reported by Hessle et al., (2008).

Entire loins were removed from the left side of the steers after chilling 24 h post-slaughter at 5°C. At processing, anterior sections of *LD* were collected from the left loin of each animal, a sample (100g) being removed, frozen at −20°C and after one month transferred to –80°C until analysis. Remaining loins were vacuum-packed and kept at 5°C. At 48 h post-slaughter, pH was measured then loins were divided into sequential 6 cm slices, vacuum packed and aged for seven days at 5°C then frozen at –20°C until shear force analysis.

Shear force

Shear force samples were thawed 24 h at 5°C before cooking 2 h in a 72°C water bath to an internal temperature of 70°C. Samples were cooled in running water to room temperature; a slice (100 g) was removed from each cooked sample and stored at –80°C until lipid extraction for analysis of cooking effects on meat lipids. Warner-Bratzler shear force (WB-SF) analysis (Honikel, 1998) was performed on cook meat by cutting the samples into 1x1x4 cm strips parallel to the muscle fibre. Maximum and total force measurements were based on the average of 12 samples from each animal measured perpendicular to the muscle fibre for using a Stable Micro Systems Texture Analyser HD 100 (Godalning, UK) equipped with a WB-SF blade. Blade dimensions include a cutting area of 11x15 mm with a blade thickness of 1-mm; blade speed was 0.83 mm s^{-1}.

Feed lipid extraction

Composite samples of individual feed ingredients collected weekly during the trial period were analysed in duplicate following a modified Folch et al. (1957) method. Briefly, concentrate samples were ground through a 0.5 mm screen, while silage samples were cut into 0.5–1 cm lengths. Feed samples (2.0 g) were soaked in 8 mL H_2O for 12 h, then homogenised (Ultra Turrax T25, Janke & Kunkel, IKA Werke, Germany) 3x30 s in 150 mL chloroform:methanol (2:1 v/v). Non-lipids were removed by adding 40 mL (0.11 M) NaCl solution, organic solvent separated, and lipid determined gravimetrically.

Tissue lipid extraction

Tissue samples were separated from visible intermuscular adipose tissue and extracted for intramuscular lipid content. Lipid extraction followed a modified Hara and Radin (1978) procedure by separately homogenising 5 g of fresh and cooked muscle tissue in 75 mL hexane: isopropanol (HIP), 3:2 v/v using an Ultra-Turrax. To remove non-lipids, 32.5 mL of 6.67% Na_2SO_4 was added to the homogenate. The solvent was evaporated and lipid determined gravimetrically.

Lipid class separation

Affect of cooking on the composition of the different fractions of total lipid from the tissue was investigated using thin layer chromatography (TLC). As a stationary phase, glass TLC plates pre-coated with silica gel (20x10 cm; Silica gel 60; 0.20 mm layer, Merck, Darmstadt, Germany) were used. Analysis was performed according to Olsen and Henderson (1989) with slight modifications. Lipid samples were applied at a concentration of 1 µg μL^{-1} on a TLC plate by a CAMAG TLC Sampler 4 (Camag, Switzerland) in 2-mm bands with an application speed of 250 nL sec^{-1}; 10 mm between each band, using nitrogen as the spray gas. Using a Camag TLC scanner 3 (Camag, Switzerland), plates were scanned at a speed of 20 mm s^{-1} and a data resolution of 100 µm $step^{-1}$ with a slit dimension of 6.0x0.45 mm at a wavelength of 350 nm. Identification of lipid classes were made by comparison to external standard TLC 18-4A (Nu-Chek Prep, MN, USA). The Savatisky-Golay 7 mode and manual baseline correlation were used for data filtering.

Polar and neutral lipid separation

Neutral lipids (NLs) and polar lipids (PLs) were separated by solid phase extraction (SPE). Tissue total lipids (5 mg) were solved in 3 mL chloroform. Samples were applied to 500 mg silica SPE columns (Isolute SI, IST, UK) pre-developed with 6 mL hexane. NLs were extracted by applying 18 mL chloroform:methanol (50:1 v/v), followed by extraction of PLs using 18 mL methanol. Solvent was evaporated under N_2, remaining lipid was re-suspended in hexane and stored at –80°C until analysis.

Preparation of Fatty Acid Methyl Esters (FAME)

Lipid fractions were methylated following the procedures of Appelqvist (1968). To each sample, 10 µg of methyl 15-methylheptadecanoate (Larodan Fine Chemicals, Malmö, Sweden) was added as an internal standard for quantification. First, 2 mL of 0.01 M NaOH in dry methanol was added, shaken and placed in a 60°C heating block for 10 min. Next, 3 mL of 20% BF_3 reagent (Merck, Darmstadt, Germany) was added and the samples were reheated for 10 min. Once cooled to room temperature, 2 mL of 20% NaCl and 2 mL hexane were added. Test tubes were shaken and allowed to stand for 20 min to separate the layers. The lipid was evaporated under nitrogen gas, solved in hexane and stored at −80°C until analysed.

Gas chromatography

FAME were analysed with a gas chromatograph CP3800 (Varian AB, Stockholm, Sweden) equipped with flame ionisation detector (FID) using a 50 m

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fused silica capillary column BPX 70 (id. 0.22 mm, 0.25 μm film thickness; SGE, TX, USA) equipped with an auto-injector. The split injector was set at a ratio of 1:10 for total and NLs, 1:1 for PLs. Column temperature was programmed to start at 158°C and hold for 5 min, then increase 2°C min^{-1} from 158 to 220°C then hold for 8 min. Injector and detector temperature were 230°C and 250°C, respectively. Identification of the FAs was achieved by comparing the sample retention times to that of standard sample GLC-68-A (Nu-check Prep Inc., MN, USA). Peak areas were integrated using Star Chromatography Workstation software version 5.5 (Varian AB, Stockholm, Sweden). The carrier gas was helium (22 cm sec^{-1}, flow rate 0.8 mL min^{-1}) and nitrogen was used as the make-up gas.

α-Tocopherol analysis

α-Tocopherol content of fresh muscle was determined following a modified Högberg et al. (2002) procedure. Briefly, 20 mg of lipid was dissolved in 2 mL of 99% ethanol while on ice. To each tube, 1.2 mL of 20% ascorbic acid, 0.6 mL methanol and 1.2 mL of 17.9 M potassium hydroxide was added, vortexing thoroughly between additions. Samples were placed in a 70°C agitating water bath for 20 min. Samples were cooled on ice then extracted twice using 3 mL hexane flushes. Samples were evaporated then suspended in 300 μL mobile phase and applied directly to the high performance liquid chromatography (HPLC). Mobile phase consisted of 95% acetonitrile:methanol (1:1 v/v) and 5% chloroform with a flow rate of 1.2 ml min^{-1}. The HPLC column was a 4.0x250 mm RP-18 LiChroCART (Merck KGaA, Germany). Quantification of α-tocopherol was performed by comparison to an external standard (Calbiochem, La Jolla, USA). α-Tocopherol was identified at an excitation wavelength of 290 nm and emission wavelength of 327 nm.

Statistical analysis

Data analysis followed the Mixed Procedure for analysis of variance that included diet and cooking treatment as main effects, significance set at ($P<0.05$), with individual animal as a random effect in the model (SAS v9.1, 2002). Interactions between diet and cooking treatment were investigated and found to be non-significant ($P>0.05$). Values are reported as least square means (LSM) with standard error of the means (SEM), or main means with standard deviation (SD).

Results

Meat quality

Hot carcass weights of HC steers (322 ± 11 kg) and SM steers (328 ± 33 kg) were similar ($P>0.05$, Table I). Diet did not have an effect on the pH of the meat (data not shown). The maximum and total shear force values were measured on cooked *LD* from each steer. WB-SF measurements did not differ between dietary groups ($P>0.05$, Table I).

Lipid composition

Lipid class analysis by TLC indicated a decrease in proportion of triacylglycerols (TAGs) ($P<0.01$) and a subsequent increase in cholesterol and free FAs in the cooked meat ($P<0.05$, Table II). No differences between lipid classes could be attributed to dietary treatments. There were no interactions between diet and cooking treatment (data not shown). No differences were noted for α-tocopherol content between dietary treatments (Table I).

Polar and neutral lipids

The SFA, MUFA and PUFA in the polar fraction, each represented roughly a third of the total PLs (Table III). The proportions of MUFA were similar between dietary treatments. However, the higher SFA proportion in SM steers, accounted for by the difference in 16:0 ($P<0.05$), is reflective of the barley and SM profiles (Table IV). A higher proportion of SFA in the SM steers depressed the PUFA proportion enough to show that the PUFA proportion of HC steers was higher ($P<0.05$). The larger proportion of PUFA within the HC steers can be largely attributed to the level of 18:2n-6, being

Table I. Carcass weight, WB-SF measurements and α-tocopherol content least square means comparison from hempseed cake (HC) or soybean meal (SM) supplemented steers with the standard error of the mean (SEM).

		HC ($n=8$)	SM ($n=8$)	SEM	p
Carcass weight (kg, –2%)		321.5	327.8	6.83	n.s.
Shear force (N)	Maximum	32.9	35.8	2.06	n.s.
	Total	199.7	224.1	11.68	n.s.
α-Tocopherol (mg $100g^{-1}$ lipid)		2.55	3.39	0.54	n.s.

n.s. = not significant ($p>0.05$).

Table II. Total lipid class profile (in%) least square means comparison of fresh and cooked *M. longissimus dorsi* from hempseed cake (HC) or soybean meal (SM) supplemented steers by cooking treatment (C) or diet (D), with pooled standard error of the mean (PSEM).

	HC ($n=8$)		SM ($n=8$)			p	
Lipid class	Fresh	Cooked	Fresh	Cooked	PSEM	C	D
Phospholipids	8.71	9.82	10.9	11.9	2.18	n.s.	n.s.
Cholesterol	4.80	6.12	5.72	6.55	1.58	*	n.s.
Free fatty acid	1.42	3.09	1.24	2.94	0.09	**	n.s.
Triacylglycerols	85.1	81.0	82.2	78.5	3.72	**	n.s.

$p<0.05$ =*, $p<0.01$ =**, n.s. =not significant ($p>0.05$).

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higher in HC steers compared to SM steers ($P<0.05$). In addition, the 18:1 *trans*-11, 18:2 *c*-9, *t*-11 and 18:3n-3 were all higher in the HC steers ($P<0.05$), which could be attributed to the HC FA profile (Table IV). In the HC steers there was a slight non-significant increase in 20:5n-3, 22:5n-3, 22:6n-3 and overall n-3 proportion ($P>0.05$).

The NL profile was comprised predominantly of SFA and MUFA, making up >95% of the profile, being mainly 18:1 *cis*-9, 16:0 and 18:0 FA (Table III). HC steers had a larger proportion of 18:1 *cis*-9 within this fraction ($P<0.05$). SM steers had a larger proportion of 14:0 and 16:0 ($P<0.05$). SM steers also had a larger proportion of 20:1 *cis*-11 ($P<0.05$).

Total lipids

The comparison of the total lipid profile between treatments indicates the influence of diet on the lipid composition (Table V). Although the proportion of SFA was similar between treatments, 16:0 was higher in the SM steers ($P<0.05$). MUFA levels differed, with HC steers having more than a higher proportion ($P<0.05$); 18:1 *cis*-9 is the predominant MUFA, with HC steers having a higher proportion than SM steers ($P<0.05$). The CLA level was reflected by its precursor, 18:1 *trans*-11, both being higher in HC steers ($P<0.01$). The proportion of 20:1 *cis*-11 was higher in HC steers ($P<0.05$). Neither the level of n-6 nor n-3 FA were affected by treatment, however, the n-6/n-3 ratio showed a difference, with HC steers having a lower ratio ($P<0.01$).

Comparison of the lipid profiles of fresh and cooked *LD* shows the change in the FA profile of the cooked meat including a significant increase in fat content during cooking ($P<0.01$). There were some non-significant changes to the overall SFA and 16:0 levels ($P>0.05$), but a significant decrease in 17:0 and 18:0 levels ($P<0.05$). The overall MUFA level was unaffected by cooking ($P>0.05$), but there were significant increases in 14:1 *cis*-9, 16:1 *cis*-9 and 18:1 *cis*-11 levels ($P<0.05$). 18:1 *trans*-11 was negatively affected by cooking, decreasing slightly ($P<0.05$). The PUFA level increased with cooking, showing significant increases in 18:2n-6 and 20:4n-6 levels ($P<0.05$). The n-6/n-3 ratio was slightly higher in cooked *LD* ($P>0.05$), owing to the significant increase in n-6 FA during cooking ($P<0.05$).

Discussion

Meat quality

The feeding trial was designed to investigate if supplementing finishing steers with HC has an effect on meat quality parameters compared to a SM supplement. There was no influence due to variations in carcass weights, lipid content and pH measurements on meat quality parameters. Our findings suggest that HC did not affect technological meat quality; however, a larger scale trial involving more animals and heavier supplementation with HC would be necessary to ensure no effect to animal performance or meat quality. Given the parameters controlled by the trial, no influence of dietary treatment was anticipated, except for shifts to the FA profile. Our results coincide with known influences to meat tenderness such as, animal age, carcass aging, genetics, degree of carcass finish and cooking treatment (Rhee, 2000).

Lipid class fractions

Changes to the lipid class proportions after cooking indicate that there was a loss of TAG during cooking, which is the plausible reason for the subsequently increased proportion of the other lipid classes. Similar to the findings of Scheeder et al. (2001), changes to the lipid fraction profile can be attributed to loss of moisture and TAG during the cooking process.

Table III. Fatty acid polar and neutral lipid fractions (in%) least square means comparison of fresh *M. longissimus dorsi* from hempseed cake (HC) or soybean meal (SM) supplemented steers, with standard error of the mean (SEM).

	Polar lipid fraction				Neutral lipid fraction			
Fatty acid	HC	SM	SEM	p	HC	SM	SEM	*p*
14:0	0.17	0.12	0.02	*	2.77	3.27	0.16	*
14:1 *cis*-9	trace	trace			0.88	1.02	0.08	n.s.
16:0	21.6	23.7	0.48	*	27.5	29.7	0.64	*
16:1 *cis*-9	1.63	1.88	0.14	n.s.	3.82	4.30	0.17	n.s.
18:0	12.0	12.6	0.32	n.s.	14.4	13.8	0.41	n.s.
18:1 *trans*-9	trace	trace			0.20	0.17	0.01	n.s.
18:1 *trans*-11	0.17	0.08	0.02	**	0.70	0.44	0.05	n.s.
18:1 *cis*-9	25.3	26.4	1.28	n.s.	44.5	42.3	0.78	**
18:1 *cis*-11	1.77	1.61	0.08	n.s.	1.28	1.29	0.05	n.s.
18:1 *cis*-13	0.65	0.94	0.08	*	trace	trace		
18:2 n-6	16.6	14.0	0.85	*	0.92	0.82	0.06	n.s.
18:3 n-3	1.74	1.24	0.11	*	0.23	0.18	0.02	n.s.
18:2 *c*-9, *t*-11	0.12	0.09	0.01	*	0.19	0.13	0.02	n.s.
20:3 n-6	2.26	1.89	0.10	*	trace	trace		
20:4 n-6	6.97	6.71	0.20	n.s.	trace	trace		
20:3 n-3	trace	trace		n.s.	n.d.	n.d.		
22:1 *cis*-13	0.46	0.33	0.02	**	n.d.	n.d.		
20:5 n-3	1.05	0.99	0.07	n.s.	n.d.	n.d.		
22:5 n-6	0.97	0.98	0.05	n.s.	n.d.	n.d.		
22:5 n-3	2.97	2.78	0.14	n.s.	trace	trace		
22:6 n-3	0.28	0.25	0.02	n.s.	n.d.	n.d.		
SFA	34.6	37.2	0.61	*	45.6	47.7	0.89	n.s.
MUFA	30.1	31.4	1.28	n.s.	51.8	49.8	0.80	n.s.
PUFA	33.1	29.1	1.16	*	1.43	1.23	0.10	n.s.
Unknown	2.11	2.28	0.13	n.s.	1.22	1.23	0.08	n.s.
n-6	26.9	23.7	0.96	*	1.17	1.02	0.08	n.s.
n-3	6.11	5.32	0.29	n.s.	0.26	0.21	0.02	n.s.
n-6/n-3	4.42	4.54	0.19	n.s.	4.94	4.97	0.46	n.s.

*$p < 0.05$; **$p < 0.01$; n.s. = not significant ($p > 0.05$); trace = <0.1%; n.d. = not detected.

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Fatty acid composition of polar and neutral lipids

The PL fraction comprises a major portion of membrane phospholipids and, in order to maintain membrane function, contains a higher percentage of PUFA (Wood et al., 2008). Both the 18:2n-6 and 18:3n-3 levels were higher in the PL of HC steers.

Table IV. Fatty acid profile of feed ingredients (in %) of total lipids (TL) on a dry matter (DM) basis.

	Silage	Barley	Hempseed cake	Soybean meal
%TL, DM	5.04	2.38	9.58	2.68
16:0	17.4	22.0	7.38	17.1
18:0	1.64	1.08	2.41	3.53
18:1 *cis*-9	3.15	13.0	8.62	15.2
18:2 n-6	15.3	54.4	53.2	55.0
18:3 n-3	43.5	6.10	19.2	5.99
SFA	24.4	23.8	11.2	21.6
MUFA	4.13	14.5	10.0	17.0
PUFA	58.9	60.7	72.5	61.0
Unknown	6.76	1.03	6.26	0.46
n-6/n-3	0.35	8.92	2.77	9.17

n-6 = 18:2 n-6 + 20:2 n-6 + 20:3 n-6 + 20:4 n-6 + 22:5 n-6.
n-3 = 18:3 n-3 + 20:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3.

Long chain n-3 PUFA are preferentially deposited within muscle membrane phospholipid rather than adipose deposits (Ponnampalam et al., 2002). Both n-6 and n-3 FA groups compete for the same desaturase/elongase enzymes, with a slight preference for n-3 FA as suggested by Hwang (2000). The content of 20:5n-3, 22:5n-3 and 22:6n-3 in the PL fraction indicates some elongation and desaturation of 18:3n-3 into its longer chain derivates. The total n-3 level is slightly higher and the total PUFA level is significantly higher in the HC PL. This might also be of importance for the function of the membranes in the live animal. Our findings were similar to Noci et al. (2005), where the proportion of SFA, MUFA and PUFA within the PL fraction was equivalent. The higher proportion of SFA in SM steers, as a result of a higher proportion of 16:0, presumably from the SM supplement, could be considered a less beneficial trait from a human nutrition perspective (Hu et al., 2001).

A higher proportion of 18:1 *cis*-9 within the neutral fraction of HC steers was likely influenced by the total fat present in the HC diet. Similar to the PL, the difference in 16:0 present in the NL fraction

Table V. Lipid content and fatty acid total lipid (in%) overall means with standard deviation of fresh and cooked *M. longissimus dorsi* from hempseed cake (HC) or soybean meal (SM) supplemented steers showing comparisons for diet and cooking main effects.

	HC				SM				p	
	Fresh	(SD)	Cooked	(SD)	Fresh	(SD)	Cooked	(SD)	Diet	Cooking
Lipid content	10.6	4.18	11.4	4.97	7.5	2.14	8.8	2.62	n.s.	*
14:0	2.65	0.35	2.72	0.42	2.97	0.52	2.80	0.56	n.s.	n.s.
14:1 *cis-9*	0.85	0.21	0.96	0.28	0.88	0.18	0.89	0.18	n.s.	*
16:0	28.3	1.17	28.3	1.15	31.0	2.65	30.6	2.77	*	n.s.
16:1 *cis-9*	3.36	0.40	3.77	0.72	3.73	0.42	3.95	0.38	n.s.	**
17:0	0.54	0.08	0.50	0.08	0.57	0.07	0.55	0.05	n.s.	*
18:0	13.1	0.71	12.3	1.48	12.8	0.83	12.5	1.18	n.s.	*
18:1 *trans*-11	0.58	0.18	0.51	0.14	0.34	0.08	0.31	0.07	**	*
18:1 *cis*-9	44.9	1.16	44.5	1.38	41.8	2.78	42.4	3.04	*	n.s.
18:1 *cis*-11	1.02	0.10	1.15	0.17	1.04	0.15	1.14	0.19	n.s.	**
18:1 *cis*-13	0.39	0.05	0.39	0.10	0.37	0.07	0.40	0.09	n.s.	n.s.
18:2 n-6	1.20	0.32	1.45	0.44	1.36	0.48	1.50	0.61	n.s.	*
18:3 n-3	0.25	0.03	0.26	0.04	0.21	0.05	0.22	0.07	n.s.	n.s.
18:2 *c*-9, *t*-11	0.19	0.05	0.18	0.05	0.12	0.03	0.11	0.03	**	n.s.
20:1 *cis*-11	0.22	0.06	0.23	0.05	0.17	0.04	0.17	0.03	*	n.s.
20:4 n-6	0.21	0.13	0.32	0.21	0.32	0.15	0.40	0.24	n.s.	*
20:5 n-3	trace		trace		trace		trace			
22:5 n-3	0.11	0.06	0.14	0.12	0.14	0.08	0.18	0.13	n.s.	n.s.
SFA	44.6	1.09	43.8	1.78	47.4	3.46	46.2	3.83	n.s.	n.s.
MUFA	51.4	1.26	51.7	1.93	48.4	2.75	49.4	3.21	*	n.s.
PUFA	2.08	0.56	2.55	0.95	2.28	0.85	2.58	1.19	n.s.	*
Unknown	1.90	0.27	1.89	0.25	1.88	0.23	1.84	0.27	n.s.	n.s.
n-6	1.52	0.49	1.92	0.77	1.80	0.70	2.05	0.95	n.s.	*
n-3	0.37	0.11	0.44	0.22	0.36	0.15	0.42	0.26	n.s.	n.s.
n-6/n-3	4.12	0.48	4.45	0.37	5.04	0.47	5.07	0.58	**	n.s.

$*p < 0.05$; $**p < 0.01$; n.s. =not significant ($p > 0.05$); trace = <0.1%.

of SM steers corresponds to the higher 16:0 proportion within the dietary ingredients. The higher proportion of both 14:0 and 16:0 could suggest differences in digestibility between the diets (Hessle et al., 2008). Differences in the proportion of 20:1 *cis*-11 could be traced back to elongation products of the 18:1 *cis*-9 within HC steers. The significance of this finding is unknown and may need to be further investigated in future trials.

Total lipid FA profile

Proportions of FA within total lipids between treatments are more representative of the total effects of the animals' dietary treatments. The higher 16:0 content within SM likely contributed to the increased incorporation of 16:0 into the tissue of the SM steers; however, *de nova* synthesis could also be a significant factor. In regard to human health, high consumption of 12:0, 14:0 and 16:0 SFA have been linked to increased levels of low density lipoproteins in hypercholesterolaemic diets, leading to an increased risk of cardiovascular disease for humans (Hu et al., 2001). A higher MUFA level in the HC steers could be a result of higher desaturase activity within the tissue of fatter animals, as suggested by Daniel et al. (2004). Alternatively, differences in the proportion of MUFA, particularly of 18:1 *cis*-9, in the tissue could be due to absorption of MUFA from the diet and be reflective of the uneven fat content of the diets, leading to the slight trend towards a higher fat content in the HC steers.

Comparison of the treatments showed that the PUFA proportion was not influenced by the trial parameters suggesting that both supplements in their present form are highly susceptible to rumen hydrogenation.

The higher proportion of 18:1 *trans*-11 within the HC total lipid profile suggesting a build-up of biohydrogenation intermediates leaving the rumen. This could be as a result of more PUFA in the diet or the 18:3n-3 was partly protected from biohydrogenation, preventing complete conversion to 18:0. 18:1 *trans*-11 is predominantly responsible for endogenous production of CLA *c*-9, *t*-11 (Bauman et al., 2003), which is supported by the subsequent accumulation of CLA *c*-9, *t*-11 within the tissue of HC steers ($P < 0.05$). The importance of CLA *c*-9 *t*-11 is based on its inhibition of atherogenesis and potential as a natural anticarcinogenic (De La Torre et al., 2006).

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The n-6/n-3 ratio of food is a concern to human health due to its influence on the body's inflammatory response (Bruckner, 2000). Although the HC steers' n-6/n-3 ratio was lower, having a more favourable ratio, the actual biological significance between the trial results is small. Although present in only trace amounts, long chain n-3 PUFA such as 20:5n-3 and 22:6n-6, have all exhibited positive effects for reducing the risk of cardiovascular disease (Wijendran & Hayes, 2004) and suppression of inflammatory response (Kelley & Rudolph, 2000). This is of importance when considering the entire human diet and especially that animal products in general are the only source of long chain n-3 PUFA.

The shifts in the FA profile after cooking from pooled dietary treatment results gives an indication to how the FA profile changes during cooking and the impact of the product on human health. Shifts in the FA profile confirm our findings from the lipid fraction shifts, cooking results in a loss of moisture and TAGs from adipocyte deposits of the meat. Decreases in TAGs are mostly associated with decreases in SFA, as signified by the changes in 16:0, 17:0 and 18:0. This loss due to cooking results in a proportional increase in the FA which are bound tighter to the tissue as indicated by the small changes to the MUFA and PUFA proportions.

The value of beef as an alternative and complementary source for long chain n-3 PUFA has been reported in numerous studies and reviews (Noci et al., 2005, Givens et al., 2006, Scollan et al., 2006, Wood et al., 2008). Oil and oilseed inclusion remain the most effective means of altering the FA profile of beef animals fed conserved diets (Dewhurst et al., 2006). We have demonstrated the potential of HC to alter the FA profile of beef *LD* by decreasing the n-6/n-3 ratio in comparison to the control diet. Higher inclusion rates of HC or alternative processing of the hempseed may result in a more pronounced shift in the FA profile than observed within our study.

Oil can be included up to 7% of dietary DM without affecting rumen function (Bauman et al., 2003). High oil content within the diet can increase bypass efficiency of long chain PUFA associated with feed particles by inhibiting microbial attachment, thus lipolysis. Beam et al. (2000) increased the soy oil content from 2 to 10% in an *in vitro* experiment, resulting in a decrease in lipolysis from 44% h^{-1} to 30% h^{-1}. Increasing the long chain PUFA content towards the upper safe limits of the diet will also disrupt the microbial environment, reducing the overall biohydrogenation, allowing greater bypass and subsequent integration of PUFA as suggested by Mir et al. (2003). Processing of the oilseeds, either by extrusion or roasting, offer varying degrees of rumen protection. McNiven et al. (2004) investigated *LD* and showed a 130% increase in the 18:3n-3 proportion by feeding roasted soybean compared to whole soybean. The value of hempseed as an animal feed supplement justifies investigations into how processed hempseed will affect the beef lipid profile in relation to human health.

Conclusion

Shear force and lipid content were unaffected by the dietary treatments. Compared to SM supplementation, HC supplementation is suggested to be a viable option for decreasing the n-6/n-3 FA ratio and to increase the proportion of CLA. Slight differences in the PL show modest increases in PUFA levels with hempseed supplementation. Minor lower proportions of SFA and concurrent higher proportions of MUFA within the HC supplemented steers total lipid profile would have positive implications regarding the risk of cardiovascular disease in humans. The lipid fractions as determined by TLC were influenced more by loss during cooking than by diet. Further investigations into the optimal inclusion rates and delivery form of hempseed are warranted to maximise the benefits to both the animal and for human health factors.

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