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Conjugated linoleic acid concentration in *M. Longissimus dorsi* from heifers offered sunflower oil-based concentrates and conserved forages

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Abstract

Dietary inclusion of polyunsaturated fatty acid (PUFA)-rich plant oils is one approach to improving the fatty acid profile of ruminant meat and meat products from a human health perspective. Whole crop wheat silages represent a possible alternative forage to grass silage for beef production, however, they may adversely impact the fatty acid profile of ruminant muscle since grass silage is rich in C18:3$n-3$. The first objective of this experiment was to investigate the relationship between an increase in the dietary supply of C18:2$n-6$ from sunflower oil (SFO) and conjugated linoleic acid (CLA) concentration in the muscle tissue of beef cattle. The second objective was to investigate the effect of the basal forage type on the muscle fatty acid composition and its response to increasing inclusion of SFO. One hundred and five heifers were blocked according to initial bodyweight and assigned to one of seven silage treatments. The silage treatments were: (1) grass silage (GS), (2) whole crop wheat silage with 38% dry matter (DM) (W1), (3) GS and W1 at a ratio of 1:2 (DM basis) (W1GS) (4) GS and W1 at a ratio of 2:1 (DM basis) (GSW1), (5) whole crop wheat silage with 52% DM (W2), (6) GS and W2 at a ratio of 1:2 (DM basis) (W2GS), (7) GS and W2 at a ratio of 2:1 (DM basis) (GSW2). Within each silage treatment, 5 animals were assigned to one of three concentrate rations, differing in the content of SFO. The levels of inclusion of SFO in the concentrate were 0, 55, 110 g/kg concentrate. Inclusion of SFO in the diet led to an increase in the $n-6$:$n-3$ fatty acid ratio in muscle. In animals fed grass silage or mixed silages the $n-6$:$n-3$ ratio was lower in muscle compared with those fed whole crop wheat silages, with the exception of animals fed 55 g SFO/kg, for which feeding W1GS led to a higher ratio than W1. Other than the $n-6$:$n-3$ ratio there were no significant interactions between the effect of type of silage and the level of SFO on the concentration of fatty acids in intramuscular fat. Increasing the inclusion of SFO led to a linear increase in the CLA-*cis-9*,*trans-11* and PUFA concentration in intramuscular fat ($P < 0.001$). This study confirmed the potential for modification, and improvement from a human health perspective, of the fatty acid composition of beef muscle by dietary manipulation.

Keywords: Conjugated linoleic acid; Fatty acids; Silage; Muscle

1. Introduction

In recent years there has been extensive research on the potential benefits for human health of consumption of conjugated linoleic acid (CLA). Some isomers of this fatty acid (in particular *cis-9*,*trans-11* and *trans*-10,*cis*-12) have been associated with inhibition of carcinogenesis (Ip, Singh, Thompson, & Scimeca, 1994), reduction of atherosclerosis (Lee, Kritchevsky, & Pariza, 1994), modification of the immune response (Cook, Miller, Park, & Pariza, 1993) and body fat

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repartitioning (Park et al., 1997). Biohydrogenation by the ruminal microbial flora of dietary C18:2$n-6$ to C18:0 produces CLA as an intermediate (Harfoot & Hazelwood, 1988). Consequently, milk and meat from ruminants are among the richest dietary sources of CLA (Chin, Liu, Storkson, Ha, & Pariza, 1992; Jiang, Bjoerck, Fonden, & Emanuelson, 1996). The supplementation of ruminant diets with fat sources rich in C18:2$n-6$, such as sunflower oil (SFO) or soybeans, has led to an increase in the proportion of CLA*cis-9*,*trans-11* in muscle (Mir et al., 2003; Madron et al., 2002), however there is less information available on oil supplementation with different forage sources.

Dietary fatty acid composition is considered one of the main factors regulating serum lipoprotein concentration (Kromhout, Menotti, Kesteloot, & Sans, 2002). Plasma low-density lipoprotein (LDL) level, a risk factor for coronary heart disease, has been shown to be increased by consumption of short and medium chain saturated fatty acids (SFA) (Williams, 2000). Current medical recommendations suggest that no more than 35% of human dietary energy should be derived from fat, with no more than 10% coming from SFA (Department of Health, 1994). The current daily intake of $n-3$ polyunsaturated fatty acids (PUFA) relative to $n-6$ PUFA is low in western countries (Gregory, Foster, Tyler, & Wiseman, 1990). This is of concern, since increasing the intake of $n-3$ PUFA appears to lower the risk of platelet aggregation and blood clotting, therefore decreasing the risk of thrombosis (Vanschoonbeek, de Maat, & Heemskerk, 2003). Nutritional guidelines therefore recommend a higher consumption of $n-3$ PUFA, suggesting a $n-6$:$n-3$ ratio at 4:1 or lower for the total diet (Department of Health, 1994).

Ruminant meats are generally low in PUFA and rich in SFA due to the biohydrogenation action of rumen bacteria on fat consumed by the animal (Enser et al., 1998). Nevertheless, the type of forage offered to animals may play an important role in determining the fatty acid composition of ruminant muscle. Thus, grass silages, which are rich in C18:3$n-3$ (Givens et al., 2000) may contribute to an increase in the proportion of $n-3$ PUFA in muscle. Although fish and eggs are richer in $n-3$ PUFA than meat products, food products of ruminant origin are often the main sources of $n-3$ fatty acids for humans (Ponnampalam, Sinclair, Egan, Blakeley, & Leury, 2001) and information is needed on the impact of replacement of grass silage with alternative forages on the fatty acid composition of beef. The objectives of this study were to investigate (a) the effect of replacing lard with dietary SFO in concentrate feed rations and (b) the effect of dietary forage source on the intramuscular fatty acid composition of beef heifers.

2. Materials and methods

2.1. Experimental design and animal management

One hundred and five continental crossbred heifers (mean live weight of 426 kg, SD = 34.3 kg) were blocked in groups of seven animals each on a descending and body weight basis and within block, assigned randomly to one of seven silage treatments. The treatments were (1) grass silage (GS), (2) whole crop wheat silage with 38% dry matter (DM) (W1), (3) GS andW1 at a ratio of 1:2 (DM basis) (W1GS) (4) GS and W1 at a ratio of 2:1 (DM basis) (GSW1), (5) whole crop wheat silage with 52% DM (W2), (6) GS and W2 at a ratio of 1:2 (DM basis) (W2GS), (7) GS and W2 at a ratio of 2:1 (DM basis) (GSW2). Silage W2 was made with addition of urea at the ensiling. Within each silage treatment, the 5 animals were randomly assigned to one of three concentrate rations, formulated to contain 110 g lard/kg, 55 g lard/kg plus 55 g SFO/kg or 110 g SFO/kg, in a 7×3 factorial arrangement. The concentrate rations were formulated to be isonitrogenous and isoenergetic and to have similar total fat content and consisted (g/kg), in addition to the fat, of rolled barley (360 g), citrus pulp (360 g), soyabean meal Hipro (140 g), molasses (10 g) and a proprietary mineral/vitamin mix (20 g) containing 20,000 IU of vit E/kg. The animals were housed indoors on concrete slatted floors, arranged in groups of five or six animals per pen and were individually offered the silages ad libitum plus 3 kg of concentrates once daily via Calan electronic gates (American Calan Inc., Northwood, NH, USA). They had free access to clean drinking water. The animals were fed the experimental diets for 142 days, from March until early July. At the end of the experiment the animals were weighed, transported for 3 h to a commercial abattoir (Meadow Meats, Rathdowney, Co. Laois, Ireland) and slaughtered within 60 min of arrival. Perirenal adipose tissue and hot carcass weights were recorded. Carcasses were hung by the Achilles tendon and chilled for 48 h at 4 °C.

2.2. Fatty acid analysis

Two steaks of the *M. Longissimus dorsi* (25 mm thick) were taken at the seventh rib, vacuum-packed and stored at −20 °C. Before extraction of intramuscular fat, the thawed steaks were trimmed of subcutaneous fat and connective tissue. Each steak was chopped coarsely and blended in a food processor (Robot Coupé, R301 Ultra processor, Robot Coupe S.N.C., Vincennes, France). The intramuscular fat was extracted using a modified version of the method used by Folch, Lees, and Stanley (1957). A 2 g sample of each blended steak was homogenised for 3 min in 1-min long intervals with 1 min pause between intervals, using an Ultra Turrax T25 (Janke and Kunkel, IKA Labortechnik), in a test

tube (25 × 200 mm) containing 36 ml of dichloromethane and methanol mix (2:1, v/v) and 0.05% (w/v) of butylated hydroxytoluene as antioxidant. The homogenised tissue was held at 4 °C overnight and filtered through Whatman filter paper no.4 into a second 25 × 200 mm test tube. The test tubes and the material left on the filter were washed with solvent and the filtrates combined. Calcium chloride (0.02% in distilled water, w/v) was added to the filtrate so that the proportions of dichloromethane, methanol and the aqueous phase were 8:4:3 (on a volume basis) and the test tube contents were held overnight at 4 °C for phase separation. The upper aqueous phase was removed by vacuum, and the remaining lower phase was poured through a funnel containing Whatman no.4 filter paper with approximately 5 g of anhydrous Na_2SO_4 to remove any traces of the aqueous phase. The filtrate was collected in a screw-cap test tube (25 × 100 mm) and dried under a stream of nitrogen. Methylation by acid-catalysed methanolysis was performed as described by Stanton et al. (1997). The fatty acid methyl esters (FAME) were extracted and analysed following the procedure described by French et al. (2000). On removal of the samples from the water bath after methylation and cooling, 2 ml of distilled water saturated with hexane (95 ml de-ionised water and 5 ml hexane) were added, followed by 5 ml of hexane. The tubes were shaken and then centrifuged (800*g*) before removing and collecting the upper layer containing FAME. Distilled water was added to the collected FAME, the centrifugation step was repeated, the top layer was collected and this step was repeated once again. The top layer was then removed and poured into a tube containing approximately 0.75 g of anhydrous Na_2SO_4. An aliquot of FAME (500 μl) was transferred into a 2 ml vial, diluted in an equal volume of hexane and analyzed with a Varian 3500 capillary GC (Varian, Harbor City, CA) equipped with a Varian 8035 autosampler and flame ionisation detector. The column was a Supelcowax-10 capillary column (Supelco, Bellefonte, PA) (60 m × 0.32 mm i.d., 0.25 μm film thickness). Helium was the carrier gas, the initial temperature was 50 °C, programmed to increase by 20 °C/min to 220 °C,with a final hold time of 46.5 min. The injection mode was an automatic sample injection on septum-equipped programmable injector (SPI) in splitless mode with an initial injector temperature of 80 °C, increasing by 100 °C/min up to 200 °C with a final hold time of 15 min. The temperature of the detector was set at 250 °C. The data collected were analyzed on a Minichrom PC system (VG Data System, Manchester, UK). Individual fatty acids were identified by retention times with reference to fatty acid standards. The CLA isomers (CLA*cis-9*,*trans-11* and *trans*-10,*cis*-12) were identified by retention time with reference to a CLA mix generously provided by M. Pariza (The Food Research Institute, University of Wisconsin, Madison, USA). The reference peak, to which a response factor of 1.00 was assigned, was C16:0. Fat for fatty acid analysis was extracted from silages and concentrates according to Folch et al. (1957) and methylated as described by Slover and Lanza (1979).

2.3. General feed composition

The DM concentration of the feeds was determined by drying at 98 °C (15 h) as described by Moloney, Read, and Keane (1996). Concentrates and silages were analysed for crude protein concentration (CP) as described by AOAC (1990), for ash concentration as described by Moloney et al. (1996) and for total fat content using the acid hydrolysis/ether extraction procedure described in EC (1984).

2.4. Statistical analysis

Data were subjected to analysis of variance using Genstat 5.0 (VSN International Ltd., Oxford, UK), and a model appropriate for a split-plot design. Block and type of silage were in the main plot and the concentration of SFO and the type of silage by level of SFO interaction were in the sub-plot. The pattern of response to increasing level of SFO in the concentrate was tested using orthogonal polynomials.

3. Results

3.1. Feed chemical composition and fatty acid profile

The chemical composition of the silages and the concentrates is shown in Table 1. Grass silage had a higher oil content than either of the whole crop wheat silages, which were similar to each other. The fatty acid composition of GS, W1, W2 and the concentrates is summarised in Table 2. Fatty acids of shorter chain length than C14:0 were detected only in trace amounts. Grass silage had the highest proportion of PUFA while W2 had the highest proportion of SFA. In all silages the main SFA was C16:0. In GS, the main PUFA was C18:3$n-3$, accounting for 50% of the total fatty acids. The main PUFA in W1 and W2 was C18:2$n-6$, accounting for 41% and 40% of the total fatty acids, respectively. The W1 silage had a higher proportion of C18:3$n-3$ and a lower proportion of C16:0 compared to W2, resulting in lower SFA and higher PUFA than W2. In the concentrate rations, the proportion of SFA decreased as the level of inclusion of SFO increased. The main SFA found in all three concentrates were C18:0 and C16:0, while the proportion of C18:2$n-6$ increased with increasing level of inclusion of SFO. Inclusion of SFO in the concentrates increased the proportion of C18:1 (and subsequently total monounsaturated fatty

Table 1
Chemical composition of individual and mixed silages, and concentrates (mean (SD))

Composition	Silages[a]							Concentrate rations (gSFO[b]/kg)		
	GS	W1	W1GS	GSW1	W2	W2GS	GSW2	0	55	110
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 7$	$n = 6$	$n = 7$
Dry matter (DM) (g/kg)	191 (7.0)	381 (7.9)	282 (10.3)	241 (7.4)	519 (31.8)	329 (19.0)	262 (11.1)	889 (4.2)	893 (2.9)	893 (4.9)
In vitro DM digestibility (g/kg)	732 (22.4)	724 (15.4)	727 (13.2)	738 (14.3)	716 (13.8)	736 (10.4)	732 (8.6)	–	–	–
Crude protein (g/kg DM)	183 (5.4)	116 (3.0)	150 (4.5)	164 (4.3)	176 (9.6)	190 (11.6)	191 (9.1)	131 (8.5)	126 (6.4)	134 (7.8)
Ash (g/kg DM)	99 (4.2)	60 (5.6)	81 (4.5)	90 (6.7)	56 (12.5)	71 (5.7)	85 (3.9)	64 (2.0)	58 (3.7)	55 (2.2)
Fat (g/kg DM)	36 (2.2)	21 (1.6)	29 (2.9)	32 (2.7)	19 (2.0)	25 (3.0)	31 (2.8)	125 (7.2)	122 (5.5)	126 (6.5)

[a] GS = grass silage; W1 = whole crop wheat silage (38% DM); W2 = whole crop wheat silage (52% DM); W1GS = GS and W1 at a ratio of 1:2 (DM basis); GSW1 = GS and W1 at a ratio of 2:1 (DM basis); W2GS = GS and W2 at a ratio of 1:2 (DM basis); GSW2 = GS and W2 at a ratio of 2:1 (DM basis).
[b] SFO = sunflower oil.

Table 2
Fatty acid composition of individual silages, and concentrates (mean (SD))

Fatty acids (g/100 g FAME[a])	Silages[b]			Concentrates (gSFO[c]/kg)		
	GS	W1	W2	0	55	110
	$n = 6$	$n = 6$	$n = 6$	$n = 4$	$n = 4$	$n = 4$
C 14:0	2.25 (0.55)	0.58 (0.05)	0.81(0.23)	1.47 (0.49)	0.87 (0.13)	0.10 (0.12)
C 16:0	16.0 (0.46)	17.3 (0.31)	24.4 (5.16)	21.0 (0.88)	15.8 (2.08)	8.9 (2.30)
C 16:1	1.76 (0.82)	1.85 (0.25)	2.37 (1.50)	2.48 (0.21)	1.38 (0.15)	0.20 (0.05)
C 18:0	1.89 (0.26)	1.02 (0.30)	2.66 (1.22)	28.1 (2.96)	23.1 (1.50)	13.4 (3.69)
C 18:1	6.4 (1.61)	12.2 (0.65)	15.9 (2.05)	17.3 (6.01)	8.6 (3.53)	10.9 (5.01)
C 18:2	18.3 (0.98)	40.9 (1.04)	39.7 (8.81)	27.9 (1.66)	48.1 (2.07)	64.9 (5.86)
C 18:3	50.40 (2.62)	23.20 (0.84)	8.99 (2.85)	0.47 (0.05)	1.07 (0.12)	0.63 (0.23)
SFA[d]	21.7 (1.23)	20.5 (0.53)	29.9 (7.09)	51.1 (3.97)	40.4 (1.34)	23.2 (1.25)
MUFA[e]	8.1 (2.39)	14.0 (0.90)	18.3 (2.99)	19.8 (5.81)	10.0 (3.39)	11.1 (5.05)
PUFA[f]	70.2 (3.59)	65.5 (1.37)	51.8 (9.74)	29.1 (2.26)	49.6 (2.14)	65.7 (5.70)

[a] FAME = fatty acid methyl esters.
[b] GS = grass silage; W1 = whole crop wheat silage (38% DM); W2 = whole crop wheat silage (52% DM).
[c] SFO = sunflower oil.
[d] SFA = saturated fatty acids (sum of all even chain fatty acids up to C24:0 + C13:0, C15:0 and C17:0).
[e] MUFA = monounsaturated fatty acids (sum of C14:1, C16:1, C17:1, all C18:1, C20:1 and C22:1).
[f] PUFA = polyunsaturated fatty acids (total, minus SFA and MUFA).

acids (MUFA)) in the 55 g SFO/kg ration, compared to no inclusion. However the proportion of C18:1 marginally increased in the ration containing 110 g SFO/kg compared to 55 g SFO/kg. A similar result was noted for the proportion of C18:3$n-3$, although the proportion of this fatty acid was small in all concentrate rations.

3.2. Feed intakes, production variables and muscle composition

The effects of the dietary treatments on feed intake, production variables and muscle composition are shown in Table 3. Silage dry matter intake (DMI) was lowest for GS and increased ($P < 0.001$) as the proportion of either W1 or W2 increased. Concentrates contributed on average 2.56 kg DM/day to the total DMI of each animal. Neither the type of silage nor the concentration of SFO in the concentrates affected final liveweight, carcass weight, average daily gain or the moisture and the fat content of the *M. Longissimus dorsi.* Averaged across the three SFO levels, daily intake of MUFA ranged from 59 g/animal for GS to 66 g/animal for W2. Daily PUFA intake ranged from 212 g/animal for W2 to 277 g/animal for the mixed silage, GSW2. The overall contribution of silage to fat intake was, on average, lower than the contribution from the concentrates. Average daily fat intake was 478 g/day, 160 g of which were derived from the silage and the remaining 318 g/day coming from the concentrates.

3.3. Effect of type of silage on fatty acid composition of intramuscular fat

The fatty acid composition of the intramuscular fat of the *M. Longissimus dorsi* is shown in Table 4. Data

Table 3
The effect of dietary treatments on dry matter intake (DMI), production variables and chemical composition of the *M. Longissimus dorsi*

	Silages[i] ($n = 15$)							SED[j]	P[j]	Concentrates (gSFO/kg) ($n = 35$)			SED	P
	GS	W1	W1GS	GSW1	W2	W2GS	GSW2			0	55	110		
DMI (kg/day)	4.81[a]	6.33[d]	6.27[d]	5.35[b]	5.96[c]	6.01[cd]	6.26[d]	0.16	***	2.54	2.57	2.57	–	–
Final liveweight (kg)	545	567	568	562	548	564	574	15.5	NS	553	566	564	10.1	NS
Carcass weight (kg)	286	304	304	303	294	306	306	8.6	NS	295	304	302	5.6	NS
KO[g] (kg/100 kg)	53.4	53.4	53.4	54.0	53.7	54.2	53.3	0.6	NS	53.5	53.6	53.7	0.4	NS
ADG[h] (g)	866	987	1019	941	869	968	1031	46.5	NS	927	976	987	45.6	NS
M. Longissimus dorsi														
Lipid (g/kg)	27.0	38.0	31.0	35.7	29.1	36.3	33.1	5.4	NS	33.7	32.5	32.5	3.2	NS
Moisture (g/kg)	734	729	741	727	733	724	730	5.8	NS	726.6	733.7	733.1	3.75	NS

[g] KO: kill out rate, calculated as cold carcass weight/liveweight.
[h] ADG: average daily liveweight gain.
[i] GS = grass silage; W1 = whole crop wheat silage (38% DM); W2 = whole crop wheat silage (52% DM);W1GS = GS and W1 at a ratio of 1:2 (DM basis); GSW1 = GS and W1 at a ratio of 2:1 (DM basis); W2GS = GS and W2 at a ratio of 1:2 (DM basis); GSW2 = GS and W2 at a ratio of 2:1 (DM basis).
[j] SED: standard error of difference. Within a row means with different superscripts differ ($P < 0.05$). NS, not significant $P > 0.05$; *, ** and *** refer to significance levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

are presented separately for the main effects of type of silage and the SFO concentration, as no interactions, unless specified, were found. There was no significant effect of the type of silage on the proportions of SFA, MUFA, PUFA, $n-6$ PUFA or on the P:S ratio. The proportion of $n-3$ PUFA was higher for GS and GSW1 than all other silages. The $n-6$:$n-3$ ratio was lower for GS, GSW1 and GSW2 and highest when the whole crop wheat silages were fed without mixing with GS ($P < 0.001$). At an individual fatty acid level, GS resulted in a higher proportion of C15:0 and C17:0 in intramuscular fat than the other silages ($P < 0.05$ and $P < 0.001$, respectively). Animals fed GS had the highest muscle proportion of C18:3$n-3$ while those fed W1 and W2 had the lowest. The mixed silages (W1GS, GSW1, W2GS and GSW2) resulted in a higher proportion of C18:3$n-3$ than either W1 or W2 alone, with GSW1 being significantly higher than the other mixed silages and similar to GS. GS resulted in a lower proportion of C20:3$n-3$ ($P < 0.001$) than W1, W2 or W2GS. The proportion of C22:1 in muscle was lower ($P < 0.01$) for animals offered W1, W2, W2GS and GSW2 than it was for those offered GS or any combination of GS and W1. The proportion of CLA was not significantly affected by the type of silage consumed.

3.4. Effect of SFO inclusion on fatty acid composition of intramuscular fat

An increase in the level of SFO inclusion in the concentrates tended to decrease ($P = 0.074$) the proportion of SFA in intramuscular fat and linearly increased ($P < 0.001$) the P:S ratio and the proportions of $n-6$ PUFA and total PUFA. A significant interaction was found between the effect of type of silage and the level of SFO inclusion for the $n-6$:$n-3$ PUFA ratio. At all levels of inclusion of SFO, the $n-6$:$n-3$ ratio decreased when W2 was replaced with W2GS, GSW2 or GS. The $n-6$:$n-3$ ratio also decreased when W1 was replaced by W1GS, GSW1 and GS when offered with concentrates containing 0 g SFO or 110 g/kg concentrate. However with the 55 g SFO/kg concentrate the $n-6$:$n-3$ ratio increased when W1 was replaced with W1GS and deceased when it was replaced with GSW1 and GS. At an individual fatty acid level, there was a linear increase ($P < 0.001$) in the proportions of C15:0, C18:2$n-6$ and CLA*cis-9,trans-11* and a linear decrease ($P < 0.001$) in the proportions of C16:0, C16:1, C17:0, C17:1, C20:1 and C18:3$n-3$ in response to increasing level of inclusion of SFO in the concentrate.

4. Discussion

The addition of plant oils or oilseeds to concentrate rations has been previously used in attempts to increase the incorporation of PUFA in milk fat (Lock & Garnsworthy, 2002) and in ruminant muscle adipose tissue (Bolte, Hess, Means, Moss, & Rule, 2002; Scollan et al., 2001). Feeding a soybean oil-based diet or extruded full fat soybean decreased C14:0, C16:0 and C16:1 in cattle muscle (Beaulieu, Drackley, & Merchen, 2002; Madron et al., 2002). The results of the present experiment are in agreement with this observation and with Mir et al. (2002), who also reported a decrease in the proportion of C16:0 and C16:1 in intramuscular fat as the addition of SFO to the diet increased. A reduction in the concentration of C16:0 in meat is desirable from a human health perspective as it has been identified as a hypercholesterolemic fatty acid (Williams, 2000). Despite the difference observed in silage DMI, the carcass

Table 4
The effect of dietary treatments on the fatty acid composition of the intramuscular lipids of the *M. Longissimus dorsi*

Fatty acids (g/100 g FAME[b])	Silages[a] ($n = 15$)							SED[e]	P[e]	Concentrates (gSFO/kg) ($n = 35$)			SED	P
	GS	W1	W1GS	GSW1	W2	W2GS	GSW2			0	55	110		
C 10:0	0.058	0.089	0.071	0.082	0.085	0.079	0.065	0.013	NS	0.071	0.077	0.078	0.008	NS
C 12:0	0.058	0.089	0.076	0.078	0.092	0.087	0.071	0.012	NS	0.073	0.079	0.083	0.008	NS
C 14:0	2.639	2.693	2.633	2.559	2.739	2.853	2.614	0.175	NS	2.626	2.688	2.713	0.114	NS
C 14:1	0.571	0.580	0.646	0.551	0.626	0.652	0.570	0.080	NS	0.621	0.598	0.580	0.053	NS
C 15:0	0.440^{h}	0.383^{g}	0.358^{g}	0.399^{g}	0.377^{g}	0.387^{g}	0.381^{g}	0.021	*	0.368	0.388	0.411	0.014	***L
C 16:0	26.36	26.19	27.16	26.44	26.72	27.02	27.45	0.480	NS	27.45	26.86	25.98	0.314	***L
C 16:1	3.248^{g}	3.580^{gh}	3.933^{i}	3.503^{gh}	3.577^{gh}	3.788^{hi}	3.670^{hi}	0.178	*	3.742	3.633	3.467	0.116	*L
C 17:0	0.881^{i}	0.748^{gh}	0.725^{g}	0.809^{h}	0.764^{gh}	0.774^{gh}	0.789^{h}	0.028	***	0.809	0.788	0.756	0.018	**L
C 17:1	0.861^{i}	0.762^{gh}	0.832^{hi}	0.852^{i}	0.724^{g}	0.763^{gh}	0.841^{i}	0.040	**	0.849	0.811	0.755	0.026	***L
C 18:0	15.08	13.86	13.43	14.52	14.16	13.86	13.87	0.559	NS	14.01	13.91	14.42	0.366	NS
C 18:1	36.55	38.51	37.64	37.61	36.65	37.25	37.81	0.842	NS	37.39	37.52	37.39	0.551	NS
C 18:2	4.73	4.62	5.12	4.67	5.34	4.66	4.23	0.467	NS	4.16	4.70	5.44	0.306	***L
CLA*cis-9, trans-11*[c]	0.661	0.781	0.610	0.657	0.651	0.662	0.566	0.068	NS	0.432	0.629	0.906	0.045	***L
C 18:3	1.000^{k}	0.567^{g}	0.820^{ij}	0.903^{jk}	0.578^{g}	0.712^{h}	0.776^{hi}	0.053	***	0.797	0.775	0.723	0.035	*L
C 20:0	0.060^{g}	0.108^{hi}	0.102^{hi}	0.140^{i}	0.080^{gh}	0.055^{g}	0.080^{gh}	0.028	*	0.102	0.075	0.090	0.018	NS
C 20:1	0.155	0.196	0.141	0.130	0.172	0.158	0.162	0.033	NS	0.188	0.165	0.125	0.022	**L
C 20:2	0.039	0.037	0.014	0.032	0.040	0.019	0.022	0.014	NS	0.026	0.033	0.028	0.009	NS
C 20:3	0.120^{g}	0.211^{h}	0.127^{g}	0.147^{gh}	0.317^{i}	0.216^{h}	0.152^{gh}	0.042	***	0.169	0.200	0.185	0.027	NS
C 20:4	1.137	1.035	1.322	1.218	1.237	1.078	1.054	0.152	NS	1.121	1.091	1.251	0.100	NS
C 22:1	0.525^{i}	0.339^{g}	0.488^{hi}	0.527^{i}	0.382^{g}	0.375^{g}	0.410^{gh}	0.049	**	0.463	0.421	0.422	0.032	NS
SFA[x]	45.66	44.23	44.61	45.11	45.07	45.19	45.39	0.754	NS	45.57	44.94	44.60	0.493	NS
MUFA[y]	41.91	43.97	43.68	43.17	42.13	42.99	43.46	0.939	NS	43.25	43.15	42.74	0.614	NS
PUFA[w]	7.93	7.66	8.31	8.00	8.78	7.83	7.17	0.636	NS	7.08	7.84	8.93	0.416	***L
$n-6$ PUFA[v]	6.09	6.05	6.68	6.22	7.16	6.18	5.63	0.605	NS	5.65	6.18	7.04	0.396	***L
$n-3$ PUFA[z]	1.182^{j}	0.834^{g}	1.018^{hi}	1.127^{ij}	0.964^{gh}	0.987^{ghi}	0.971^{ghi}	0.088	**	1.009	1.037	0.989	0.057	NS
$n-6$:$n-3$ ratio	5.19^{g}	7.73^{j}	6.97^{ij}	5.74^{gh}	7.90^{j}	6.33^{hi}	5.94^{ghi}	0.556	***	5.88	6.10	7.65	0.364	***L
P:S[d] ratio	0.175	0.173	0.187	0.179	0.196	0.174	0.158	0.015	NS	0.156	0.176	0.201	0.010	***L

[a] GS = grass silage; W1 = whole crop wheat silage (38% DM); W2 = whole crop wheat silage (52% DM); W1GS = GS and W1 at a ratio of 1:2 (DM basis); GSW1 = GS and W1 at a ratio of 2:1 (DM basis); W2GS = GS and W2 at a ratio of 1:2 (DM basis); GSW2 = GS and W2 at a ratio of 2:1 (DM basis).

[b] FAME = fatty acid, ethyl esters.

[c] CLA = conjugated linoleic acid

[d] Polyunsaturated:saturated fatty acid ratio.

[e] SED = standard error of difference. Within a row means with different superscripts differ ($P < 0.05$). NS, not significant $P > 0.05$; *, ** and *** refer to significance levels $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; L = linear effect of increasing inclusion of SFO.

[v] Total $n-6$ PUFA were (sum of C18:2$n-6$, C18:3$n-6$, C20:2, C20:3$n-6$, C20:4 and C 22:2).

[w] MUFA = monounsaturated fatty acids (sum of C14:1, C16:1, C17:1, all C18:1, C20:1 and C22:1).

[x] SFA = saturated fatty acids (sum of all even chain fatty acids up to C 24:0 + C15:0 and C 17:0).

[y] PUFA = polyunsaturated fatty acids (sum of total $n-6$, total $n-3$, CLA*cis-9,trans-11*).

[z] Total $n-3$ PUFA were (sum of C18:3$n-3$, C20:3$n-3$, C20:5, C22:5 and C22:6).

weight and the intramuscular fat concentration were not affected by the dietary treatments, possibly due to differences in the metabolisable energy concentrations of the silages, avoiding thereby the confounding effect of fatness on fatty acid composition suggested by Leat (1978).

4.1. Dietary effects on intramuscular CLA proportion

In the present study, consumption by cattle of increasing amounts of C18:2$n-6$ through the inclusion of SFO (up to 4% of the dietary DM), raised the CLA-*cis-9*,*trans-11* content of muscle lipid by up to 109% when compared with the CLA*cis-9*,*trans-11* content of muscle of heifers offered no SFO. Ivan et al. (2001) also reported an increase in CLA in the rib muscle of lambs when SFO (6% of dietary DM) was added to a 60:40 barley silage/barley grain-soybean meal diet. When dietary C18:2$n-6$ is available in the rumen, ruminal bacteria first convert it into CLA*cis-9*,*trans-11* by isomerisation of the *cis*-12 double bond into a *trans*-11 bond. CLA*cis-9*,*trans*-11 is then biohydrogenated further to C18:1*trans-11* and ultimately to C18:0 (Harfoot & Hazelwood, 1988). In agreement with the results of the present experiment, Mir et al. (2002) found an increase in the concentration of CLA*cis-9*,*trans-11* in bovine intramuscular fat as the inclusion level of SFO in the diet increased from 0 to 6% of dietary DM. In contrast, Beaulieu et al. (2002) did not find a difference in the content of this isomer in intramuscular fat in the loin of heifers fed a corn-based diet with or without a 5% soybean oil supplement. A possible explanation for that result is the nature of the basal ration, which consisted exclusively of concentrate. It has been reported (Piperova et al., 2000) that low forage:concentrate ratios decrease the formation of C18:1*trans*-11 in the rumen. There is evidence that the biohydrogenation pathway that links the production of CLA*cis-9*,*trans-11* to the presence of C18:2$n-6$ as a substrate could be of secondary importance compared to the action of the enzyme Δ^9-desaturase, present in the adipose tissue and in the mammary gland, which converts the C18:1*trans*-11 isomer into CLA*cis-9*,*trans-11* by desaturation of the $n-9$ single bond (Griinari & Bauman, 1999). Increasing the concentration of C18:2$n-6$ in the diet has been shown to increase the concentration of C18:1*trans*-11 produced in the rumen (Lock & Garnsworthy, 2002) which would explain the increase in CLA-*cis-9*,*trans-11* proportion in the muscle found in animals offered high SFO concentrates. Equally, possible low C18:1trans-11 production in the study of Beaulieu et al. (2002) would lead to lower CLA deposition in tissue.

The pathway of hydrogenation of C18:3$n-3$ also includes C18:1*trans*-11 as intermediate, However, in the current study the contribution of the concentrates to the daily intake of C18:3$n-3$ was minor, and the average daily intake of C18:3$n-3$ across the seven silages ranged from 10 to 87 g/animal (for GS and W2, respectively). It is possible that the differences in C18:3$n-3$ intake across the silage treatments were not sufficient to increase the production of C18:1*trans*-11 in the rumen in order to lead to any significant effect of silage in the proportion of CLA in muscle fatty acids. In this respect, the C18:2$n-6$ intake from the different silages could also have played a role in determining the C18:1*trans*-11 production in the rumen, masking the effect of C18:3$n-3$ intake. A possible contribution of silage treatments to the proportion of CLA*cis-9*,*trans-11* in the muscle, may be in maintaining the forage:concentrate ratio close to 70:30 on a DM basis. A high forage:concentrate ratio establishes conditions in the rumen favouring the production of CLA*cis-9*, *trans-11* and C18:1*trans*-11 as the main intermediates of biohydrogenation, while an increase in the concentrate proportion in the diet has been suggested to shift production of intermediates towards CLA*trans*-10,*cis*-12 and CLA*trans*-7,*cis*-9 with a concomitant decrease in C18:1*trans*-11 (Piperova et al., 2000). In the present experiment, CLA*trans*-10,*cis*-12, was separated in the standard mixture but was not detected in intramuscular lipid samples, suggesting a low accumulation of this particular isomer in muscle tissue similar to the findings of French et al. (2000). Other isomers, such CLA*cis*-8,*trans*-10 may co-elute with the main isomer CLA*cis-9*,*trans-11* in beef fat, under the analytical conditions used in this experiment.

4.2. Dietary effects on intramuscular SFA, MUFA and PUFA proportions

The different silages had a limited impact on SFA, MUFA and PUFA compared to the concentrate rations. The absence of a response to type of silage on intramuscular SFA proportion was not surprising, as the daily intake of SFA from the diet was similar across the seven silage treatments. Despite GS having the highest proportions of PUFA, the PUFA proportion of intramuscular fat was not influenced by the type of silage fed, but it was affected by SFO addition to the concentrates. The proportion of PUFA in this study was higher than that observed by Scollan et al. (2001) in steers of comparable intramuscular fatness. This likely reflects the higher content of dietary fat in the concentrates in the present experiment and the high concentration of dietary C18:2$n-6$ supplied by the SFO. A high concentration of C18:2$n-6$ in rumen fluid has been shown to inhibit the complete biohydrogenation of C18:2$n-6$ in vitro, leading to increased production of C18:1*trans*-11 and less C18:0, and to a higher proportion of dietary C18:2$n-6$ escaping biohydrogenation (Harfoot, Noble, & Moore, 1973).

Mir, Rushfeldt, Mir, Paterson, and Weselake (2000) observed a similar increase in PUFA and in the P:S ratio

as in the present study when lambs were fed a diet supplemented with 6% safflower oil. Dietary oil may have led to the increased PUFA. Overall the P:S ratio was substantially higher in muscle in the present experiment (0.18 on average) than the value of 0.11 reported by Enser, Hallett, Hewett, Fursey, and Wood (1996) for UK beef obtained from retail outlets and higher than that predicted (0.05–0.11) from the inverse exponential relationship between the fatty acid content and the P:S ratio described by Scollan, Enser, Gulati, Richardson, and Wood (2003).

4.3. Dietary effects on intramuscular $n-6$ and $n-3$ PUFA proportions

As expected, the inclusion of SFO in the concentrates led to an increase in the proportion of $n-6$ PUFA in intramuscular fat, mainly attributable to an increase in the proportion of C18:2$n-6$. Similar results have been reported for the inclusion of 4% soybean oil (Engle, Spears, Fellner, & Odle, 2000) and for the inclusion of 6% SFO (Mir et al., 2002, 2003) in diets of steers. An increase in $n-6$ PUFA proportion in lamb muscle due to high C18:2$n-6$ oil or oilseed supplementation was also reported in trials conducted by Bolte et al. (2002), Ivan et al. (2001) and Mir et al. (2000). In contrast, Beaulieu et al. (2002) did not find a difference between a control and 5% soybean oil diet for C18:$n-2$ in muscle from heifers.

In the present experiment the addition of SFO led to a linear decrease in C18:3$n-3$ in muscle, even though the differences were numerically small, possibly due to the decreasing proportion of C18:3$n-3$ in the diet as the inclusion of SFO was increased in the concentrate rations. This result agrees with the findings by Mir et al. (2003) and Mir et al. (2002) in cattle, and Ivan et al. (2001) in lambs. Feeding a high $n-6$ PUFA diet is not desirable from a muscle $n-6$:$n-3$ PUFA ratio perspective, as the ratio increases as the proportion of $n-6$ PUFA in the diet increases. In this experiment the proportion of $n-6$ PUFA was higher in the whole crop wheat silages (40 g/100 g FAME) than in GS (18 g/100 gFAME). However, the contribution of the oil present in the silages to the total oil intake in the diet was insufficient to induce any difference in the proportion $n-6$ PUFA in muscle due to the greater contribution to daily C18:2$n-6$ intake from the concentrate. Conversely, the proportion of $n-3$ PUFA in the different silages led to a difference in the daily intake of $n-3$ PUFA across the silage treatments, which resulted in differences in the proportion incorporated in the intramuscular fat (Fig. 1). Therefore, by feeding a range of different silages contributing to different $n-3$ PUFA intake, the highest $n-6$:$n-3$ value of 7.65 (C18:2$n-6$/C18:3$n-3$ ratio of 7.52), obtained when 110 g SFO/kg concentrates were fed to the cattle (averaged across silages), was substantially lower than the C18:2$n-6$/C18:3$n-3$ ratio obtained by Mir et al. (2003) of almost 12:1. The overall $n-6$:$n-3$ average of 6.29:1 was approximately halfway between the values indicated by Enser et al. (1998) for steers fed grass and bulls fed concentrates. The concentrate intake did not have any significant effect on the total intake of C18:3$n-3$ (2.3g C18:3$n-3$/day on average), therefore the type of silage was the main factor influencing the overall $n-3$ PUFA proportion in muscle. Overall, combinations of SFO-enriched meals with GS or predominantly GS mixed silages as forage sources were most appropriate for maintaining the $n-6$:$n-3$ PUFA ratio close to the acceptable value of 4:1 (Department of Health, 1994), since a value of 4.9:1 was observed when GS was fed with no addition of SFO, this increased to 5.5:1 when 110 g SFO/kg were supplied and averaged across the levels of inclusion of SFO, GS and GSW1 resulted in the lowest $n-6$:$n-3$ ratio.

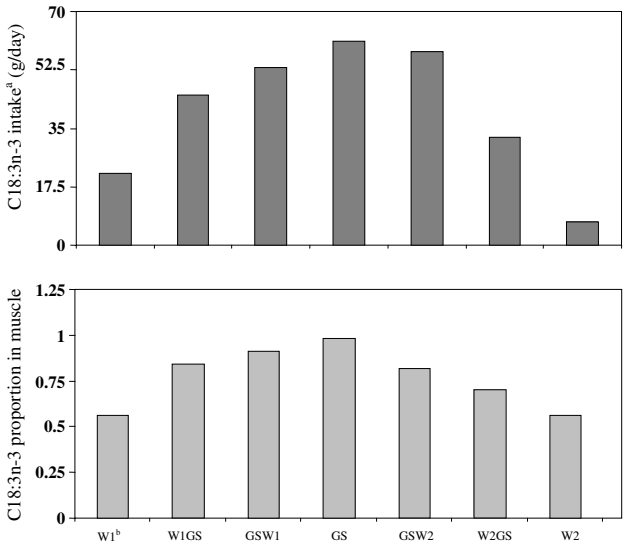

Fig. 1. Comparison of the consumption of C18:3$n-3$ from silage and the proportion of C18:3$n-3$ in intramuscular lipid. [a]Intake of individual fatty acids was estimated by multiplying the calculated total fat intake by the concentration (gFAME/100 g FAME) of C18:3$n-3$ in the feeds and assuming that forages and concentrates contained 530 g FAME/kg lipid and 750 g FAME/kg lipid, respectively (Choi et al., 2000). [b]W1 = whole crop wheat silage (38% DM); GS = grass silage; W2 = whole crop wheat silage (52% DM);W1GS = GS and W1 at a ratio of 1:2 (DM basis); GSW1 = GS and W1 at a ratio of 2:1 (DM basis); W2GS = GS and W2 at a ratio of 1:2 (DM basis); GSW2 = GS and W2 at a ratio of 2:1 (DM basis).

5. Conclusions

This study demonstrated the effectiveness of feeding finishing diets rich in PUFA to beef heifers as a means of modifying the fatty acid profile of their muscle. Replacement of lard with SFO linearly increased the

CLA*cis-9,trans-11* content of the muscle, showing that large amounts of dietary C18:2$n-6$ are an effective means for CLA*cis-9,trans-11* enhancement in intramuscular fat. An increase in dietary PUFA led to an increase in the muscle P:S ratio, which is a desirable effect from a human nutrition point of view, but a disadvantage of enhancing the dietary intake of $n-6$ PUFA was an increase in the $n-6$:$n-3$ ratio. However, the "negative" effect of feeding a C18.2$n-6$ enriched diet on the $n-6$:$n-3$ ratio can be counterbalanced by appropriate inclusion of silage high in C18.3$n-3$. Grass silage or mixed silages with a high proportion of grass silage were more effective at maintaining the $n-6$:$n-3$ ratio close to recommended values than the other whole crop wheat silages evaluated.

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