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CONJUGATED LINOLEIC ACID (CLA) IN MUSCLE FROM STEERS FED DIFFERENT DIETARY LIPIDS

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Background:
Conjugated linoleic acid (CLA) comprises the isomers of linoleic acid which contain conjugated cis, trans double bonds. Cis-9, trans-11 octadecadienoic acid is the major isomer present in ruminant milk, milk products and meat. It is produced in the rumen in the initial step of biohydrogenation of linoleic acid (Kepler and Tove, 1967). CLA isomers are metabolically active and have potential pharmacological benefits since in animals they can modify the immune response, alter energy partition between muscle and fat and inhibit carcinogenesis and atherosclerosis (Pariza, 1997). In humans the lowered risk of breast cancer resulting from milk consumption has been attributed to its CLA content (Knekt *et al* 1996). The factors regulating CLA synthesis in the rumen and its deposition are not fully understood. However, dietary polyunsaturated fatty acids increase the accumulation of trans 18:1 in ruminant tissues indicating a decrease in hydrogenation in the rumen and if this extends to the hydrogenation of CLA, increased tissue levels should occur.

Objectives:
To measure the concentration of CLA in beef muscle and to determine the effect of dietary fats containing n-3 PUFA with different degrees of unsaturation on CLA deposition. The dietary lipids were from linseed, high in α-linolenic acid with three double bonds, and fish oil with high levels of fatty acids with five and six double bonds, eicosapentaenoic acid and docosahexaenoic acid respectively. These were compared with Megalac, a palm oil based fat, as a control.

Methods:
Four groups of eight Charolais cross steers, live weight 436 ± 3.36 kg were fed for an average of 120 days on grass silage (60% of DM intake) and one of four concentrates based on sugarbeet pulp and barley (40% of DM intake). The concentrates supplied half of the total dietary fat of 6% as either 1. Megalac, 2. whole linseed, 3. fish oil, 4. linseed plus fish oil on an equal fat basis. Feed intake was recorded individually using Calan/Broadbent gates.
Forty eight hours after slaughter complete cross-sections of *m.longissimus lumborum* were blended in a food processor and the lipids extracted using Chloroform: Methanol (2:1, v/v). Fatty acids were prepared by alkaline hydrolysis, in the presence of heneicosanoic acid methyl ester as an internal standard, and were converted to methyl esters with diazomethane in diethyl ether. Feed fatty acids were prepared similarly from samples of freeze-dried silage and concentrates. Methyl ester composition was determined by glc on a CP sil 88 (50 m x 0.25 mm ID) column and quantified on the basis of the internal standard. A mixed CLA methyl ester standard was obtained from Sigma Chemical Co (Pool, UK), one component of which corresponded with a single peak on the glc trace assumed to be cis-9, trans-11 CLA.

Results and discussion:
The type of dietary fat supplement did not effect the feed intake, growth rate or cold carcass weight of the steers (Table 1). The daily intake of polyunsaturated fatty acids from the total feed is shown in Table 1. Intake of linoleic acid, the precursor of CLA, was highest for the linseed supplemented feed and lowest for the fish oil supplemented feed. The linseed supplement increased the intake of α-linolenic acid fourfold compared with the Megalac supplement. The fish oil supplement provided 46g per day of EPA plus DHA with half this amount in the mixed linseed/fish oil feed.

In accordance with our hypothesis the concentrations of CLA and trans 18:1 in the muscle fatty acids (Table 2) were 2-3 fold higher in animals consuming the unsaturated fat supplements compared to Megalac. There was also a general linear relationship between the levels of these 2 fatty acids (Figure 1). The proportion of CLA tended to follow the intake of linoleic acid and was highest in the linseed treatment. However trans 18:1 was significantly higher in the animals given fish oil and inspection of Figure 1 shows that the linseed group were all clustered below the regression line. This shows that fish oil long-chain PUFA are extremely effective in inhibiting the hydrogenation of trans 18:1 to stearic acid. 46g/d of EPA plus DHA produced a greater effect than 184g of α-linolenic acid (Linseed minus Megalac intakes). Thus, although all high-PUFA diets reduced the biohydrogenation of linoleic acid, the linseed diet favoured CLA rather than trans 18:1 formation and fish oil had the reverse effect.

Conclusion:
Feeding fat supplements containing n-3 PUFA to beef steers significantly increased the concentration of CLA in muscle. Associated increases in trans 18:1 may be less desirable from the human health aspect but these were increased least and CLA was increased most when linseed was the dietary supplement. Fish oil fatty acids increased trans 18:1 relative to CLA.

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Table 1. Animal performance and fatty acid intakes

Feed fat supplement:	Megalac	Linseed	Fish Oil	Linseed /Fish Oil	sed	P
Silage intake (DM, kg/d)	5.43	5.43	5.12	5.37	0.124	NS
Concentrate intake (DM, kg/d)	3.61	3.62	3.46	3.60	0.082	NS
Live weight gain (kg/d)	1.29	1.25	1.38	1.36	0.056	NS
Carcass weight (cold, kg)	331.1	330.1	334.4	337.3	4.56	NS
Fatty acid intake (g/day)						
18:2 linoleic	79.9	101.4	71.0	82.4	3.75	<0.001
18:3 α-linolenic	59.4	243.8	58.6	158.5	5.83	<0.001
20:5 EPA	0	1.5	28.4	15.2	0.80	<0.001
22:6 DHA	0	1.0	17.6	9.6	0.49	<0.001

Table 2 Effect of dietary fat on selected fatty acids in *m.longissimus*

Dietary fat:	Megalac	Linseed	Fish Oil	Linseed /Fish Oil	sed	P
mg/100g muscle						
CLA	13.3	35.6	24.3	29.0	6.48	<0.05
% of total fatty acids						
CLA	0.3	0.8	0.6	0.7	1.1	<.001
Trans 18:1	1.7	3.4	4.2	4.5	0.3	<.001
18:2	2.6	2.0	1.7	1.7	0.3	NS
18:3	0.7	1.1	0.6	0.8	0.1	<0.01

Figure 1 Relationship between muscle trans 18:1 (y) and CLA (x).y = 1.68 + 2.85x (r = 0.62)