EFFECT OF PROTECTED LIPID SUPPLEMENTS CONTAINING EITHER FISH OR LINSEED OIL ON THE PROFILE OF TRANS-OCTADECENOIC AND CONJUGATED LINOLEIC ACID ISOMERS IN BEEF MUSCLE

Wood, J. D.[1], Hallett, K. G.[1], Whittington, F. M.[1], Enser, M.[1], Scollan, N. D.[2] and Richardson, R. I.[1]

[1] Division of Farm Animal Science, School of Veterinary Science, University of Bristol, Langford, Bristol, BS40 5DU, UK;
[2]Institute of Grassland and Environmental Research, Plas Goggerdan, Aberystwyth, SY23 3EB, UK

Background

Conjugated linoleic acid (CLA, 9-cis,11-tr C18:2) and vaccenic acid (TVA, 11-tr C18:1) are both intermediates of ruminal biohydrogenation. Vaccenic acid is derived from dietary linoleic acid, C18:2, in the rumen and is the precursor for 9-cis, 11tr-CLA synthesis in tissues. 10-tr,12-cis CLA is an isomer that is produced only in the rumen, and has been found in milk from lactating cows. Ruminant meat and milk are especially high in CLA and this may have beneficial implications for human health, primarily due to the anti-carcinogenic effect of 9-cis,11-tr CLA as shown in rats (Corl et *al*, 2003), and the partitioning effects of 10-tr,12-cis CLA (Park *et al*, 1999). Studies involving feeding grass silage supplemented with either linseed (rich in C18:3), fish oil (rich in C20:5 and C22:6) or a mixture of the two, resulted in increases in trans- C18:1 and 9-cis, 11-tr CLA in beef muscle when compared with animals fed Megalac, a more saturated supplement containing C16:0 from palm oil (Enser *et al*, 1999, Scollan *et al*, 2001). The fish oil supplement was found to result in higher values for trans-C18:1 than linseed alone, and although the levels of long chain PUFA increased in muscle, the degree of deposition was low (Scollan et al, 2001), reflecting a significant degree of biohydrogenation still occurring. In a similar study involving lamb, fish oil was found to increase total trans-C18:1 but not CLA in muscle and adipose tissue (Enser *et al*, 2002). Although it has been shown that there is a strong relationship between total trans C18:1 and 9-cis CLA, it is of interest to evaluate the distribution of the other isomers of trans C18:1 that are not involved with CLA synthesis.

Objectives

In this study we have investigated the effect of dietary lipid on the distribution of the different conjugated C18:2 isomers and trans-C18:1 isomers in beef muscle by using protected lipid supplements to reduce the effects of biohydrogenation, and including some free fish oil to encourage CLA production in the rumen.

Materials and Methods

The profile of trans-C18:1 and conjugated C18:2 fatty acid isomers was studied in muscle from beef cattle fed grass silage *ad libitum* in conjunction with a concentrate (ratio 60:40), where the concentrate contained increasing levels of formaldehyde protected lipid supplement, containing 40% oil, either fish or linseed oil. In order to balance dietary fat intake, Megalac concentrate (high in saturated C16:0 from palm oil) was varied.

In Trial 1, protected linseed supplement (PLS) comprised soya bean, linseed and sunflower oil, with C18:2/C18:3 in a ratio of 1:1, and was fed at 0, 400, 800 and 1000g/d (LControl, PLS1, PLS2 and PLS3). In Trial 2, protected fish oil (soya bean and tuna oil, PFO) was fed at 0, 50, 100 or 200g/d and included 100g/d free fish oil (FFO) in all 4 diets (FControl, PFO1, PFO2 and PFO3).

Both trials comprised 32 Charolais crossbred steers having an initial liveweight of 507 ± 10.3kg (PLS, Trial 1) and 619.7 ± 7.9kg (PFO, Trial 2). They were fed the experimental diets for 100d.

Following slaughter, lipids were extracted from m. *longissimus thoracis et lumborum* using chloroform/methanol, followed by alkaline hydrolysis before preparation of methyl esters using diazomethane. FAME, including conjugated C18:2 isomers, were analysed on a CP Sil88 100m x 0.25mm i.d. capillary column using hydrogen as the carrier gas, and a flame ionisation detector. Fatty acids were quantified by the use of an internal standard, C23:0, and identified using standards purchased from Matreya, Inc. USA (Universal Biologicals, Cambridge Ltd) and Sigma, UK. Linearity of the detector was checked by using a C16-C24-monoenoic fatty acid mixture (FAME#5, Restek, UK).

Fatty acid methyl esters taken from animals fed PFO3 and PLS3 as well as their control –fed groups were analysed for trans-C18:1 isomers, using argentation thin-layer chromatography to isolate the trans-monoenoic fatty acid fraction (IUPAC-AOAC method 985.20, 1990), followed by GC analysis.

Statistical analysis was carried out by ANOVA using Genstat 5, Release 4.1(1995),

Results and Discussion

Three major isomers of CLA were quantified in lipid extracts (Table 1), 9-cis, 11-tr CLA, 11-tr, 13-cis CLA and 11-cis, 13-tr CLA, comprising 83%, 10.3% and 6.13% of total CLA respectively. This value for 9-cis, 11-tr CLA compares well with that of 80% found by Chin *et al* (1992) in beef muscle. Comparison with HPLC analysis suggests that this peak includes a small amount of 7-tr, 9-cis and 8-cis,10-trans CLA (Fritsche *et al*, 2000).

9-cis, 11-tr CLA was significantly higher in steers fed PLS3 ($P<0.01$, Table 1). In contrast, there was no significant effect of PFO supplementation on levels of this isomer although PFO1 appeared higher than the other groups. Loor *et al* (2002), in a study with lactating cows, suggested that linseed oil may increase endogenous synthesis of this isomer in tissues by enhancing post-absorptive availability of 11-tr C18:1. The lack of an effect in the PFO trial may be due to insufficient levels of free fish oil in the rumen, and a lack of inhibition of rumen reductase activity.

11-tr, 13-cis CLA showed a similar pattern to that of 9-cis, 11-tr CLA in the PLS trial except that PLS2 as well as PLS3 were also significantly higher than LControl fed steers ($P<0.001$). Animals fed PFO showed no change in the level of this isomer.

While 11-tr, 13-tr CLA appeared to decrease, but not significantly, in animals fed all levels of PLS compared with their LControls, inclusion of PFO had no effect on the level of this isomer in steers in Trial 2.

Levels of 10-tr, 12-cis CLA were found to be below the analytical threshold in all groups of animals, regardless of supplementation. This is not unexpected since these animals have been fed a high forage diet, which has been shown to result in lower levels of the isomer (Dannenberger *et al*, 2004)

Analysis of trans-C18:1 isomers showed that, when comparing level 3 of either protected lipid supplement with its control group, PLS3 resulted in a significant decrease in 6-8-trans, 9-trans, and 12-trans C18:1 when expressed as % of total C18:1 isomers, whereas feeding PF0 had no effect (Fig.1). In contrast, 11-trans C18:1 (TVA) was significantly raised in PLS3 fed animals ($P<0.05$). There was a small increase in PFO3-fed animals compared with their FControls but this was not significant. Overall there was no difference in the amount of total trans-C18:1 present, with levels ranging from 164-204mg/100g tissue.

There was a strong relationship between 11-tr C18:1 and 9-cis, 11-tr CLA ($y = 0.2366x + -0.0614$, $R^2 = 0.622$, Figure 2)

Conclusions

Feeding increasing levels of protected linseed supplement resulted in changes in the pattern of isomer distribution within the total trans-C18:1 fraction and the three major isomers of CLA in beef muscle.

The lack of effect of PFO supplementation on levels of all CLA and trans-C18:1 isomers may have been due to insufficient quantities of oil when compared with that given in the PLS trial.

The distribution of various isomers of trans-C18:1 was affected by dietary PLS although overall amounts of total trans-C18:1 was not different between the groups of steers. Regardless of the effects of diet on the other trans C18:1 isomers, 11-trans C18:1 (TVA) was strongly related to 9-cis, 11-trans CLA.

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Table 1. CLA isomers in beef muscle (% of total fatty acids).

Numbers within rows with different superscripts differ significantly, (P<0.01, ** P<0.001,***) § includes 7tr,9c and 8c,10tr.

	L-Control	PLS1	PLS2	PLS3	F-Control	PFO1	PFO2	PFO3	sed	P
9c,11t[§]	0.421[a]	0.497[ab]	0.495[ab]	0.639[c]	0.441[ab]	0.545[bc]	0.518[ab]	0.491[ab]	0.057	0.009
11t,13c	0.050[a]	0.057[ab]	0.072[bc]	0.076[c]	0.046[a]	0.041[a]	0.044[a]	0.049[a]	0.009	<0.001
11t,13t	0.039	0.019	0.021	0.019	0.018	0.018	0.018	0.019	0.009	0.365

Figure 1. Effect of protected oils on trans C18:1 isomers in beef muscle (% total C18:1)

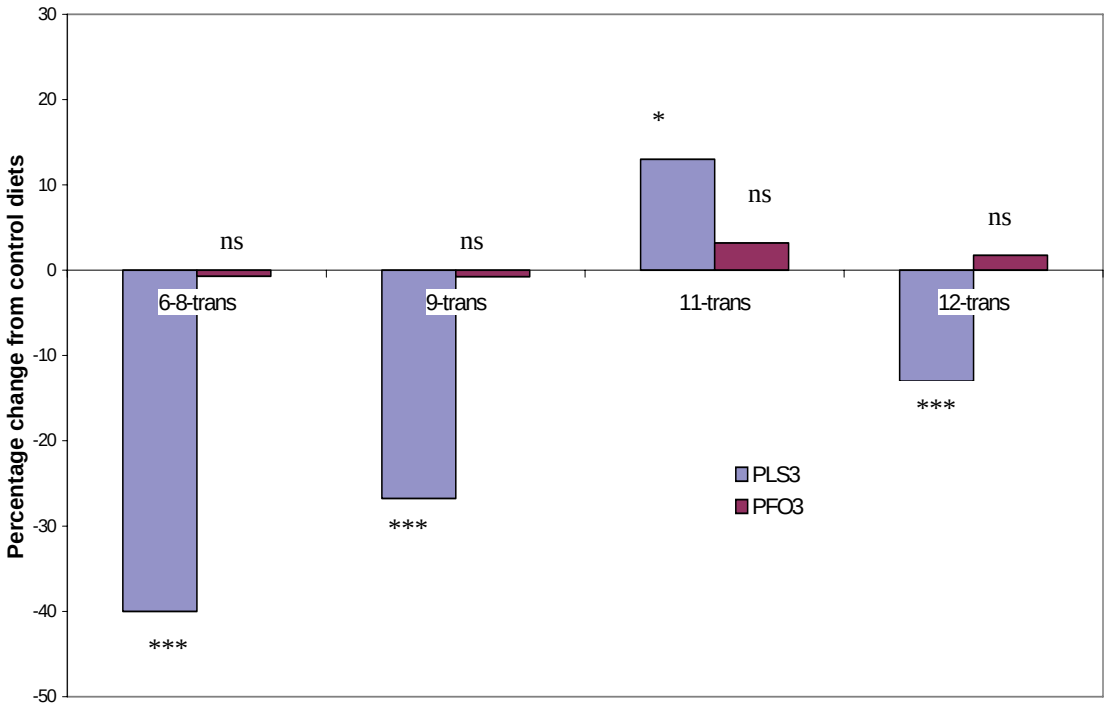

Figure 2. Relationship between 11-trans C18:1 and 9-cis,11-tr CLA in beef muscle