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Effect of incremental amounts of fish oil in the diet on ruminal lipid metabolism in growing steers

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Based on the potential benefits to human health, there is interest in developing sustainable nutritional strategies to enhance the concentration of long-chain *n*-3 fatty acids in ruminant-derived foods. Four Aberdeen Angus steers fitted with rumen and duodenal cannulae were used in a 4 × 4 Latin square experiment with 21 d experimental periods to examine the potential of fish oil (FO) in the diet to enhance the supply of 20 : 5*n*-3 and 22 : 6*n*-3 available for absorption in growing cattle. Treatments consisted of total mixed rations based on maize silage fed at a rate of 85 g DM/kg live weight$^{0·75}$/d containing 0, 8, 16 and 24 g FO/kg diet DM. Supplements of FO reduced linearly ($P<0·01$) DM intake and shifted ($P<0·01$) rumen fermentation towards propionate at the expense of acetate and butyrate. FO in the diet enhanced linearly ($P<0·05$) the flow of *trans*-16 : 1, *trans*-18 : 1, *trans*-18 : 2, 20 : 5*n*-3 and 22 : 6*n*-3, and decreased linearly ($P<0·05$) 18 : 0 and 18 : 3*n*-3 at the duodenum. Increases in the flow of *trans*-18 : 1 were isomer dependent and were determined primarily by higher amounts of *trans*-11 reaching the duodenum. In conclusion, FO alters ruminal lipid metabolism of growing cattle in a dose-dependent manner consistent with an inhibition of ruminal biohydrogenation, and enhances the amount of long-chain *n*-3 fatty acids at the duodenum, but the increases are marginal due to extensive biohydrogenation in the rumen.

Biohydrogenation: *n*-3 Fatty acids: *trans*-Fatty acids: Conjugated linoleic acid: Fish oil

The long-chain PUFA 20 : 5*n*-3 and 22 : 6*n*-3 are known to exert beneficial physiological effects including a reduction in CVD risk, which are at least in part related to the hypotriacylglycerolaemic potential of these fatty acids[1,2]. Even though clinical and biomedical studies have provided evidence that low intakes of 20 : 5*n*-3 and 22 : 6*n*-3 in the human diet are associated with increased chronic disease risk[3], consumption of long-chain *n*-3 PUFA within European populations is often suboptimal[4]. Ruminant-derived foods are a major source of fat in typical Western diets[5,6], indicating the potential of enriching the 20 : 5*n*-3 and 22 : 6*n*-3 content of milk and meat as a nutritional strategy to enhance the *n*-3 PUFA status of human populations.

Inclusion of fish oil (FO) in the diet of ruminants is known to enhance the concentration of 20 : 5*n*-3 and 22 : 6*n*-3 in milk[7,8] and meat[9,10], but enrichment is limited due to the metabolism of long-chain *n*-3 PUFA in the rumen. While a number of studies have examined the potential of FO to increase post-ruminal 20 : 5*n*-3 and 22 : 6*n*-3 supply in lactating and growing ruminants[8,11,12], there is evidence to suggest that the extent of ruminal metabolism of *n*-3 fatty acids varies according to the composition of the basal diet. This comes in part from observations that increases in the ratio of starch to fibre in the diet decrease the rate of lipolysis of dietary lipids *in vitro* [13], that ruminal biohydrogenation of 18 : 3*n*-3 is reduced on high concentrate starch-rich diets[14,15] and that metabolism of 20 : 5*n*-3 in the rumen is more extensive in cattle fed red clover (*Trifolium pratenses*) silage compared with grass (*Lolium perenne*) silage[16]. Furthermore, 18 : 2*n*-6 was shown to inhibit metabolism of 20 : 5*n*-3 and 22 : 6*n*-3 by mixed rumen microbes *in vitro* [17]. These findings suggest that the extent of ruminal metabolism of long-chain *n*-3 fatty acids is potentially lower in ruminants fed diets containing maize (*Zea mays*) silage rich in starch and 18 : 2*n*-6 as the basal forage, but there are no data to substantiate this hypothesis. In the present experiment, the effects of incremental amounts of FO as a source of 20 : 5*n*-3 and 22 : 6*n*-3 on ruminal lipid metabolism and the flow of fatty acids at the duodenum in steers fed maize silage-based diets were examined.

Material and methods

Animals and experimental design

All experimental procedures used were licensed, regulated and inspected by the UK Home Office under the Animals

Abbreviations: CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; FO, fish oil; NDF, neutral-detergent fibre; OM, organic matter; VFA, volatile fatty acids.

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(Scientific Procedures) Act of 1986. Four Aberdeen Angus steers of 555 (SE 12·0) kg live weight at the start of the experiment fitted with rumen cannula (internal diameter 100 mm) and a simple duodenal cannula located within 50 mm of the pylorus were used in a 4 × 4 Latin square design with 21 d experimental periods. Steers were housed in individual tie stalls and offered daily rations as equal meals at 06.00 and 18.00 hours. Animals had continuous access to water and trace-mineralised salt blocks (Baby Red Rockies, Winsford, Cheshire, UK). Steers were weighed at the beginning of the experiment and at the end of each experimental week at 12.00 hours. At the end of the 84 d experiment, steers weighed 636 (SE 10·6) kg.

Experimental diets

Steers were offered total mixed rations based on maize silage (forage:concentrate ratio 60:40 on a DM basis) containing 0, 8, 16 or 24 g/kg DM of refined herring (*Clupea* spp.) and mackerel (*Scomber* spp.) oils (Napro Pharma, AS, Brattvaag, Norway; Table 1) fed at a rate of 85 g DM/kg live weight$^{0·75}$/d equivalent to 95 % of *ad libitum* intake measured at the start of the experiment. Experimental treatments were designed to be within the range of FO doses evaluated in cattle fed grass silage[16], grass silage-based diets[12,18,19] or red clover silage[16], allowing inferences to be drawn on the role of forage species on the potential to enhance the supply of 20 : 5*n*-3 and 22 : 6*n*-3 for incorporation into meat and milk in ruminants. Diets were fed as total mixed rations at a restricted intake to avoid selection of dietary components and maintain a constant forage:concentrate ratio across treatments. Ration mixes were adjusted weekly for changes in component DM content. Supplements of FO were mixed with concentrate ingredients immediately before the addition of maize silage to optimise oil dispersal in the diet. FO was stored in the dark at 4°C before inclusion in daily rations.

Forage maize (cv. Hudson) was harvested using a forage harvester fitted with grain crackers and ensiled directly without additive. Concentrates were formulated[20] to meet the nutrient requirements of growing cattle.

Measurements and sampling

Individual animal intakes were recorded daily, but only measurements collected during the last 5 d of each experimental period were used for statistical analysis. During this period, samples of fresh maize silage, concentrate ingredients and feed refusals were collected daily, and DM content was determined by drying in a forced draught oven at 100°C for 24 h. Feed samples collected daily were added to a composite sample for each experimental period and stored at −20°C. Frozen samples of maize silage were analysed for volatile fatty acids (VFA), ethanol, lactic acid and ammonia nitrogen using accredited and Parliamentary approved procedures for feedstuff analysis (Statutory Instruments, 1982; 1985) by a commercial laboratory (Natural Resources Management, Bracknell, UK), and used to correct the DM content of maize silage for volatile losses during drying[21]. Organic matter (OM) content of maize silage and concentrates was determined by ashing at 550°C for 16 h. Neutral-detergent fibre (NDF) concentrations in maize silage and concentrates corrected for residual ash were measured in the presence of SDS and α-amylase using an ANKOM Fibre analyser (ANKOM-Technology, Fairport, NY, USA)[21]. Feed starch content was measured using the amyloglucosidase technique[22] followed by the determination of total reducing substances and correction for water-soluble carbohydrates, nitrogen was assessed by the Kjeldahl technique and water-soluble carhohydate content was determined by spectrophotometry according to standard procedures[21]. Samples (40 ml) of rumen fluid (*n* 9) were collected on day 20 of each period from each steer at 1·5 h intervals starting at 06.00 hours. Following removal, pH was measured

Table 1. Ingredient and chemical composition of experimental diets (g/kg DM)

	Fish oil in the diet (g/kg DM)			
	0	8	16	24
Ingredient (g/kg DM)				
Maize silage*	600	600	600	600
Soyabean meal	150	147	143	140
Rapeseed meal†	150	147	143	140
Wheat feed	62·5	61·1	59·7	58·3
Fish oil‡	0	8·0	16·0	24·0
Blended cane molasses and urea§	12·5	12·2	11·9	11·6
Minerals and vitamins‖	15·6	15·3	15·0	14·7
Limestone	9·4	9·2	9·0	8·8
Chemical composition (g/kg DM)				
OM	910	911	912	913
NDF	299	298	296	295
Starch	262	262	261	261
WSC	40·6	39·8	39·0	38·2
N	32·0	31·5	31·1	30·6
14 : 0	0·1	0·6	1·2	1·7
16 : 0	4·1	5·2	6·3	7·4
cis-9 16 : 1	0·1	0·7	1·3	1·9
18 : 0	0·6	0·8	1·0	1·2
cis-9 18 : 1	6·6	7·4	8·2	8·9
cis-11 18 : 1	0·9	1·1	1·3	1·5
18 : 2*n*-6	9·9	9·9	9·9	10·0
18 : 3*n*-3	1·3	1·3	1·4	1·5
18 : 4*n*-3	0·0	0·2	0·4	0·7
20 : 5*n*-3	0·0	1·3	2·5	3·8
22 : 5*n*-3	0·0	0·1	0·3	0·4
22 : 6*n*-3	0·0	0·8	1·6	2·4
Σ Fatty acids	25·5	32·9	40·2	47·6

OM, organic matter; NDF, neutral-detergent fibre; WSC, water-soluble carbohydrate.

* Maize silage contained (g/kg) 12 : 0 (0·06), 14 : 0 (0·07), 16 : 0 (3·92), *cis*-9 16 : 1 (0·08), 17 : 0 (0·04), 18 : 0 (0·58), *cis*-9 18 : 1 (5·03), *cis*-11 18 : 1 (0·21), 18 : 2*n*-6 (11·18), 18 : 3*n*-3 (1·47), 20 : 0 (0·30), *cis*-9 20 : 1 (0·04), *cis*-11 20 : 1 (0·06), 20 : 2*n*-6 (0·04), 22 : 0 (0·13), *cis*-13 22 : 1 (0·02), *cis*-15 24 : 1 (0·02) and total fatty acids (23·7).

† Solvent-extracted rapeseed meal of low glucosinolate content.

‡ Fish oil contained (g/kg) 12 : 0 (1·26), 14 : 0 (69·0), *cis*-9 14 : 1 (0·52), 16 : 0 (143), *cis*-9 16 : 1 (74·5), 16 : 2*n*-4 (10·9), 16 : 3*n*-4 (13·5), 16 : 4*n*-1 (23·5), 16 : 4*n*-3 (1·49), 17 : 0 (3·85), 18 : 0 (24·7), *cis*-9 18 : 1 (105), *cis*-11 18 : 1 (25·7), *cis*-12 18 : 1 (0·55), 18 : 2*n*-6 (11·1), 18 : 3*n*-3 (8·67), 18 : 3*n*-6 (2·37), 18 : 4*n*-3 (28·0), 20 : 0 (1·65), *cis*-9 20 : 1 (1·50), *cis*-11 20 : 1 (11·8), *cis*-13 20 : 1 (2·26), 20 : 2*n*-3 (0·49), 20 : 2*n*-9 (1·84), 20 : 3*n*-6 (1·42), 20 : 4*n*-3 (6·82), 20 : 4*n*-6 (7·54), 20 : 5*n*-3 (157), 21 : 5*n*-3 (6·56), 22 : 0 (0·66), *cis*-11 22 : 1 (6·88), *cis*-15 22 : 1 (2·24), 22 : 5*n*-3 (16·7), 22 : 5*n*-6 (2·36), 22 : 6*n*-3 (99·4), 24 : 0 (0·31), *cis*-15 24 : 1 (4·40) and total fatty acids (950).

§ Regumaize 44 (SvG Intermol Limited, Bootle, Merseyside, UK); declared composition (g/kg DM) crude protein (440), water-soluble carbohydrate (550) and metabolisable energy content (11·8 MJ/kg DM).

‖ Proprietary mineral supplement (Dairy direct, Bury St. Edmonds, UK) declared as containing (g/kg) Ca (270), Mg (60), Na (40), P (40), Zn (5·0), Mn (4·0), Cu (1·5); (mg/kg) iodine (500), Co (50), Se (15), retinyl acetate (150), cholecalciferol (2·50) and DL-α-tocopheryl acetate (500).

(pH meter HI8520; Hanna Instruments Ltd., Leighton Buzzard, UK), and samples were stored at −20°C. At the end of the experiment, samples of rumen fluid collected at each time point were bulked on an equal volume basis, and daily composite samples were analysed for VFA by a commercial laboratory (Natural Resources Management) using the same procedures applied to feeds.

Digesta flow was determined using LiCoEDTA and Cr-mordanted straw as indigestible markers for liquid and particulate phases, respectively[23]. Coarsely chopped barley straw was soaked in tap water overnight, rinsed with neutral detergent and labelled with chromium[24]. Cr-mordanted straw containing 40·5 (SE 0·30) mg Cr/g DM was administered (20 g/d) twice daily on top of the rumen contents via the cannula at 12 h intervals starting at 18.00 hours on day 14 of each experimental period. LiCoEDTA (6 g) prepared according to standard procedures[24] was dissolved in 3 litres of distilled water and infused at 18.00 hours on day 14 into the rumen at a constant rate (2·1 ml/min). Ruminal infusions were made using polyamide tubing (internal diameter 4 mm) that passed through the rumen fistula and a peristaltic pump (Model 202; Watson-Marlow, High Wycombe, UK). Markers were administered to each animal to provide daily doses of 0·8 and 0·9 g/d of Cr and Co, respectively. At the start of each marker administration, steers were given priming doses of Cr-mordanted straw and LiCoEDTA supplying 1·0 and 1·35 g of Cr and Co, respectively, to facilitate rapid equilibration of the marker concentrations in the rumen.

Spot samples (250 ml) of digesta at the duodenum were collected three times daily at 4 h intervals over the last 4 d of each experimental period starting at 06.00 hours on day 18. Immediately after collection, 2·5 ml of 2,6-di-tert-butyl-4-methoxyphenol in 80 % (v/v) methanol (1 mg/1 ml) were added, and samples were stored under nitrogen at −20°C. At the end of the study, digesta from each animal was thawed at room temperature, and pooled on an equal volume basis across sampling times to provide a composite sample for each experimental period. Composite digesta samples were stirred vigorously and split into two equal subsamples. One subsample was frozen and lyophilised as whole duodenal digesta, while the remainder was separated into liquid and solid phases by centrifugation at 200 ***g*** for 10 min at 4°C. The supernatant was decanted and stored at −20°C, while the solid phase was frozen immediately, lyophilised and stored at −20°C. Samples of solid and whole digesta were analysed for DM, OM, N, ammonia N, starch and NDF using the same methods used for feed ingredients. Concentrations of Cr and Co in digesta were measured by atomic absorption spectroscopy (SpectrAA-10 analyser, Varian Limited, Walton-On-Thames, UK) using reference procedures[25] and samples of duodenal digesta and faeces collected from one steer before the start of the experiment for calibration purposes.

Whole-tract apparent digestibility coefficients were determined by total faecal collection. Faeces were collected over 120 h starting at 10.00 hours on day 17 of each experimental period. Total faeces excreted were weighed, thoroughly mixed, subsampled (10 %, w/w) and stored at −20°C until analysed for DM, OM, NDF, starch, N, Cr and Co contents using the same methods used for the analysis of duodenal digesta. Flows of digesta at the duodenum were calculated after mathematical reconstitution of true digesta[26]. Marker administration was based on faecal excretion. Appearance of Co in faeces was not corrected for potential absorption from the gastrointestinal tract.

Lipid analysis

Fatty acid methyl esters (FAME) of lipids in FO and freeze-dried samples of maize silage and concentrates were prepared in a one-step extraction–transesterification procedure using chloroform[27] and 2 % (v/v) sulphuric acid in methanol[8]. Feed fatty acid content was determined using trinonadecanoin (T-165; Nu-Chek-Prep, Elysian, MN, USA) as an internal standard. Following the addition of 100 μl of internal standard (heneicosanoate in chloroform (15 mg/ml)), lipid in solid and whole digesta samples was extracted in triplicate using a mixture of chloroform–methanol (2:1; v/v). Organic extracts were combined, dried under nitrogen at 50°C, dissolved in hexane and converted to FAME using a base–acid-catalysed transesterification procedure by incubation with freshly prepared 0·5 M-sodium methoxide in methanol at 50°C for 15 min followed by reaction with 5 % (v/v) hydrochloric acid in methanol at 50°C for 60 min[28].

The FAME were separated and quantified using a gas chromatograph (3800 CP, Varian Instruments, Walnut Creek, CA, USA) equipped with a flame ionisation detector, automatic injector, split injection port and a 100 m fused silica capillary column (CP-SIL 88 for FAME; Chrompack, Middelburg, The Netherlands) with helium as the carrier gas and hydrogen as the fuel gas. Total FAME profile in a 1 μl sample at a split ratio of 1:30 was determined using a temperature gradient programme[18]. Peaks were identified by comparison of retention times with authentic FAME standards (ME61, Larodan fine chemicals, Malmo, Sweden; S37, Supelco, Poole, Dorset, UK). Methyl esters in feed ingredients and duodenal digesta not contained in commercially available standards were formally identified by GC-MS analysis of 4,4-dimethyloxazoline fatty acid derivatives prepared from selected samples of FAME by incubation overnight with 2-amino, 2-methyl-1-propanol under a nitrogen atmosphere at 150°C[29]. Impact ionisation spectra of 4,4-dimethyloxazoline fatty acid derivatives were recorded under an ionisation energy of 70 eV using a gas chromatograph (Model 6890; Hewlett-Packard, Wilmington, DE) equipped with a selective quadrupole mass detector (Model 5973N, Agilent Technologies Inc., Wilmington, DE) and a 100 m fused silica capillary column (internal diameter 0·25 mm) coated with 0·2 μm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using a temperature gradient and helium as the carrier gas[29]. Double bond geometry was determined based on atomic mass unit distances, with an interval of twelve atomic mass units between the most intense peaks of clusters of ions containing n and n-1 carbon atoms being interpreted as cleavage of the double bond between carbon n and $n + 1$ in the fatty acid moiety.

Samples of FAME were evaporated under nitrogen, dissolved in heptane and analysed for conjugated linoleic acid (CLA) methyl ester composition by HPLC using four silver-impregnated silica columns (ChromSpher 5 lipids, 250 × 4·6 mm; 5 μm particle size, Varian Ltd., Walton-on-Thames, UK) coupled in series and 0·1 % (v/v) acetonitrile in heptane as the mobile phase[8]. Isomers were identified using an authentic CLA methyl ester standard (O-5632,

Sigma-Aldrich) and chemically synthesised *trans*-9,*cis*-11 CLA[30]. Identification was verified by cross-referencing with the elution order reported in the literature[31] using *cis*-9,*trans*-11 CLA as a landmark isomer.

Statistical analysis

Experimental data were subjected to ANOVA using the mixed linear model procedure of Statistical Analysis Systems software package version 8.2 (SAS Institute, Cary, NC, USA) with a model that included the random effects of animal and fixed effects of period and treatment. Sums of squares for treatment effects were further separated using orthogonal contrasts into single degree of freedom comparisons to test for the significance of linear, quadratic and cubic components of the response to experimental treatments. Least-square means are reported, and treatment effects were declared significant at $P<0{\cdot}05$. Treatments effects at $P<0{\cdot}10$ were considered as a trend towards significance.

Table 2. Effect of incremental amounts of fish oil in the diet on feed and nutrient intake in growing cattle (Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Nutrient intake (g/d)	0	8	16	24	SEM†	*L*	*Q*
Silage DM	6319	6404	6230	5896	56·8	0·001	0·010
Oil	0·0	82·3	163	245	3·3	<0·001	0·971
Concentrate DM	4131	4106	3921	3625	35·7	<0·001	0·009
Total DM	10 450	10 592	10 314	9767	90·8	0·001	0·009
OM	9514	9572	9248	8678	83·5	<0·001	0·009
NDF	3128	3157	3065	2871	30·8	<0·001	0·011
Starch	2750	2786	2706	2552	25·3	0·001	0·009
WSC	419	417	399	369	3·6	<0·001	0·009
N	332	332	319	297	2·9	<0·001	0·010
12 : 0	0·4	0·5	0·6	0·7	<0·01	<0·001	0·010
14 : 0	0·6	6·3	11·9	17·5	0·25	<0·001	0·918
16 : 0	42·7	54·7	64·6	73·8	0·45	<0·001	0·022
cis-9 16 : 1	1·3	7·4	13·4	19·4	0·28	<0·001	0·872
16 : 2*n*-4	0·0	0·9	1·8	2·7	0·04	<0·001	0·981
16 : 3*n*-4	0·0	1·1	2·2	3·3	0·05	<0·001	0·960
16 : 4*n*-1	0·0	1·9	3·8	5·8	0·09	<0·001	0·981
18 : 0	6·5	8·6	10·3	12·0	0·08	<0·001	0·035
cis-9 18 : 1	69·2	77·9	83·7	88·3	0·59	<0·001	0·013
cis-11 18 : 1	9·2	11·3	12·9	14·5	0·10	<0·001	0·032
18 : 1 *trans*	0·8	0·8	0·7	0·7	<0·01	<0·001	0·007
18 : 2*n*-6	103	105	103	97·4	0·9	0·003	0·011
18 : 3*n*-3	13·5	14·3	14·5	14·5	0·11	<0·001	0·009
18 : 3*n*-6	0·0	0·2	0·4	0·6	0·01	<0·001	0·976
18 : 4*n*-3	0·0	2·3	4·6	6·9	0·09	<0·001	0·974
20 : 0	2·4	2·6	2·6	2·6	0·02	<0·001	0·008
cis-9 20 : 1	0·3	0·4	0·5	0·7	0·01	<0·001	0·227
cis-11 20 : 1	1·1	2·1	3·0	3·9	0·04	<0·001	0·450
cis-13 + 14 + 15 20 : 1	0·0	0·2	0·4	0·6	0·01	<0·001	0·991
20 : 2*n*-9	0·0	0·2	0·3	0·5	0·01	<0·001	0·977
20 : 3*n*-6	0·0	0·1	0·2	0·3	<0·01	<0·001	0·999
20 : 4*n*-3	0·0	0·6	1·1	1·7	0·02	<0·001	0·977
20 : 4*n*-6	0·0	0·6	1·2	1·8	0·03	<0·001	0·982
20 : 5*n*-3	0·0	12·9	25·6	38·5	0·52	<0·001	0·970
21 : 5*n*-3	0·0	0·5	1·1	1·6	0·02	<0·001	0·956
22 : 0	1·4	1·4	1·4	1·4	0·01	0·051	0·010
cis-11 22 : 1	0·0	0·6	1·1	1·7	0·03	<0·001	0·978
cis-13 22 : 1	0·4	0·6	0·8	0·9	0·01	<0·001	0·214
22 : 5*n*-3	0·0	1·4	2·7	4·1	0·05	<0·001	0·967
22 : 5*n*-6	0·0	0·2	0·4	0·6	0·01	<0·001	0·999
22 : 6*n*-3	0·0	8·2	16·2	24·3	0·33	<0·001	0·966
24 : 0	6·5	6·5	6·2	5·8	0·04	<0·001	0·003
Summary							
Σ Saturates	62·7	84·4	103	121	0·82	<0·001	0·049
Σ Monounsaturates	83·9	105	122	138	0·9	<0·001	0·027
Σ Polyunsaturates	118	153	183	210	1·5	<0·001	0·036
Σ Fatty acids	266	346	413	475	3·1	<0·001	0·033

OM, organic matter; NDF, neutral-detergent fibre; WSC, water-soluble carbohydrate.

* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P>0{\cdot}05$).

† SEM for *n* 16 measurements; error df 6.

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Results

Food composition

Maize silage had the following chemical composition and fermentation characteristics (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 372 (SE 4·7); OM, 936 (SE 2·0); N, 14·1 (SE 0·13); NDF, 371 (SE 24·3); starch, 396 (SE 10·7); pH, 3·84 (SE 0·024); lactic acid, 27·9 (SE 6·11); VFA, 15·5 (SE 1·90); ethanol, 2·91 (SE 1·186); water-soluble carbohydrate, 3·28 (SE 0·744); ammonia N (g/kg total N), 50·0 (SE 4·22). The basal concentrate contained (g/kg DM, unless otherwise stated): DM (g/kg fresh weight), 877 (SE 3·2); OM, 871 (SE 2·5); N, 58·8 (SE 0·52); NDF, 192 (SE 5·9); starch, 61·8 (SE 2·13); water-soluble carbohydrate, 96·5 (SE 0·90).

Maize silage contained 23·7 g fatty acids/kg DM with relatively high amounts (g/kg DM) of 16 : 0 (3·92), *cis*-9 18 : 1 (5·03) and 18 : 2*n*-6 (11·2). The basal concentrate (total fatty acids 28·2 g/kg DM) was relatively abundant in 16 : 0, *cis*-9 18 : 1 and 18 : 2*n*-6 (4·36, 9·02 and 7·93 g/kg DM, respectively), while FO contained (g/kg) 20 : 5*n*-3 (157), 22 : 5*n*-3 (16·7) and 22 : 6*n*-3 (99·4) with a total fatty acid content of 950 g/kg. FO also contained several fatty acids not present in the other feed ingredients including 18 : 4*n*-3 (28·0), 20 : 4*n*-3 (6·82), 20 : 4*n*-6 (7·55) and 21 : 5*n*-3 (6·56).

Nutrient intake

Incremental inclusion of FO in the diet decreased ($P<0·05$) DM, OM, N, NDF, starch and water-soluble carbohydrate intakes in a quadratic manner with the effects being greater at the highest amount of FO (Table 2). Supplementing the diet with FO increased linearly ($P<0·01$) the intake of most fatty acids including 14 : 0, 16 : 0, 18 : 0, *cis*-9 18 : 1, 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3, while the highest FO dose decreased ($P=0·003$) 18 : 2*n*-6 ingestion (Table 2).

Rumen fermentation

Inclusion of FO in the diet had no effect ($P>0·05$) on rumen pH or ammonia N concentrations, but tended ($P=0·06$) to decrease linearly rumen VFA content (Table 3). Supplements of FO increased linearly ($P < 0·01$) molar proportions of propionate and reduced linearly ($P < 0·01$) molar butyrate proportions, with a trend ($P=0·08$) towards a linear decrease in molar proportions of acetate in rumen VFA (Table 3).

Nutrient flow at the duodenum

Increasing amounts of FO in the diet decreased linearly ($P<0·05$) duodenal DM, OM, N and non-ammonia N flow, and tended to reduce ($P=0·07$) post-ruminal NDF flow, but had no effect ($P>0·05$) on the amount of starch or total fatty acids at the duodenum (Table 4). Supplements of FO altered the composition of fatty acids flowing into the duodenum, changes that were characterised by linear or quadratic increases in 14 : 0, 15 : 0, 16 : 0, 3, 7, 11, 15-tetra-methyl-16 : 0, 16 : 1, 17 : 0, 18 : 1, 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 and linear reductions in 18 : 0 and 18 : 3*n*-3 at the duodenum (Table 4). FO enhanced linearly ($P<0·001$) the flow of all *trans* 16 : 1 isomers (Δ6–13), while changes in the amount of 18 : 1 at the duodenum to FO were isomer dependent (Table 5). Duodenal flow of *trans*-4, -5 and -16 18 : 1 was independent of treatment, whereas FO in the diet increased linearly ($P<0·05$) the flow of *trans*-6 to -15 18 : 1 at the duodenum, with most of the increase being related to elevated levels of *trans*-11 18 : 1. FO in the diet also altered the profile of 18 : 2 of duodenal digesta, increasing linearly ($P<0·05$) *trans*-11,*cis*-15 18 : 2, *trans*-9,*trans*-12 18 : 2 and *trans*-8,*trans*-10 CLA and decreasing linearly ($P<0·05$) *trans*-11,*cis*-13 CLA flow at the duodenum (Table 6). Supplements of FO also tended ($P=0·07$) to reduce the amount of *cis*-9,*cis*-12 18 : 2 at the duodenum and altered *trans*-9,*trans*-11 CLA flow (Table 6) in a quadratic manner ($P<0·01$). Incremental amounts of FO in the diet increased linearly ($P<0·05$) the extent of *cis*-9 18 : 1, 18 : 3*n*-3, 20 : 5*n*-3 and 22 : 6*n*-3 biohydrogenation in the rumen (Table 7).

Nutrient digestibility

Inclusion of FO in the diet had no effect ($P>0·05$) on forestomach (defined as rumen, reticulum, omasum and abomasum)

Table 3. Effect of incremental amounts of fish oil in the diet on rumen fermentation characteristics in growing cattle

(Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					
	0	8	16	24	SEM†	*P**
pH	6·59	6·56	6·57	6·50	0·031	0·116
Volatile fatty acids (mmol/l)	58·6	56·5	58·8	52·9	1·21	0·062
Molar proportions (mmol/mol)						
Acetate	671	661	657	643	9·7	0·081
Propionate	169	183	184	210	6·7	0·006
Butyrate	152	147	145	138	2·2	0·005
Isovalerate	8·5	9·8	14·3	10·0	0·85	0·769
Molar ratios						
Acetate:propionate	4·00	3·64	3·59	3·08	0·166	0·009
(Acetate+butyrate):propionate	4·91	4·45	4·38	3·74	0·201	0·007

* Significance of linear (*L*) components of the response to fish oil in the diet. Quadratic and cubic responses to fish oil in the diet were NS ($P>0·05$), with the exception of a trend ($P=0·097$) towards a cubic decrease in rumen volatile fatty acid concentrations to fish oil supplementation.

† SEM for *n* 16 measurements; error df 6.

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Table 4. Effect of incremental amounts of fish oil in the diet on the flow of nutrients at the duodenum in growing cattle (Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Flow (g/d)	0	8	16	24	SEM†	*L*	*Q*
DM	5696	5477	5371	5076	131·2	0·015	0·781
OM	4730	4494	4472	4219	125·7	0·033	0·949
NDF	1735	1695	1594	1476	89·4	0·070	0·676
Starch	446	445	431	406	62·5	0·649	0·861
N	240	234	227	202	6·8	0·007	0·210
NAN	187	184	182	158	7·2	0·031	0·185
12 : 0	0·62	0·69	0·83	0·81	0·069	0·066	0·501
13 : 0	0·14	0·14	0·16	0·16	0·013	0·278	0·982
14 : 0	2·7	5·1	8·2	11·0	0·42	<0·001	0·655
15 : 0	3·7	4·4	4·8	4·9	0·35	0·037	0·432
15 : 0 *iso*	2·1	2·3	2·4	2·4	0·19	0·345	0·729
15 : 0 *anteiso*	4·5	4·9	4·4	4·4	0·44	0·659	0·720
16 : 0	58·4	88·4	105	109	5·72	<0·001	0·059
16 : 0 *iso*	1·8	2·3	2·1	2·1	0·28	0·689	0·490
3, 7, 11, 15-tetra-methyl-16 : 0§	0·43	0·57	0·68	0·73	0·037	<0·001	0·281
Σ 16 : 1	1·3	3·4	7·1	10·3	0·61	<0·001	0·413
17 : 0	2·5	3·4	3·7	3·9	0·26	0·008	0·189
17 : 0 *iso*	1·0	1·2	1·3	1·3	0·09	0·046	0·576
17 : 0 *anteiso*	2·1	2·5	2·3	2·4	0·19	0·479	0·525
cis-8 17 : 1	0·03	0·09	0·24	0·19	0·063	0·067	0·423
18 : 0	227	219	169	120	20·7	0·007	0·365
18 : 1 *cis*	28·1	33·7	35·2	33·2	1·94	0·105	0·099
18 : 1 *trans*	40·3	68·8	107	116	9·02	<0·001	0·335
Σ 18 : 1	68·4	103	142	149	10·5	0·001	0·255
Σ 18 : 2‡	23·5	24·3	20·6	19·3	2·96	0·266	0·743
Σ CLA	0·39	0·52	0·47	0·37	0·037	0·506	0·023
18 : 3*n*-3	2·8	2·7	2·3	2·0	0·15	0·008	0·505
20 : 0	3·8	11·5	9·4	6·2	1·24	0·393	0·005
cis-11 20 : 1	0·7	2·5	4·4	5·3	0·21	<0·001	0·067
20 : 2*n*-6	0·09	0·28	0·42	0·53	0·028	<0·001	0·183
20 : 3*n*-3	0·04	0·40	1·51	3·17	0·228	<0·001	0·029
20 : 3*n*-6	0·19	0·24	0·24	0·35	0·051	0·088	0·603
20 : 4*n*-6	0·25	0·26	0·17	0·20	0·042	0·256	0·756
20 : 5*n*-3	0·30	0·89	1·16	1·26	0·116	<0·001	0·077
22 : 0	2·1	4·2	3·2	2·6	0·42	0·776	0·019
cis-13 22 : 1	0·37	0·33	0·51	0·71	0·110	0·048	0·324
22 : 2*n*-6	0·0	0·03	0·18	0·30	0·026	<0·001	0·162
22 : 4*n*-6	0·35	0·25	0·25	0·27	0·024	0·076	0·039
22 : 5*n*-3	0·4	0·7	1·0	1·5	0·18	0·005	0·663
22 : 6*n*-3	0·21	0·69	0·95	1·04	0·082	<0·001	0·054
23 : 0	0·44	0·48	0·41	0·29	0·039	0·027	0·082
24 : 0	2·2	2·7	2·6	2·4	0·16	0·484	0·079
Summary							
Σ Saturates	316	354	321	274	28·2	0·256	0·185
Σ Monounsaturates	70·9	109	155	167	11·3	<0·001	0·287
Σ Polyunsaturates	29·4	32·7	32·1	33·7	3·62	0·477	0·821
Σ Fatty acids	417	496	508	475	31·2	0·227	0·122

OM, organic matter; NDF, neutral-detergent fibre; N, nitrogen; NAN, non-ammonia nitrogen; CLA, conjugated linoleic acid.
* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P>0·05$).
† SEMfor *n* 16 measurements; error df 6.
‡ Total 18 : 2 excluding isomers of CLA.
§ Phytanic acid, all isomers of 3, 7, 11, 15-tetra-methyl-hexadecanoic acid.

or whole-tract nutrient digestibility coefficients, with the exception of a trend ($P=0·06$) towards an increase in total-tract DM digestibility (Table 8).

Live weight

Supplementing the diet with FO had no effect ($P<0·05$) on mean animal live weight (604, 612, 605 and 602 kg for diets containing 0, 8, 16 and 24 g FO/kg DM, respectively).

Discussion

A number of experiments have examined the potential of FO to increase the supply of 20 : 5*n*-3 and 22 : 6*n*-3 available for absorption in growing cattle fed grass silage[16], red clover silage[16] or grass silage-based diets[12,18,19], but measurements in ruminants fed diets containing maize silage are limited[32]. In the present experiment, the effects of incremental amounts of FO on ruminal lipid metabolism and the flow of fatty acids at the duodenum in growing cattle fed maize

Table 5. Effect of incremental amounts of fish oil in the diet on the flow of 16 : 1 and 18 : 1 isomers at the duodenum in growing cattle

(Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Flow (g/d)	0	8	16	24	SEM†	*L*	*Q*
cis-9 16 : 1	0·93	1·52	2·49	3·05	0·222	<0·001	0·945
trans-6 16 : 1	0·02	0·21	0·29	0·32	0·023	<0·001	0·013
trans-7 + 8 16 : 1	0·01	0·15	0·34	0·62	0·066	<0·001	0·319
trans-9 16 : 1	0·05	0·20	0·44	0·65	0·047	<0·001	0·472
trans-10 16 : 1	0·01	0·21	0·55	0·85	0·054	<0·001	0·393
trans-11 16 : 1	0·02	0·38	1·39	2·62	0·160	<0·001	0·036
trans-12 16 : 1	0·24	0·56	1·01	1·33	0·072	<0·001	0·952
trans-13 16 : 1	0·0	0·21	0·58	0·87	0·059	<0·001	0·510
cis-9 18 : 1	22·5	26·1	26·1	23·2	1·44	0·755	0·065
cis-11 18 : 1	3·7	4·5	5·8	6·7	0·39	0·001	0·817
cis-12 18 : 1	1·4	1·9	1·6	1·4	0·16	0·851	0·078
cis-13 18 : 1	0·17	0·33	0·50	0·55	0·036	<0·001	0·190
cis-15 18 : 1	0·35	0·88	1·19	1·23	0·114	0·001	0·073
trans-4 18 : 1	0·41	0·47	0·52	0·42	0·036	0·632	0·078
trans-5 18 : 1	0·30	0·39	0·39	0·34	0·015	0·176	0·003
trans-6 + 7 + 8 18 : 1	2·6	4·2	5·6	5·7	0·42	0·001	0·128
trans-9 18 : 1	1·8	3·3	4·4	4·7	0·29	<0·001	0·100
trans-10 18 : 1	3·3	4·3	5·0	6·0	0·67	0·025	0·962
trans-11 18 : 1	21·6	38·7	69·5	79·1	7·16	<0·001	0·618
trans-12 18 : 1	2·8	5·9	7·8	7·9	0·50	<0·001	0·027
trans-13 + 14 18 : 1	2·1	3·3	4·3	4·3	0·47	0·011	0·257
trans-15 18 : 1	2·5	4·7	5·5	5·1	0·37	0·002	0·015
trans-16 18 : 1‡	2·8	3·7	3·5	2·7	0·37	0·768	0·052

* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P > 0·05$).
† SEM for *n* 16 measurements; error df 6.
‡ Contains *cis*-14 18 : 1 as a minor component.

silage-based diets were examined. Experimental treatments were designed to be within the range of FO doses evaluated previously in growing cattle[12,16,18,19], allowing inferences to be drawn on the possible role of dietary forage type on the potential of FO to enhance 20 : 5*n*-3 and 22 : 6*n*-3 supply in ruminants.

Nutrient intake and digestion

Even though steers were fed diets at a restricted intake in the present experiment, inclusion of 24 g/kg DM of FO lowered DM intake, whereas lower rates of supplementation had no effect compared with the control. Previous studies have demonstrated that incremental inclusion of FO in the diet

Table 6. Effect of incremental amounts of fish oil in the diet on the flow of 18 : 2 isomers at the duodenum in growing cattle

(Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Flow (mg/d)	0	8	16	24	SEM†	*L*	*Q*
cis-9,*cis*-12 18 : 2	21 848	21 410	16 279	14 463	2745·9	0·068	0·810
trans-11,*cis*-15 18 : 2	1337	2340	3291	3618	293·5	0·001	0·293
trans-9,*trans*-12 18 : 2	323	568	993	1254	95·8	<0·001	0·940
cis-9,*trans*-11 CLA	230	284	278	212	23·4	0·579	0·043
cis-12,*trans*-14 CLA	2·5	3·1	2·6	4·0	1·80	0·652	0·847
trans-8,*cis*-10 CLA	5·9	12·2	12·9	16·3	5·45	0·237	0·799
trans-10,*cis*-12 CLA	10·4	16·6	6·9	7·5	5·07	0·453	0·602
trans-11,*cis*-13 CLA	26·1	33·4	25·3	13·1	3·92	0·036	0·047
trans-8,*trans*-10 CLA	12·0	13·2	14·9	18·5	1·51	0·020	0·461
trans-9,*trans*-11 CLA	48·4	75·3	72·0	50·5	6·38	0·922	0·009
trans-10,*trans*-12 CLA	25·6	29·3	19·9	16·1	4·82	0·210	0·451
trans-11,*trans*-13 CLA	21·9	28·5	22·0	14·9	8·53	0·498	0·453
trans-12,*trans*-14 CLA	15·2	24·5	18·2	14·0	3·40	0·550	0·094

CLA, conjugated linoleic acid.
* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P > 0·05$).
† SEM for *n* 16 measurements; error df 6.

Table 7. Effect of incremental amounts of fish oil in the diet on the apparent ruminal biohydrogenation of unsaturated fatty acids in growing cattle

(Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Biohydrogenation (%)	0	8	16	24	SEM†	*L*	*Q*
cis-9 18 : 1	67·0	65·9	68·4	73·6	1·78	0·032	0·124
18 : 2*n*-6	78·8	79·8	84·1	85·4	2·66	0·091	0·953
18 : 3*n*-3	79·6	81·6	83·9	86·4	1·07	0·003	0·823
20 : 5*n*-3	–	93·1	95·5	96·7	0·35	<0·001	<0·001
22 : 6*n*-3	–	91·6	94·1	95·7	0·58	<0·001	<0·001

* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P>0·05$).
† SEM for *n* 16 measurements; error df 6.

from 0 to 39 g/kg DM had no effect on DM intake in growing cattle fed grass silage diets[18,19,33]. In contrast, FO in the diet at a rate of 30 g/kg DM was reported to reduce DM intake of steers fed red clover silage, but not grass silage[16], while studies in lactating cows have shown that FO in the diet depresses nutrient intake in a dose-dependent manner[7,34,35]. Comparisons of responses to ruminal or duodenal infusions of FO indicate that the effects on DM intake are related to the effects arising from changes in rumen function[32,35], possibly mediated via an increase in the amount of unsaturated fatty acid leaving the rumen[29]. The extent to which FO alters nutrient intake appears to be related to several factors including the overall composition of the diet and level of DM intake, as well as the amount and composition of FO supplements.

Negative effects of lipids rich in PUFA on DM intake have often been attributed to reductions in ruminal digestion and a shift towards more extensive digestion of nutrients in the intestine, as well as to increases in the amount of lipid reaching the small intestine[36]. Even though the highest rate of FO supplementation in the present experiment lowered DM intake, this was not accompanied by a reduction in ruminal DM, OM or NDF digestion, when expressed in relation to the amounts consumed, with no evidence of a greater proportion of nutrient digestion occurring in the hindgut. In growing cattle fed grass silage or grass silage-based diets, FO has been reported to either decrease[16] or increase the extent of OM and NDF digestion in the rumen[19], whereas the proportion of these nutrients digested in the forestomach was shown to be independent of FO in the diet in steers fed red clover silage[16]. In lactating cattle, FO tends to improve ruminal OM digestion due to increases in ruminal N digestibility[8] and increase whole-tract DM, OM and NDF digestibility coefficients[8,32,34]. Alterations in the site and extent of nutrient digestion due to FO in the diet probably reflect associated changes in nutrient intake, since reductions in DM intake generally improve all of these parameters[37].

Rumen fermentation

Incremental inclusion of FO in the diet had no effect on rumen pH, but altered rumen fermentation towards propionate at the expense of butyrate with a trend towards decreased molar proportions of acetate. Lipolysis of ingested TAG liberates glycerol that serves as a substrate for propionate production in the rumen[38], but this would not in isolation explain the changes in rumen fermentation observed in the present experiment. Earlier studies have shown that FO had no major effect on rumen pH or fermentation characteristics in steers fed grass silage[16], red clover silage[16] or grass silage-based diets[12,18,19], whereas FO has been reported to enhance the ratio of glucogenic:lipogenic precursors in the rumen of lactating cows fed grass silage[8], lucerne hay[38] and maize silage-based diets[32]. Furthermore, inclusion of herring and mackerel oils in concentrate supplements for growing cattle fed grass silage[33] had no effect on rumen VFA, while the addition of a FO premix of different fatty acid composition decreased the molar proportion of acetate and increased that of propionate. Overall evidence from the literature suggests the impact on rumen fermentation being dependent on several factors including the source and inclusion rate of FO supplements, as well as the intake potential and composition of the diet. It is possible that variation in rumen fermentation patterns between studies is, at least in part, related to FO inducing changes in the relative abundance of specific microbial populations in the rumen. Recent reports on the 16S ribosomal RNA-based denaturing gradient gel electrophoresis profiling of ruminal digesta have provided the first indications that FO alters total ruminal eubacteria and *Butyrivibrio* populations in growing cattle[19].

Rumen lipid metabolism

Comparison of the intake and duodenal flow of fatty acids indicated a net synthesis of fatty acids in the rumen of

Table 8. Effect of incremental amounts of fish oil in the diet on rumen and whole-tract apparent digestibility coefficients in growing cattle

(Mean values with their standard errors)

	Fish oil in the diet (g/kg DM)					*P**	
Digestibility coefficient	0	8	16	24	SEM†	*L*	*Q*
Rumen							
DM	0·456	0·483	0·478	0·481	0·0103	0·170	0·275
OM	0·503	0·531	0·515	0·514	0·0117	0·746	0·277
NDF	0·442	0·465	0·471	0·489	0·0273	0·269	0·926
Starch	0·837	0·841	0·839	0·842	0·0231	0·913	0·987
N	0·279	0·286	0·288	0·319	0·0178	0·174	0·526
Whole tract							
DM	0·742	0·741	0·741	0·764	0·0062	0·062	0·111
OM	0·765	0·763	0·760	0·779	0·0060	0·169	0·125
NDF	0·584	0·585	0·578	0·610	0·0116	0·218	0·213
Starch	0·972	0·967	0·976	0·978	0·0032	0·108	0·387
N	0·801	0·795	0·785	0·802	0·0041	0·648	0·029
Proportion occurring in the rumen							
DM	0·613	0·651	0·645	0·629	0·0151	0·561	0·128
OM	0·658	0·695	0·678	0·660	0·0167	0·868	0·151
NDF	0·757	0·799	0·814	0·809	0·0583	0·536	0·710
Starch	0·861	0·869	0·859	0·861	0·0251	0·918	0·909
N	0·348	0·359	0·367	0·398	0·0233	0·179	0·695

OM, organic matter; NDF, neutral-detergent fibre; N, nitrogen.
* Significance of linear (*L*) and quadratic (*Q*) components of the response to fish oil in the diet. Cubic responses to fish oil in the diet were NS ($P>0·05$).
† SEM for *n* 16 measurements; error df 6.

steers fed maize silage-based diets containing 0, 8 and 16 g/kg DM of FO (151, 150 and 95 g/d, respectively), whereas FO at 24 g/kg DM resulted in comparable intake and flow of fatty acids at the duodenum. Measurements in growing cattle fed grass silage or red clover silage reported a net synthesis of fatty acids to moderate amounts of FO (10 g/kg DM), whereas higher amounts (20–30 g/kg DM) of FO in the diet resulted in a net disappearance[16]. On typical diets, 75–80 % of ingested fatty acids are recovered at the duodenum, but the balance between post-ruminal fatty acid flow relative to intake can be expected to be negative in ruminants fed diets containing more than 40–50 g lipid/kg DM[39,40]. Data from the present and earlier experiments[8,16] indicate that at relatively high rates of supplementation, FO can be expected to decrease the contribution of bacterial lipid to total post-ruminal fatty acid flow.

Supplementing maize silage-based diets with FO enhanced the flow of 20 : 5*n*-3 (mean recovery 4·57, 3·36 and 2·49 % for diets containing 8, 16 and 24 g/kg DM of FO, respectively) and 22 : 6*n*-3 (mean recovery 5·85, 4·57 and 3·42 %) at the duodenum, but the increases were marginal relative to the intake of these fatty acids, confirming previous reports[16,18,19] that the potential to enhance the flow of long-chain *n*-3 fatty acids leaving the rumen in growing cattle is limited. Indirect comparisons with reports in the literature provide little support that the extent of ruminal long-chain *n*-3 fatty acid metabolism is lower in growing cattle fed diets containing maize silage than in those fed diets containing grass silage or red clover silage when comparisons of biohydrogenation are made based on the concentration of 20 : 5*n*-3 and 22 : 6*n*-3 in the diet (Fig. 1).

By analogy with the known pathways of C18 unsaturated fatty acid metabolism in the rumen[41], complete biohydrogenation of 20 : 5*n*-3 and 22 : 6*n*-3 would be expected to yield 20 : 0 and 22 : 0 as final end products, respectively. Measurements in the present experiment indicated that in low amounts FO enhanced the flow of 20 : 0 and 22 : 0 at the duodenum, but at inclusion rates above 8 g FO/kg DM, the magnitude of increase relative to the control diet declined. Since changes in duodenal flow of 20 : 0 and 22 : 0 are not explained by the intake of these fatty acids from the diet, it appears that 20 : 5*n*-3 and 22 : 6*n*-3 or other C20 and C22 unsaturated fatty acids in FO can be completely hydrogenated in the rumen, but at higher rates of FO inclusion, the biohydrogenation of long-chain unsaturated fatty acids becomes progressively incomplete.

Recovery of dietary fatty acids at the duodenum for total C20 (141, 82, 37 and 34 %) and C22 (191, 52, 27 and 19 %) fatty acids in diets containing 0, 8, 16 and 24 g FO/kg DM, respectively, could be interpreted as evidence of the removal of long-chain fatty acids from the rumen via β-oxidation or diffusion across the rumen epithelium[39,40]. However, under normal circumstances, losses of fatty acids in the rumen are thought to be of minor importance owing to the association of non-esterfied fatty acids with feed particles[41]. A low recovery of long-chain fatty acids at high levels of FO in the diet is most probably explained by the formation of C20 and C22 biohydrogenation intermediates that were unable to be identified and quantified in the present experiment. It has been speculated that metabolism of 20 : 5*n*-3 and 22 : 6*n*-3 involves the formation of intermediates with five or six

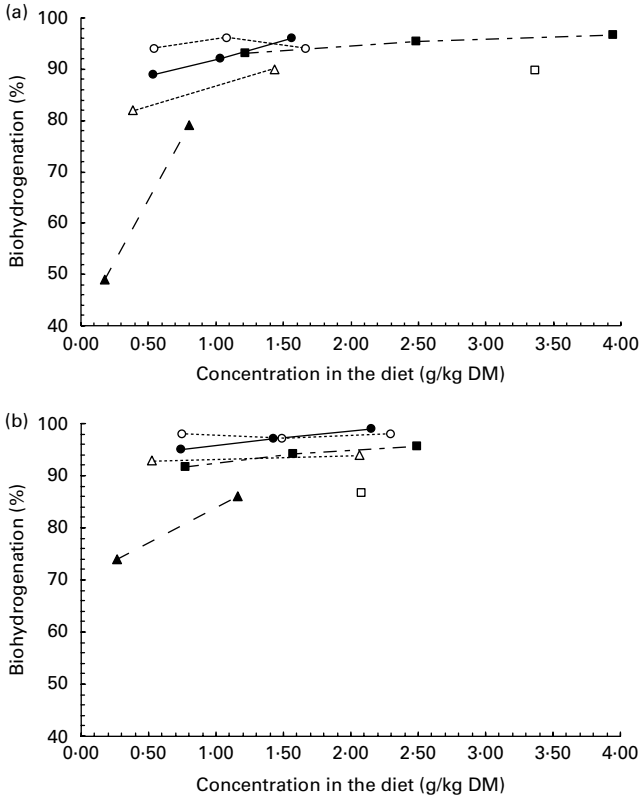

Fig. 1. Relationship between the concentration in the diet and biohydrogenation of 20 : 5*n*-3 (a) and 22 : 6*n*-3 (b) in the rumen of growing cattle. Data derived from studies reporting the effects of fish oil in the diet in steers fed red clover silage (○)[16], grass silage (●)[16], grass silage-based diets (□[12], ▲[18], △[19]) or maize silage-based diets determined in the present experiment (■).

double bonds with at least one in the *trans* configuration[42], while two-dimensional GC analysis of milk fat has provided the first indications that ruminal biohydrogenation of 22 : 6*n*-3 results in the formation of numerous C22 metabolites[43].

Supplements of FO also altered ruminal metabolism of C18 unsaturated fatty acids, resulting in an increase in the flow of *trans* 18 : 1 and *trans* 18 : 2 and reduction in 18 : 0 and 18 : 3*n*-3 at the duodenum. Earlier studies have demonstrated that FO inhibits the complete biohydrogenation of C18 unsaturated fatty acids in the rumen, leading to the accumulation of 18 : 1 and 18 : 2 biohydrogenation intermediates, *trans*-11 18 : 1 in particular[8,16,18,19]. In the present experiment, dose-dependent changes in the flow of 18 : 0 and C18 biohydrogenation intermediates were also associated with increases in the extent of ruminal *cis*-9 18 : 1, 18 : 2*n*-6 and 18 : 3*n*-3 metabolism. Previous studies have reported that FO has no effect[8] or increases the extent of C18 unsaturated fatty acid metabolism[16,18], or in some circumstances, can decrease ruminal *cis*-9 18 : 1 biohydrogenation[19] *in vivo*. Differences in the impact of FO or long-chain *n*-3 fatty acids on the extent of C18 fatty acid biohydrogenation are difficult to reconcile, but they may reflect alterations in the rumen microbial community and the proliferation of rumen bacterium involved in lipolysis and biohydrogenation.

Even though FO altered the flow of CLA at the duodenum and the distribution of specific CLA isomers in a quadratic manner, the magnitude of the changes in *cis*-9,*trans*-11

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CLA, *trans*-11,*cis*-13 CLA and *trans*-9,*trans*-11 CLA while significant on diets containing 8 or 16 g FO/kg DM, was relatively minor in absolute terms. In ruminants fed 18 : 3*n*-3-rich diets, FO enhanced ruminal outflow of *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA and *trans*-9,*trans*-11 CLA and decreased *cis*-12,*trans*-14 CLA accumulation[8,16]. Results from the present experiment confirmed that *cis*-9,*trans*-11 CLA is the major isomer at the duodenum in cattle fed maize silage-based diets[44], and that irrespective of diet composition, *trans*-7,*cis*-9 CLA is not produced in the rumen[8,16,44]. Furthermore, there was no clear effect on the flow of *trans*-10,*cis*-12 CLA at the duodenum, indicating that the inhibitory effects of FO in the diet on mammary lipogenesis in ruminants must be related to increased formation of other biohydrogenation intermediates and/or other mechanisms[45].

In addition to the effects on ruminal C18 biohydrogenation, the present experiment also revealed that FO inhibits the metabolism of C16 unsaturated fatty acids in the rumen. Supplements of FO increased the flow of *trans* (Δ6–13) 16 : 1 isomers at the duodenum, with the changes in *trans*-11 16 : 1 being quantitatively the most important. It is probable that the increased accumulation and outflow of these isomers in response to FO addition are explained by incomplete metabolism of 16 : 2*n*-4, 16 : 3*n*-4, 16 : 4*n*-1 and 16 : 4 *n*-3 in the rumen. It has been suggested that the effects of FO on ruminal lipid metabolism are related to a reduction in the number and activity of bacteria capable of metabolising C18 unsaturated fatty acids to 18 : 0 or due to the direct inhibition of reductases which catalyse the penultimate step of biohydrogenation in the rumen[8,16–18]. Thus far, the only rumen bacterium known to metabolise 18 : 2*n*-6 and 18 : 3*n*-3 to 18 : 0 is *Clostridium proteoclasticum*[46,47], but data to support that FO alters ruminal lipid metabolism via a direct effect on this rumen bacterium are equivocal[19].

Conclusions

Supplementing maize silage diets with FO to high amounts reduced DM intake of growing cattle, and shifted rumen fermentation towards propionate at the expense of acetate and butyrate. Incremental inclusion of FO in the diet enhanced the flow of 20 : 5*n*-3 and 22 : 6*n*-3 at the duodenum, but the magnitude of increase was marginal relative to the intake from the diet due to extensive metabolism of these fatty acids in the rumen. Supplementing the diet with FO altered ruminal lipid metabolism leading, to dose-dependent increases in the flow of *trans* 16 : 1, *trans* 18 : 1 and *trans* 18 : 2, and a decrease in 18 : 0 at the duodenum. In conclusion, current data offered no support that ruminal metabolism of long-chain *n*-3 fatty acids is lower in growing cattle fed diets based on maize silage compared with other forages.

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