Biohydrogenation of dietary *n*-3 PUFA and stability of ingested vitamin E in the rumen, and their effects on microbial activity in sheep

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The present study investigated the susceptibility of dietary *n*-3 PUFA to ruminal biohydrogenation, the stability of ingested vitamin E in the rumen and the subsequent uptake of PUFA and vitamin E into plasma. Six cannulated sheep were assigned to six diets over five 33 d periods, in an incomplete 6×5 Latin square. The diets, based on dried grass, were formulated to supply 50 g fatty acids/kg DM using three lipid sources: Megalac® (calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK), linseed (formaldehyde-treated; Trouw Nutrition, Northwich, Ches., UK) and linseed–fish oil (formaldehyde-treated linseed + fish oil). The diets were supplemented with 100 or 500 mg α-tocopheryl acetate/kg DM. Fat source or level of vitamin E in the diet did not alter microbial activity in the rumen. Biohydrogenation of linoleic acid ($18:3n$-6; 85–90 %), linolenic acid ($18:3n$-3; 88–93 %), docosahexaenoic acid ($22:6n$-3; 91 %) and EPA ($20:5n$-3; 92 %) was extensive. Feeding formaldehyde-treated linseed elevated concentrations of $18:3n$-3 in plasma, whilst $22:6n$-3 and $20:5n$-3 were only increased by feeding the linseed–fish oil blend. Duodenal recovery of ingested vitamin E was high (range 0·79–0·92 mg/mg fed). High dietary vitamin E was associated with increased plasma α-tocopherol (2·57 *v.* 1·46 μg/ml for 500 and 100 mg α-tocopheryl acetate/kg DM respectively), although all concentrations were low. Plasma vitamin E levels, however, tended to decrease as the type and quantity of PUFA in the diet increased. The present study illustrates that nutritionally beneficial PUFA in both fish and linseed oils are highly susceptible to biohydrogenation in the rumen. Although α-tocopheryl acetate resisted degradation in the rumen, plasma vitamin E status remained deficient to borderline, suggesting either that uptake may have been impaired or metabolism post-absorption increased.

Polyunsaturated fatty acids: Vitamin E: Microbial protein synthesis

Sheep meat is high in saturated fatty acids, such that its PUFA:saturated fatty acid ratio is lower than the value recommended for human diets (0·45; Enser *et al.* 1996). High intake of saturated fat is a major predisposing factor to CHD in man (Department of Health, 1994). Whilst the beneficial effects of PUFA, especially those of the *n*-3 series, such as α-linolenic acid ($18:3n$-3), EPA ($20:5n$-3) and docosahexaenoic acid ($22:6n$-3; DHA), in lessening the risk of CHD are now widely recognised (Department of Health, 1994). The low PUFA:saturated fatty acid ratio of sheep meat is a consequence of the extensive biohydrogenation of ingested PUFA by rumen micro-organisms, leading to the formation of *trans*-monounsaturated and saturated fatty acids, which are subsequently incorporated into lipids in the muscle (Jenkins, 1993). In addition, dietary PUFA have several inter-related effects (positive and negative) on rumen metabolism that affect fermentation pattern, protozoal numbers, nutrient digestion, efficiency

of microbial growth, and the site and kinetics of digestion (Demeyer & Van Nevel, 1995).

The nutritional quality of lipids in sheep meat may be enhanced by dietary manipulation strategies that minimise biohydrogenation of ingested PUFA in the rumen. Traditionally this has been achieved by treatment of protein-rich lipid supplements with formaldehyde (Scott *et al.* 1971). Long-chain PUFA ($20:5n$-3 and $22:6n$-3) present in fish oils may possess a natural protection from biohydrogenation by rumen micro-organisms (Ashes *et al.* 1992; Palmquist & Kinsey, 1994). However, increased incorporation of *n*-3 PUFA is known to reduce the oxidative stability of muscle lipids. Therefore, a concomitant increase in antioxidant capacity is essential to counteract the resultant negative effects on meat quality caused by oxidative deterioration. High concentrations of vitamin E in muscle can delay post mortem lipid peroxidation and enhance the shelf-life of sheep meat (Wulf *et al.* 1995). However,

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Abbreviations: CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; VFA, volatile fatty acid.

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deficiencies in vitamin E have been demonstrated to occur even when sheep consume diets with supra-nutritional amounts of the vitamin (Enser *et al.* 1999*a*; Kasapidou *et al.* 2001). On the other hand, it is uncertain if the deficiencies arise as a result of degradation of vitamin E in the rumen, impaired intestinal uptake of the vitamin or increased metabolism post absorption. For example, there are conflicting reports in the literature regarding the susceptibility of orally administered vitamin E to degradation by rumen microbes. Some studies indicate that pre-intestinal vitamin E losses may be as high 42 % in sheep (Alderson *et al.* 1971) and up to 52 % in cattle (Shin & Owens, 1990). In contrast, other studies indicate that vitamin E is not catabolised to any significant extent (Astrup *et al.* 1974; Leedle *et al.* 1993).

The objectives of the present study were twofold. First, to determine the extent of biohydrogenation of *n*-3 PUFA contained in formaldehyde-treated whole linseed and fish oil and their effects on microbial activity; second, to assess the stability of ingested α-tocopheryl acetate in the rumen of sheep and its subsequent uptake into plasma.

Materials and methods

Animal management

This experiment was performed after approval by the UK Animal Ethics Inspectorate in accordance with the Animal (Scientific Procedures) Act, 1986. Six Suffolk cross wether sheep fitted with permanent rumen cannula and T-piece cannula in the proximal duodenum were used in a 6 × 5 incomplete Latin square design. The experiment lasted 165 d. The mean live weight of the sheep at the start of the experiment was 40 (SD 2·9) and 51 (SD 4·4) kg at the end of the trial. Each period was 33 d, with 24 d of adaptation and 9 d of sampling. During the first 20 d of each treatment period the animals were penned individually on raised slatted floors under continuous lighting. The sheep were offered 1·2 kg pelleted feed (fresh weight), supplied in two equal meals at 09.00 and 17.00 h each day. On day 20 of each period, the animals were moved into metabolism crates, where daily feed allocation for each animal was supplied in twelve equal meals, one every 2 h, using an automatic mechanical feeder. Water was available *ad libitum*.

Experimental diets

Six isoenergetic and isonitrogenous diets based on milled dried grass were formulated to supply a similar fatty acid level of 50 g/kg DM. Three fat sources were used: Megalac® (a calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK), linseed (formaldehyde-treated whole linseed, 4 g formaldehyde/kg seed; Trouw Nutrition UK, Northwich, Ches., UK) and linseed–fish oil (50:50, w/ w). The fish oil was from South American herring (Isaac Spencer & Co. Ltd, Fleetwood, Lancs., UK) and was preserved with the antioxidant butylated hydroxytoluene (also known as toluene 2,6-di-*tert*-butyl-*p*-cresol; 500 mg/ kg). Vitamin E (α-tocopheryl acetate; Roche Vitamins (UK) Ltd, Heanor, Derby., UK) was added to the three

fat-supplemented diets before pelleting at the rate of either 100 (low vitamin E) or 500 (high vitamin E) mg/kg DM. The six treatments diets were: Megalac with low vitamin E, Megalac with high vitamin E, linseed (formaldehyde treated) with low vitamin E, linseed (formaldehyde treated) with high vitamin E, linseed–fish oil with low vitamin E and linseed–fish oil with high vitamin E. Diets were offered as pellets (10 mm diameter and 25 mm long). The raw material composition of the experimental diets is shown in Table 1.

Experimental procedure

Daily digesta DM flow at the proximal duodenum was estimated using a dual-phase marker method (Faichney, 1975), using Cr-EDTA and ytterbium acetate as the liquid- and solid-phase markers respectively. The Cr-EDTA solution was prepared according to Binnerts *et al.* (1968) and infused into the reticulo-rumen to provide 140 mg Cr/kg DM intake, while ytterbium acetate was infused at a rate of 50 mg Yb/kg DM intake. The two digesta flow markers were infused continuously for 10 d at a rate of 4·65 ml/h via separate infusion lines using a twelve-channel Watson Marlow-205U peristaltic pump (Watson Marlow, Falmouth, Cornwall, UK). Commencing on day 1 of digesta marker infusion, total faecal collection was made using faecal harnesses for 6 d. Faeces were weighed daily and thoroughly mixed, and then a portion (10 %) was frozen at $-20°C$ for subsequent chemical analysis. On day 3 of digesta flow marker infusion, $[^{15}NH_4]_2SO_4$ (10 % enriched; Isotec Inc., Miamisburg, OH, USA, supplied by CK Gas Products Ltd, Workingham, Berks., UK) was incorporated into the Cr-EDTA solution and infused at the rate of 25 mg ^{15}N per animal per d concurrently with the two digesta flow markers.

On days 2 and 3 of the collection period, blood samples of approximately 10 ml were taken at 11.00 and 15.00 hours by jugular venepuncture into two sterile vacutainer tubes containing potassium oxalate and then centrifuged (2000 *g* for 15 min at 4°C) immediately. Plasma was decanted and frozen at $-20°C$ for fatty acid and vitamin E analysis. On days 7 to 9 of the collection period, approximately 200 ml duodenal digesta was collected (twice daily at 09.00 and at 15.00 hours). A two-way latex Foley bladder catheter (Rusch UK Ltd, High Wycombe, Bucks., UK) was used to divert digesta into a collection bag by using the catheter balloon to block the duodenum at the distal foot of the cannula. Immediately after digesta collection, a mineral solution (g/l: Na_2HPO_4 2·57, $C_2H_3O_2Na$ 1·76, NaCl 2·99, KCl 2·00, $CaCl_2$ 0·75, $MgCl_2.6H_2O$ 0·83, $C_2H_4O_2$ 1·30, adjusted to pH 3 by adding HCl (4 g/l)) was infused via the catheter into the duodenum to replenish minerals withdrawn during sampling, and to maintain the steady-state conditions. Immediately after sampling, 40 g aliquots of whole duodenal digesta were transferred into plastic cups containing 2 g ascorbic acid; 2 ml butylated hydroxytoluene (100 mg/ml ethanol) was added. The cups were capped, contents mixed and they were frozen at $-20°C$ for vitamin E analysis. A further portion (60 ml) of the digesta was frozen and lyophilised as whole duodenal digesta. The remainder (100 ml) was separated into liquid and solid phases by centrifugation at 650 *g* for 5 min

Table 1. Raw materials of experimental diets containing three different fat sources and two levels of vitamin E, and the daily intakes of fatty acids and vitamin E by sheep

Fat source...	Megalac®*		Linseed		Linseed–fish oil	
Vitamin E level...	Low	High	Low	High	Low	High
Ingredients (g/kg fresh weight)						
Dried grass	759	759	739	739	754	754
Sugarbeet pulp (molassed)	105	105	105	105	105	105
Megalac®	35	35	-	-	-	-
Formaldehyde-treated linseed	-	-	85	85	42	42
Fish oil	-	-	-	-	15	15
Soyabean meal	46	46	16	16	29	29
Molasses	25	25	25	25	25	25
Vitamin and mineral premix	20	20	20	20	20	20
NaCl	5	5	5	5	5	5
Ammonium chloride	5	5	5	5	5	5
Fatty acid and vitamin E intake (g/d)						
14 : 0 (myristic)	0·7	0·7	0·2	0·2	1·4	1·4
16 : 0 (palmitic)	17·8	18·8	5·8	5·5	7·7	7·4
16 : 1 (palmitoleic)	0·4	0·4	0·4	0·4	1·8	1·8
18 : 0 (stearic)	1·7	1·8	1·8	1·8	1·6	1·5
18 : 1*n*-9 (oleic)	10·7	11·4	6·4	6·2	5·7	5·3
18 : 1*n*-7 (*cis*-vaccenic)	0·4	0·4	0·5	0·5	0·8	0·8
18 : 2*n*-6 (linoleic)	6·0	6·3	6·3	7·4	6·3	6·1
18 : 3*n*-3 (linolenic)	8·8	9·5	27·5	26·5	18·8	17·3
20 : 5*n*-3 (eicosapentaenoic)	-	-	-	-	2·6	2·6
22 : 6*n*-3 (docosahexaenoic)	-	-	-	-	1·4	1·4
Remaining fatty acids	3·0	3·0	3·0	3·7	5·7	5·6
Total fatty acids	49·4	52·3	51·9	52·1	53·8	51·3
Vitamin E (mg/d)	151	572	144	478	158	554

* Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

at 4°C. The solid phase or particulate duodenal digesta was frozen immediately and lyophilised. The liquid fraction was used for isolation of bacterial cells by a modified method of Mathers & Miller (1980). After centrifugation at 28 600 *g* for 20 min at 4°C the supernatant fraction was discarded, and the isolated microbial cells were rinsed by re-suspending them in McDougall's buffer (g/l: NaHCO$_3$ 9·80, Na$_2$HPO$_4$ 3·70, NaCl 0·47, KCl 0·57, CaCl$_2$ 0·04, MgCl$_2$ 0·06). The re-suspended cells were then re-centrifuged at 28 600 *g* for 20 min at 4°C. The resultant microbial cell isolates were lyophilised and pulverised before chemical analysis.

Rumen contents samples (approximately 100 ml) were collected on the last day of each collection period at 09.00, 13.00 and at 15.00 hours using a manual vacuum pump. Each sample was strained through two layers of muslin, the pH measured immediately and then the sample was acidified to pH < 2 by the addition of concentrated HCl. The samples were then frozen at −20°C for volatile fatty acid (VFA) analysis. Protozoal enumeration in rumen contents was performed using the procedure of Newbold *et al.* (1987).

Chemical analyses

DM and organic matter were determined according to the methods of the Association of Official Analytical Chemists (1995). N was determined using the Kjeldahl procedure with a Tecator Kjeltec-1035 analyser (Tecator AB, Hoganas, Sweden). Neutral-detergent fibre, acid-detergent fibre and acid-detergent lignin were determined according to Van Soest *et al.* (1991). Cellulose was calculated as the difference between acid-detergent fibre and acid-detergent lignin, and hemicellulose as the difference between neutral-detergent fibre and acid-detergent fibre. Cr and Yb concentrations were determined by atomic absorption spectrophotometry (Smith-Hieftje 1000; Thermo Jarrel Ash Corporation, Franklin, MA, USA) using the technique described by Siddons *et al.* (1985). VFA were determined using GLC on a Perkin-Elmer 8500 using a Dura-Bond free fatty acids and phenols (DB-FFAP) capillary column of 30 m length and 0·25 mm internal diameter (J & W Scientific, Folsom, CA, USA) with phenol as the internal standard. The purine contents of the isolated microbial cells and duodenal digesta were assayed according to Zinn & Owens (1986). The ^{15}NH$_3$ not incorporated into microbial protein, but adsorbed onto lyophilised whole duodenal digesta and particulate digesta was removed according to Firkins *et al.* (1992). The abundance of ^{15}N in samples was determined by isotope ratio MS by Rowett Research Services (Bucksburn, Aberdeen, UK).

Methyl esters of fatty acids contained in feed, plasma and duodenal digesta were obtained as follows: samples were spiked with an internal standard (methyl heneicosanoate, 21 : 0) and directly saponified using KOH, then acid-hydrolysed to release NEFA. After methylation using diazomethane, the composition of the methyl ester mixtures was analysed using the method of Enser *et al.* (1996) by GLC (Carlo Erba Strumentazione HRGC 5160 Mega Series; Carlo Erba, Milan, Italy) using a 50 m × 0·25 mm internal diameter CP-Sil 88 WCOT column for fatty acid methyl esters (catalogue no. 7488; Chrompak Ltd, Middelburg, The Netherlands). The GC conditions were: carrier gas He; split mode injection; injector and flame ionisation

detector temperature 250°C; initial oven temperature 180°C for 15 min, then increased at 1·5°C per min to 220°C, held at 220°C for 10 min. Saturated (fatty acid methyl ester 4) and monounsaturated (fatty acid methyl ester 5) fatty acid methyl ester standard mixtures (Thames Restek UK Ltd, Windsor, Berks., UK) were used to establish the response linearity of the system. As a result of incomplete resolution, the *trans*-18 : 1 isomers are reported as a single value that does not include minor isomers (*trans*-13,*trans*-16-18 : 1) which were not resolved from cis-18 : 1*n*-9 and cis-18 : 1*n*-7. The vitamin E content of the feeds was determined according to the method of Pocklington & Dieffenbacher (1988).

Calculations and statistical analyses

Digesta flow at the proximal duodenum was calculated after mathematical reconstitution of true digesta as described by Faichney (1975). Microbial N flow was estimated using purine bases and ^{15}N, and calculated as:

$$\text{microbial N (g/d)} = \left(\frac{\text{Marker : NAN (duodenal digesta)}}{\text{Marker : N (microbial isolate)}} \right) \times \text{NAN flow (g/d)}$$

where NAN is non-NH$_3$-N.

Ruminal biohydrogenation of individual *n*-3 PUFA was calculated as the amount of *n*-3 PUFA disappearing in the rumen (loss between mouth and proximal duodenum) divided by the amount of individual PUFA ingested. All data were analysed as an incomplete 6 × 5 Latin square design, with a 2 × 3 factorial treatment structure, using Genstat 5 Statistical Software (1998, release 4.1; Lawes Agricultural Trust, Rothamsted, Herts., UK).

Results

Composition of experimental diets

The composition of experimental diets and the intakes of individual and total fatty acids and vitamin E are presented in Table 1. The mean concentrations of crude protein (N × 6·25), neutral-detergent fibre, acid-detergent fibre, cellulose, hemicellulose and total fatty acids in the diets were similar in all six diets and averaged 174, 416, 227, 167, 188 and 48 g/kg DM respectively. The Megalac diets contained the highest concentrations of palmitic acid (16 : 0). The mean intake of 16 : 0 in sheep offered the Megalac diet was 18·3 g/d; this was two and three times greater than values for the linseed−fish oil and linseed diets respectively. The intake of stearic acid (18 : 0) was approximately 1·7 g/d and was similar for all experimental diets. The intake of oleic acid (18 : 1*n*-9) by sheep given the Megalac diets (average 11·1 g/d) was approximately twice that of sheep consuming any of the other diets. The mean intakes of α-linolenic acid (18 : 3*n*-3) were 9·2, 18·1 and 27·0 g/d for the Megalac, linseed−fish oil and linseed diets respectively. The intakes of the long-chain PUFA EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) by sheep receiving the linseed−fish oil diets were on average 2·6 and 1·4 g/d respectively, whilst their intakes were negligible for the Megalac- and

linseed-supplemented diets. Total fatty acid intake was similar across all diets, with a mean value of 51·8 g/d. The mean vitamin E intakes were 151 and 534 mg/day for low- and high-vitamin E diets respectively.

Rumen biohydrogenation and duodenal flow

Total fatty acid flow at the proximal duodenum did not differ between diets (Table 2). Nevertheless, the profiles of duodenal digesta fatty acids were altered substantially in response to dietary fat source. The flow of 18 : 0 (stearic acid) was approximately doubled ($P < 0.001$) in sheep consuming the linseed or Megalac diets compared with those given the linseed−fish oil diets (18·7, 15·8 and 8·6 g/d respectively). The flow of *trans*-18 : 1 increased significantly in response to source of fat ($P < 0.001$) in the sequence: linseed−fish oil (10·3 g/d) > linseed (6·7 g/d) > Megalac (2·8 g/d). Supplementation with linseed−fish oil quadrupled the duodenal flow of 18 : 1*n*-7 ($P < 0.001$) relative to either linseed or Megalac diets (mean values 0·4, 0·1 and 0·1 g/d for the linseed−fish oil, linseed and Megalac diets respectively). The flow of 18 : 1*n*-9 to post-ruminal sites in sheep receiving either of the Megalac diets exceeded ($P < 0.001$) the flow observed in sheep offered any of the other two fat sources (3·0, 1·9 and 1·9 g/d for Megalac, linseed and linseed−fish oil diets respectively). Formaldehyde-treated linseed elicited a twofold increase in the flow of 18 : 3*n*-3 at the duodenum relative to Megalac ($P < 0.001$), whilst rates of flow were intermediate in the linseed−fish oil diet. The concentration of long chain n-3 PUFA (20 : 5*n*-3 and 22 : 6*n*-3) in the duodenal digesta of sheep consuming either the Megalac or linseed diets were negligible (below detection limits). However, average flows of 0·2 and 0·1 g/d respectively were observed in animals offered the two linseed−fish oil diets.

No effect of dietary treatment on the biohydrogenation of 18 : 2*n*-6 was observed (range 85·1−89·8 %, Table 2). The biohydrogenation of 18 : 3*n*-3 in the rumen of sheep receiving the Megalac diets was lower ($P < 0.001$) compared with values observed in animals offered either the linseed or linseed−fish oil diets (mean values 89·7, 92·7 and 91·6 % respectively). The long-chain n-3 fatty acids (20 : 5*n*-3 and 22 : 6*n*-3) present in the linseed−fish oil diet were biohydrogenated at rates of 91·9 and 91·0 % respectively.

Plasma fatty acid composition

In blood plasma lipids (Table 3), the concentrations of 16 : 0 were lower ($P < 0.001$) when either the linseed or linseed−fish oil diets were offered compared with Megalac (mean values 20·8, 11·8 and 13·1 g/100 g total fatty acids for the Megalac, linseed and linseed−fish oil diets respectively). The concentrations of *trans*-18 : 1 in plasma of sheep offered the linseed (6·1 g/100 g total fatty acids) and linseed−fish oil (9·4 g/100 g total fatty acids) diets were greater ($P < 0.001$) than those observed in sheep receiving the Megalac diets (2·9 g/100 g total fatty acids). Supplementing with either linseed or linseed−fish oil was associated with substantial reductions ($P < 0.001$) in the concentrations of 18 : 1*n*-9 compared with Megalac (mean

Table 2. Effects of dietary fat source and vitamin E level on the daily flow of fatty acids in duodenal digesta and the biohydrogenation of PUFA in sheep†

(Mean values for six sheep)

Fat source...	Megalac®‡		Linseed		Linseed–fish oil			Statistical significance of effect		
Vitamin E level...	Low	High	Low	High	Low	High	SED	Fat	Vitamin	Fat × Vitamin
Duodenal flow (g/d)										
12:0	0·4	0·4	0·2	0·2	0·2	0·2	0·04	***	NS	NS
14:0	0·7	0·6	0·4	0·3	1·1	1·2	0·10	***	NS	NS
16:0	17·0	16·0	6·4	4·8	7·7	8·3	1·34	***	NS	NS
16:1	0·1	0·1	0·2	0·2	0·7	0·8	0·12	***	NS	NS
18:0	16·4	15·2	18·2	19·1	9·0	8·1	2·16	***	NS	NS
trans-18:1	2·8	2·7	6·9	6·5	9·5	11·0	1·68	***	NS	NS
18:1*n*-9	3·3	2·6	1·9	1·8	1·7	2·1	0·26	***	NS	NS
18:1*n*-7	0·1	0·1	0·1	0·1	0·4	0·4	0·06	***	NS	NS
18:2*n*-6	0·9	0·7	0·8	0·8	0·7	0·7	0·10	NS	NS	NS
18:3*n*-3	1·0	0·9	2·0	1·9	1·5	1·6	0·22	***	NS	NS
20:5*n*-3	-	-	-	-	0·2	0·2	-	-	-	-
22:6*n*-3	-	-	-	-	0·1	0·1	-	-	-	-
Remaining fatty acids	3·1	3·0	5·1	5·3	9·5	10·7	0·77	***	NS	NS
Total fatty acids	46·7	42·0	42·3	41·0	42·4	45·4	3·87	NS	NS	NS
Biohydrogenation (%)										
18:2*n*-6	85·1	88·6	87·8	89·8	89·0	88·2	1·56	NS	NS	NS
18:3*n*-3	88·2	91·1	92·7	92·7	92·2	91·0	1·10	***	NS	NS
20:5*n*-3	-	-	-	-	92·6	91·2	-	-	-	-
22:6*n*-3	-	-	-	-	90·5	91·5	-	-	-	-

***P<0·001.
† For details of diets and procedures, see Table 1 and p. 541.
‡ Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

values 15·3, 13·2 and 8·6 g/100 g total fatty acids for Megalac, linseed and linseed–fish oil respectively). Similarly, concentrations of 18:2*n*-6 in plasma decreased (*P*<0·001) when either the linseed or linseed–fish oil diets were offered (15·8, 12·1 and 8·9 g/100 g total fatty acids for Megalac, linseed and linseed–fish oil diets respectively). In contrast, the linseed diets significantly increased (*P*<0·001) 18:3*n*-3 compared with animals fed either of the Megalac diets. Linseed–fish oil led to a marginal increase in the concentration of 18:3*n*-3 in plasma (5·6, 8·8 and 6·0 g/100 g total fatty acids for Megalac, linseed and linseed–fish oil respectively). A weak interaction (*P*=0·054) between source of dietary fat and level of vitamin E on the concentration of 20:5*n*-3 in plasma was observed. When vitamin E was supplied in the diet at 100 mg/kg DM, there were no differences in the concentrations of 20:5*n*-3 between animals on the Megalac and linseed diets. However, those offered

Table 3. Effects of dietary fat source and vitamin E level on the fatty acid composition (g/100 g total fatty acids) of blood plasma lipids in sheep†

(Mean values for six sheep)

Fat source...	Megalac®‡		Linseed		Linseed–fish oil			Statistical significance of effect		
Vitamin E level...	Low	High	Low	High	Low	High	SED	Fat	Vitamin	Fat × Vitamin
Fatty acid composition										
14:0	0·8	1·0	0·8	0·7	1·3	1·0	0·31	NS	NS	NS
16:0	20·6	20·9	11·7	11·8	13·4	12·7	0·44	***	NS	NS
16:1	1·0	0·6	0·7	0·9	0·9	1·0	0·37	NS	NS	NS
18:0	19·4	19·4	23·8	21·3	16·2	14·3	2·32	**	NS	NS
trans-18:1	2·9	2·9	5·5	6·7	9·2	9·6	1·80	***	NS	NS
18:1*n*-9	15·5	15·1	13·7	12·6	8·6	8·5	1·22	***	NS	NS
18:2*n*-6	15·9	15·7	12·1	12·0	8·6	9·2	0·91	***	NS	NS
18:3*n*-3	5·5	5·7	8·6	9·0	5·6	6·4	0·83	***	NS	NS
20:3*n*-3	2·5	2·3	2·3	2·6	2·7	3·2	0·32	**	NS	NS
20:5*n*-3	2·3	2·2	2·8	3·7	6·1	8·0	0·76	***	*	NS
22:6*n*-3	2·0	2·1	2·2	2·6	3·2	3·9	0·36	***	NS	NS
Remaining fatty acids	10·5	11·0	14·6	14·5	23·5	21·6	2·29	***	NS	NS
Total fatty acids (mg/ml)	1·3	1·3	1·2	1·1	1·0	1·0	0·12	*	NS	NS

*P<0·05, **P<0·01, ***P<0·001.
† For details of diets and procedures, see Table 1 and p. 541.
‡ Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.

linseed–fish oil had a threefold increase in plasma $20:5n$-3 (mean values for Megalac, linseed and linseed–fish oil were 2·3, 2·8 and 6·1 g/100 g total fatty acids). In contrast, when vitamin E was included in the diet at 500 mg/kg, both linseed and linseed–fish oil significantly increased the concentrations of $20:5n$-3 in plasma compared with Megalac (2·2, 3·7 and 8·0 g/100 g total fatty acids for Megalac, linseed and linseed–fish oil respectively). Linseed–fish oil increased ($P<0.001$) the concentration of $22:6n$-3 in plasma lipids compared with the other two fat supplements (2·1, 2·4 and 3·6 g/100 g total fatty acids for Megalac, linseed and linseed–fish oil respectively). The concentration of total fatty acids in plasma was similar in sheep offered either the Megalac or linseed diets, but was reduced significantly ($P=0.019$) in animals given the linseed–fish oil diets (mean values for Megalac, linseed and linseed–fish oil were 1·3, 1·2 and 1·0 mg/ml respectively).

Vitamin E metabolism

The effects of source of dietary fat and level of vitamin E on the metabolism of vitamin E are illustrated in Figs 1 and 2. Supplying α-tocopheryl acetate at 500 rather than at 100 mg/kg DM consistently increased the flow of vitamin E at the proximal duodenum ($P<0.001$, Fig. 1). The proportions of ingested vitamin E recovered at the duodenum were 0·79 and 0·92 for sheep receiving low- and high-vitamin E diets respectively. High concentrations of vitamin E in the diet led to elevated concentrations of vitamin E in the plasma ($P<0.001$, Fig. 2), with mean plasma concentrations of 2·57 and 1·46 μg/ml in sheep offered the high- and low-vitamin E diets respectively. There was a significant interaction of source of dietary fat × vitamin E content on the concentration of vitamin E in blood plasma ($P<0.05$, Fig. 2). Sheep offered the high-vitamin E diet had reduced concentrations of vitamin E in plasma as the level and type of PUFA in the diet increased (mean values 3·29, 2·61 and 1·81 μg/ml for Megalac, linseed and linseed–fish oil respectively; SED 0·290, $P<0.001$). Although the concentration of vitamin E in plasma declined in a similar fashion in the sheep receiving low vitamin E in their diet, the differences between the fat sources were not significant
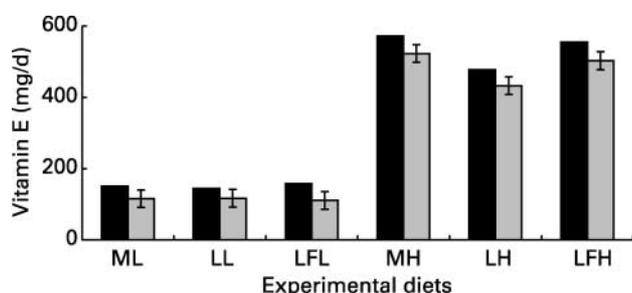

Fig. 1. Vitamin E intake (■) and flow (▨) at the duodenum in sheep fed diets containing Megalac® (M; calcium soap of palm fatty acid, Volac Ltd, Royston, Herts., UK), formaldehyde-treated linseed (L; Trouw Nutrition UK, Northwich, Ches., UK) or formaldehyde-treated linseed + fish oil (LF) and supplemented with 100 (L) or 500 (H) mg α-tocopheryl acetate/kg DM. For details of diets and procedures, see Table 1 and p. 541. Values are means with standard errors shown by vertical bars (six sheep per group).
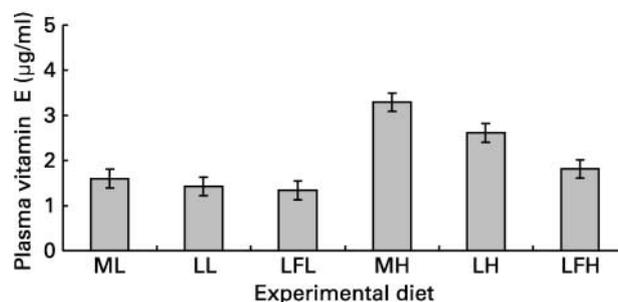

Fig. 2. Plasma vitamin E concentrations in sheep fed diets containing Megalac® (M; calcium soap of palm fatty acid, Volac Ltd, Royston, Herts., UK), formaldehyde-treated linseed (L; Trouw Nutrition UK, Northwich, Ches., UK) or formaldehyde-treated linseed + fish oil (LF) and supplemented with 100 (L) or 500 (H) mg α-tocopheryl acetate/kg DM. For details of diets and procedures, see Table 1 and p. 541. Values are means with standard errors shown by vertical bars (six sheep per group).

(mean values 1·60, 1·43 and 1·34 μg/ml for Megalac, linseed and linseed–fish oil respectively, SED 0·290).

Microbial activity

Apparent digestibilities of organic matter, DM, neutral-detergent fibre and acid-detergent fibre in the rumen were not significantly altered when the sheep were offered any of the treatment diets (mean values 424, 375, 533 and 536 g/kg respectively; Table 4). Similarly, total tract digestibilities of organic matter, DM, neutral-detergent fibre and acid-detergent fibre were not changed by the treatments (mean values 660, 637, 592 and 546 g/kg respectively). Ruminal digestion of cellulose and hemicellulose did not change when the treatments were imposed (Table 4). However, feeding linseed–fish oil resulted in an increase in the total tract digestion of hemicellulose compared with either Megalac or linseed (mean values 637, 645 and 661 g/kg for Megalac, linseed and linseed–fish oil respectively; SED 13·1, $P=0.045$, Table 4). There was a significant interaction of fat source × level of dietary vitamin E on the total tract digestibility of cellulose (Table 4). The total tract digestibility of cellulose decreased significantly in the order: Megalac > linseed > linseed–fish oil in sheep offered diets with high vitamin E, but this was not the case in sheep receiving low vitamin E. In fact, on the low-vitamin E diets, the total tract digestibility of cellulose was lowest in sheep on Megalac.

Rumen pH was low (mean value 6·0) and was not altered substantially by any of the treatments (Table 4). Numbers of protozoa cells in rumen contents were also not affected by treatment (average value 1.07×10^6 cells per ml). The concentrations of total VFA in rumen contents decreased ($P<0.05$) when linseed–fish oil was offered relative to Megalac or linseed (mean values 103·8, 94·8 and 88·4 mM for Megalac, linseed and linseed–fish oil diets, Table 4). However, the molar proportions of major individual VFA in rumen contents did not change in response to dietary treatment (Table 4).

Microbial growth data are presented in Table 4. When $[^{15}NH_4]_2SO_4$ or total purines were used to estimate microbial growth, microbial-N yield and efficiency of

Table 4. Effects of dietary fat source and vitamin E level on rumen pH, total volatile fatty acids (VFA), molar proportions of major VFA (mmol/mol total VFA), ruminal and total tract digestibility of plant fibres, microbial nitrogen yield (estimated using ^{15}N and purine bases) and efficiencies of microbial nitrogen synthesis in the rumen of sheep†

(Mean values for six sheep)

Fat source…	Megalac®‡		Linseed		Linseed–fish oil			Statistical significance of effect		
Vitamin E level…	Low	High	Low	High	Low	High	SED	Fat	Vitamin	Fat × Vitamin
Rumen pH	6·0	6·0	6·0	6·1	6·0	6·0	0·07	NS	NS	NS
Total VFA (mM)	108·0	99·6	93·9	95·7	84·3	92·5	6·42	*	NS	NS
Molar proportions of VFA										
Acetate	610	618	611	607	594	592	19·1	NS	NS	NS
Propionate	219	230	223	208	250	240	29·2	NS	NS	NS
Butyrate	143	128	142	161	131	141	17·0	NS	NS	NS
Apparent rumen digestibility (g/kg)										
Hemicellulose§	512	513	466	534	572	572	59·5	NS	NS	NS
Cellulose‖	559	613	652	587	614	540	40·2	NS	NS	NS
Whole tract digestibility (g/kg)										
Hemicellulose§	640	634	645	645	675	646	13·1	*	NS	NS
Cellulose‖	625	637	661	598	636	532	23·9	*	**	*
Microbial growth using [15]N										
N yield (g/d)	14·0	14·0	13·7	13·5	13·8	13·2	0·78	NS	NS	NS
Efficiency (g N/kg TDOMR)	25·2	24·9	23·6	22·9	23·8	22·7	1·91	NS	NS	NS
Microbial growth using purines										
N yield (g/d)	11·5	13·4	11·6	11·4	10·2	12·6	1·03	NS	*	NS
Efficiency (g N/kg TDOMR)	20·9	23·4	20·3	19·3	17·6	21·8	2·07	NS	NS	NS

TDOMR, truly digested organic matter in the rumen.
*$P<0.05$, **$P<0.01$.
† For details of diets and procedures, see Table 1 and p. 541.
‡ Calcium soap of palm fatty acid distillate; Volac Ltd, Royston, Herts., UK.
§ Neutral-detergent fibre − acid-detergent fibre.
‖ Acid-detergent fibre − acid-detergent lignin.

microbial-N synthesis were consistent and not altered by fat source. However, when total purines were used, vitamin E content had a significant effect ($P=0.04$) on microbial-N synthesis. High-vitamin E diets tended to increase the quantity of microbial-N arriving at the proximal duodenum relative to low-vitamin E diets (mean values 12·5 and 11·1 g N/d respectively).

Discussion

Duodenal fatty acid flows

The profiles of fatty acids of digesta arriving at the proximal duodenum were different from those of the diets ingested. This is partly the result of rumen biohydrogenation and partly the contribution of fatty acids from *de novo* synthesis by micro-organisms in the rumen. For all diets used in the present trial, the balances of fatty acids at the duodenum were negative. Positive and negative fatty acid balances have been reported in the literature. In the ninety-one studies summarised by Doreau & Ferlay (1994), the majority of the negative balances were associated with diets containing >50 g fatty acids/kg DM. However, negative balances have also been reported when diets with concentrations as low as 20 g fatty acids/kg DM were consumed. There are several possible explanations for negative balances at the duodenum. Theoretically, dietary fatty acids can be catabolised or absorbed across the rumen epithelium. The true extent to which these two processes occur is not known. Jenkins (1993) has suggested that there is very little, if any, catabolism of dietary long-chain NEFA in the rumen, and argued

that fatty acid degradation is an aerobic process that is unlikely to occur to any significant extent in the anaerobic environment of the rumen. There is, however, ample evidence to suggest that NEFA are catabolised to ketone bodies by the rumen epithelium *in vitro* (Hird *et al.* 1966; Cook *et al.* 1967). Doreau & Chilliard (1997a) proposed that this oxidative catabolism of fatty acids might be accomplished by micro-organisms that adhere to the rumen wall, and derive their O_2 from epithelial cells. Absorptive losses of long-chain NEFA across the rumen epithelium are generally thought to be insignificant (Moore & Christie, 1984), as the majority of NEFA are associated with particulate digesta (Harfoot *et al.* 1973). However, Doreau & Ferlay (1994) suggested that it is possible for significant absorption of NEFA to occur if large amounts of fatty acids are available in the rumen.

Negative and positive fatty acid balances at the duodenum have also been associated with the variable contribution of fatty acids originating from *de novo* synthesis (Wu *et al.* 1991). The rate of *de novo* synthesis of microbial long-chain fatty acids tends to diminish as the amount of exogenous lipid increases (Demeyer *et al.* 1978). Thus, rumen micro-organisms preferentially assimilate lipids of dietary origin at the expense of *de novo* synthesis, as this is an energetically more efficient strategy (Demeyer & Van Nevel, 1995). The quantity of microbial fatty acids can be estimated, since it constitutes about 30 g/kg truly digested organic matter in the rumen (Demeyer & Van Nevel, 1995), and thus would represent approximately 13 g of the 45 g arriving at the proximal duodenum (Table 2) or about 30 % of fatty acid flow, a value often underestimated.

Biohydrogenation of PUFA

The proportion of 18 : 3n-3 biohydrogenated in the current study was high, and comparable with values reported in the study of Wachira *et al.* (2000) in which untreated whole linseed was fed. Our present findings, therefore, imply that formaldehyde treatment may not be an effective strategy for protecting 18 : 3n-3 in whole linseed. Successful protection of whole linseed with formaldehyde depends on the permeability of the seed coat to formaldehyde, a factor essential for the formation of the rumen-degradation resistant formaldehyde–protein matrix. Supplying dietary lipids as oil seeds with intact seed coats as opposed to free oils may also impart partial protection against biohydrogenation (Rule *et al.* 1989). However, in the current trial, destruction of the seed coat may have occurred during pelleting and mastication, thus partly contributing to the observed extensive losses of 18 : 3n-3 in the rumen.

The biohydrogenation of 18 : 3n-3 appeared to be slightly lower in animals offered the Megalac diets compared with those on the other treatments. The main source of 18 : 3n-3 in the Megalac diets was dried grass. It is conceivable that 18 : 3n-3 in dried grass, by virtue of its location in the cell structure, may be less accessible to ruminal lipases and to subsequent biohydrogenation compared with that in linseed. Wood & Enser (1997) suggested that natural protection of PUFA may be provided by the flow of intact organelles to the small intestine, for instance chloroplasts in grasses. However, despite the extensive biohydrogenation of 18 : 3n-3 in the rumen, feeding formaldehyde-treated linseed doubled the flow of α-linolenic acid (Table 2) to the proximal duodenum and also increased the concentration of 18 : 3n-3 in plasma fatty acids by 57 % (Table 3) relative to Megalac. This increase in the flow of 18 : 3n-3 at the duodenum was also translated into a doubling in muscle 18 : 3n-3 content in growing lambs when fed similar diets (Demirel *et al.* 2004).

In the present study, both EPA and DHA in linseed–fish oil diets were extensively biohydrogenated. Previous studies have reported contradictory results in relation to the biohydrogenation of EPA and DHA in fish oil. Some *in vitro* studies using rumen contents have reported that the biohydrogenation of EPA and DHA is negligible (Ashes *et al.* 1992; Palmquist & Kinsey, 1994). In contrast, other *in vitro* studies reported that their biohydrogenation was extensive, with 61·7 and 77·7 % EPA and DHA respectively being biohydrogenated after 24 h of incubation (Fievez *et al.* 2000). In some *in vivo* studies, the results were consistent with the present findings, that long-chain PUFA are extensively biohydrogenated in the rumen (Choi *et al.* 1997; Doreau & Chilliard, 1997b), whilst others (e.g. Wachira *et al.* 2000) found EPA and DHA to be moderately susceptible to biohydrogenation.

Chilliard *et al.* (2001) suggested three possible explanations for the contradictory rates of biohydrogenation of EPA and DHA reported in the literature. First, duration of exposure or acclimatisation of rumen microbes to fish oil appears to affect their ability to biohydrogenate these fatty acids. In general, higher rates of biohydrogenation tended to be associated with medium term (3–4 weeks) rather than short-term exposure (3–4 d). Second, there

may also be significant differences between species (i.e. bovine *v.* ovine), as EPA and DHA appear to be less extensively biohydrogenated by small ruminant animals compared with cattle (comparison of results from Wachira *et al.* (2000) *v.* Choi *et al.* (1997)). Third, *in vitro* studies have tended to produce lower estimates of biohydrogenation than *in vivo* studies (comparison of results from Ashes *et al.* (1992) and Palmquist & Kinsey (1994) *v.* Wachira *et al.* (2000), Choi *et al.* (1997) and Doreau & Chilliard (1997b)). Despite the high levels of biohydrogenation reported for EPA and DHA in the current work, feeding similar diets to growing lambs was observed to increase the concentration in the semimembranosus muscle by between 1·8 to 2·1 times (Demirel *et al.* 2004).

Trans fatty acids in duodenal digesta and blood plasma

In the present study, animals offered linseed or linseed–fish oil supplemented diets had two- to threefold increases in total *trans*-18:1 (mainly vaccenic, *trans*-18 : 1n-7) in duodenal digesta and in blood plasma compared with those given Megalac. The increased production of *trans*-18 : 1 following the feeding of oils of marine origin to ruminant animals has also been reported in other studies (Wonsil *et al.* 1994; Doreau & Chilliard, 1997b; Wachira *et al.* 2000). Vaccenic acid is the penultimate intermediate in the biohydrogenation pathways of both α-linolenic acid (18 : 3n-3) and linoleic acid (18 : 2n-6) to stearic acid (Harfoot & Hazzlewood, 1997). In the pathways, group B bacteria exclusively perform the transformation of *trans*-18 : 1n-7 to 18 : 0. The long-chain fatty acids contained in fish oil (i.e. 20 : 5n-3 and 22 : 6n-3) have been implicated as possible inhibitors of group B bacteria (Wachira *et al.* 2000), partly explaining the accumulation of *trans*-18:1 in duodenal digesta and blood plasma following the ingestion of linseed–fish oil. The accumulation of vaccenic acid has also been speculated to occur via the inhibition of bacterial reductase enzyme systems that are responsible for converting vaccenic acid to stearic acid (Offer *et al.* 1999). Another conjecture is that *trans*-18 : 1 acids that accumulate after feeding fish oil may be remnants arising from oxidative shortening of EPA and DHA, although this has never been demonstrated (Bessa *et al.* 2000). In addition, the possibility of *trans*-18 : 1 formation from *cis*-18 : 1 should be considered (Mosley *et al.* 2002).

The accumulation of *trans*-18 : 1 in duodenal digesta and plasma that occurs in response to offering fish oil, may be a useful way of increasing levels of conjugated linoleic acid (CLA) in ruminant-animal food products. It is now well established that endogenous synthesis of CLA via desaturation of vaccenic acid by the enzyme Δ^9-desaturase in the mammary gland of lactating dairy cows is far greater than the *de novo* synthesis of CLA in the rumen (Griinari *et al.* 2000). Raes *et al.* (2001) and Enser *et al.* (1999b) also reported that endogenous synthesis of CLA occurred in muscle of beef cattle. However, in beef animals, the quantitative significance of endogenous synthesis relative to *de novo* synthesis remains to be verified. In sheep, studies with growing lambs that have examined the effects of diets containing fish oil have not demonstrated an increase in muscle CLA content (Wachira *et al.* 2002).

The potential beneficial effects of CLA to human subjects are widely recognised. CLA exhibits a diverse array of metabolic effects such as anti-carcinogenesis, anti-athero-genesis, immuno-modulation, body fat reduction and growth promotion (Bessa *et al.* 2000). In addition, any benefits of an increase in both CLA and *n*-3 PUFA levels in ruminant-animal food products need to be weighed against any possible deleterious consequences of increased dietary intake of vaccenic acid by human subjects. However, if the transformation of vaccenic acid to CLA via Δ^9-desaturase in human subjects was demonstrated, as in mice (Santora *et al.* 2000), then increased intake of vaccenic acid may be indirectly beneficial to increasing availability of CLA to human subjects (Santora *et al.* 2000).

Vitamin E metabolism

In the present study, losses of vitamin E in the rumen were 21 and 9 % of intake for low- and high-vitamin E diets respectively. These losses are within the ranges reported in other *in vivo* studies in the literature. Losses of the administered dose of vitamin E as high as 42 % in sheep (Alderson *et al.* 1971) and up to 52 % in cattle (Shin & Owens, 1990) have been reported. On the other hand, some *in vitro* studies have suggested that vitamin E is stable in rumen contents (Astrup *et al.* 1974; Leedle *et al.* 1993). There appears to be little evidence that vitamin E is absorbed across the rumen epithelium (Alderson *et al.* 1971; Hidiroglou & Jenkins, 1974) and rumen micro-organisms are implicated as the main cause of vitamin E loss. However, there is no known mechanism by which vitamin E is degraded in the rumen. According to Leedle *et al.* (1993), the double bonds of tocopherols are stable within the aromatic ring, and are not readily hydrogenated or degraded under anaerobic conditions. Instead, methodological inadequacies, such as the incomplete extraction of tocopherols, are thought to account for the disappearance of tocopherols in the rumen reported in some studies (Leedle *et al.* 1993).

The vitamin E status of ruminant animals is routinely ascertained from plasma or serum concentrations. The concentration of α-tocopherol in plasma has been observed to be correlated with α-tocopherol levels in the liver and to the amounts administered in the diet (Hidiroglou *et al.* 1992). However, the validity of plasma values as a means of assessing vitamin E status in sheep has been questioned. Hidiroglou & Charmley (1990) noted that vitamin E concentration in plasma could be increased by supplementation, but no direct relationship existed between graded levels of intake and plasma vitamin E content. Despite this, Hidiroglou *et al.* (1992) indicated that plasma vitamin E concentrations of $<2\,\mu g/ml$ in ruminant animals implies a deficiency, $2–3\,\mu g/ml$ is marginal, $3–4\,\mu g/ml$ is minimal, while values $>4\,\mu g/ml$ are normally considered adequate. In the present study, despite dietary α-tocopheryl acetate being offered at concentrations of 100 and 500 mg/kg DM, significantly exceeding the 15 mg/kg DM recommended by Agricultural Research Council (1980), the concentrations of vitamin E in plasma ranged from only 1·34 to 1·60 and from 1·81 to 3·29 μg/ml for sheep on the low- and high-vitamin E

diets respectively. Given the high rate of recovery of ingested vitamin E at the proximal duodenum, this implies either an impaired intestinal uptake of vitamin E or increased metabolism post absorption. Growing lambs fed diets similar to those used here were reported to have even lower concentrations of vitamin E in plasma and in many cases were undetectable, although no clinical deficiency cases were reported (Demirel *et al.* 2004).

The concentration of vitamin E in plasma in the current study decreased as both the amount and level of polyunsaturation in the diet increased with Megalac > linseed > linseed–fish oil (Fig. 2). It is well established that ruminant animal diets need to be supplemented with vitamin E in relation to their content of PUFA (Agricultural Research Council, 1980; Putnam & Comben, 1987). Putnam & Comben (1987) suggested that 3 mg vitamin E should be supplied per g added PUFA. However, the susceptibility of PUFA to peroxidation is also related to the number of double bonds in the long-chain PUFA molecules (Murray & Rice, 1982). Sheep offered Megalac had the lowest load of PUFA, which is consistent with the highest plasma α-tocopherol levels being observed in this group relative to sheep given linseed or linseed–fish oil. On the other hand, although sheep offered linseed and linseed–fish oil had comparable quantities of total PUFA in digesta and in plasma, the composition of the constituent PUFA was different. Therefore, the α-tocopherol concentrations in the plasma of sheep on linseed–fish oil, which were lower than those of animals offered linseed, are indicative of an effect of type of PUFA on vitamin E requirements.

Rumen metabolism

In the present study, the different supplements of PUFA had no effect on several rumen variables such as fibre digestion, protozoal numbers, molar proportions of individual VFA and microbial growth. In other studies, unsaturated fatty acids have generally been demonstrated to have a greater inhibitory effect on ruminal fermentation than saturated fatty acids (Chalupa *et al.* 1984). They have been demonstrated to have direct toxic effects on cellulolytic microbes (Hino & Nagatake, 1993). It has been suggested that unsaturated fatty acids affect the fluidity of cytoplasmic membranes of microbial cells by impairing permeability (Jenkins, 1993). According to Doreau & Chilliard (1997*a*), the type and nature of the PUFA is important, and α-linolenic acid-rich linseed oil causes the greatest disturbances (Ikwuegbu & Sutton, 1982; Broudiscou *et al.* 1994). The current observation that feeding formaldehyde-treated linseed did not significantly reduce the biohydrogenation of 18 : 3*n*-3, but was not associated with an alteration in rumen metabolism or digestibility, may indicate an effect of protection on reducing the rate, but not the extent, of 18 : 3*n*-3 release in the rumen.

The concentration of total VFA in the rumen increased significantly with Megalac compared with the other fat supplements, although this effect was less pronounced in animals offered the high-vitamin E diets. These differences in VFA concentrations were probably related to differences in rumen volume rather than to a disturbance in rumen fermentation, because no concomitant effects

on fibre digestion were observed. In contrast to the results presented in the present paper, other studies have generally reported that inclusion of either linseed oil (Ikwuegbu & Sutton, 1982; Broudiscou *et al.* 1994) or fish oil (Nicholson & Sutton, 1971) increases the production of propionate in the rumen. This increased production of propionate is elicited by changes in the species composition of rumen microbes, with unsaturated fatty acids being toxic to cellulolytic and methanogenic bacteria (Demeyer & Van Nevel, 1995). Anaerobic fermentation of carbohydrates and proteins to acetic acid and CO_2 in the rumen is typically accompanied by the removal of electrons and H to CH_4, propionic acid and butyric acid, whilst the inhibition of methanogenesis stimulates propionate production (Demeyer & Van Nevel, 1995).

The average rumen pH reported in this experiment was 6·0. This low pH may have affected the manner in which diet digestion responded to the different fat supplements. Cellulolytic bacteria are sensitive to low rumen pH, with their activities being impaired when pH is $<6\cdot2$ and complete inhibition at pH $<6\cdot0$ (Shi & Weimer, 1992; Weimer, 1993). The toxicity to cellulolytic bacteria of low rumen pH is due to the metabolic inhibition by VFA anions (Russell & Wilson, 1996), impairment of glucose transport (Chow & Russell, 1992) and reduced numbers of cellulolytic bacteria adhered to feed particles (Hoover, 1986). The effect of treatments observed in the total tract digestibilities of cellulose and hemicellulose were probably concomitant with compensatory increases in hindgut fermentation in animals fed the Megalac or linseed diets. Rumen pH $<6\cdot3$ is also thought to reduce lipolysis (Demeyer & Van Nevel, 1995). It is not certain whether the impairment of lipolysis that occurs at low pH in the rumen is caused by the inhibition of lipolytic micro-organisms or their lipases. In the present study, the possibility that lipolysis may have been reduced cannot be sustained given the extensive biohydrogenation of ingested dietary PUFA.

Conclusions

The results reported in our present study illustrate that nutritionally desirable *n*-3 PUFA in fish oil and linseed are susceptible to biohydrogenation in the rumen, and that formaldehyde-treatment of whole linseed may not be an effective means in protecting $18:3n$-3 from biohydrogenation. Nevertheless, feeding linseed substantially increased both the flow of $18:3n$-3 at the proximal duodenum and its concentration in blood plasma. Although the majority of PUFA contained in fish oil were biohydrogenated, feeding a blend of fish oil and linseed markedly enhanced concentrations of $18:3n$-3, $20:5n$-3 and $22:6n$-3 in plasma lipids. Ingested α-tocopheryl acetate appeared to be resistant to microbial degradation in the rumen. However, despite feeding supra-nutritional amounts of vitamin E, concentration of vitamin E in plasma still ranged from deficient to borderline. It is speculated that the uptake of vitamin E may have been impaired.

Acknowledgements

References

Agricultural Research Council (1980) *The Nutrient Requirements of Ruminant Livestock*. (Slough, Bucks.: Commonwealth Agricultural Bureaux).

Alderson NE, Mitchell JR, Little GE, Warner RE & Tucker RE (1971) Pre-intestinal disappearance of vitamin E in ruminants. *J Nutr* **101**, 655–660.

Ashes JR, Siebert BD, Gulati SK, Cuthbertson AZ & Scott TW (1992) Incorporation of *n*-3 fatty acids of fish oil into tissue and serum lipids. *Lipids* **27**, 629–631.

Association of Official Analytical Chemists (1995) *Official Methods of Analysis*, 16th ed., Arlington, VA: AOAC.

Astrup HN, Mills SC, Cook LJ & Scott TW (1974) Stability of α-tocopherol in rumen liquor of sheep. *Acta Vet Scand* **15**, 451–453.

Bessa RJB, Santos-Silva J, Ribeiro JMR & Portugal AV (2000) Reticulo-rumen biohydrogenation and the enrichment of ruminant edible products with linoleic acid conjugated isomers. *Livest Prod Sci* **63**, 201–211.

Binnerts WT, van't Klooster ATh & Frens AM (1968) Soluble chromium indicator by atomic absorption in digestion experiments. *Vet Rec* **82**, 470.

Broudiscou L, Pochet S & Poncet C (1994) Effect of linseed oil supplementation on feed degradation and microbial synthesis in the rumen of ciliate-free and refaunated sheep. *Anim Feed Sci Technol* **49**, 189–202.

Chalupa W, Rickabaugh B, Kronfeld DS & Sklan D (1984) Rumen fermentation *in vitro* as influenced by long chain fatty acids. *J Dairy Sci* **67**, 1439–1444.

Chilliard Y, Ferlay A & Doreau M (2001) Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livest Prod Sci* **70**, 31–48.

Choi NJ, Kim EJ, Maeng WJ, Neville MA, Enser M, Wood JD & Scollan ND (1997) Rumen biohydrogenation of fatty acids from different sources of fat. *Proc Br Soc Anim Sci* **1997**, 19 Abstr.

Chow JM & Russell JB (1992) Effect of pH and monensin on glucose transport by *Fibrobacter succinogenes*, a cellulolytic ruminal bacterium. *Appl Environ Microbiol* **58**, 1115–1120.

Cook DA, McGilliard AD & Richard M (1967) *In vitro* conversion of long-chain fatty acids to ketones by bovine rumen mucosa. *J Dairy Sci* **51**, 715–720.

Demeyer DI, Henderson C & Prins RA (1978) Relative significance of exogenous and *de novo* synthesised fatty acids in the formation of rumen microbial lipids *in vitro*. *Appl Environ Microbiol* **35**, 24–31.

Demeyer DI & Van Nevel CJ (1995) Transformations and effects of lipids in the rumen: Three decades of research at Ghent University. *Arch Anim Nutr* **48**, 119–134.

Demirel G, Wachira A, Sinclair LA, Wilkinson RG, Wood JD & Enser M (2004) Effects of dietary *n*-3 polyunsaturated fatty acids, breed and dietary vitamin E on the fatty acids of lamb muscle, liver and adipose tissue. *Br J Nutr* **91**, 000–000.

Department of Health (1994) *Nutritional Aspects of Cardiovascular Disease*. Report on Health Social Subjects no. 46. London: H. M. Stationery Office.

Doreau M & Chilliard Y (1997a) Digestion and metabolism of dietary fat in farm animals. *Br J Nutr* **78**, S15–S35.

Doreau M & Chilliard Y (1997b) Effects of ruminal or post-ruminal

fish oil supplementation on intake and digestion in dairy cows. *Reprod Nutr Dev* **37**, 113–124.

Doreau M & Ferlay A (1994) Digestion and utilisation of fatty acids by ruminants. *Anim Feed Sci Technol* **45**, 379–396.

Enser M, Demirel G, Wood JD, Nute GR, Wachira AM, Sinclair LA & Wilkinson RG (1999*a*) Impaired deposition of vitamin E in lambs of two breeds fed a dry pelleted complete diet and its effects on meat quality. *Proc Br Soc Anim Sci* **1999**, 40 Abstr.

Enser M, Hallett K, Hewitt B, Fursey GAJ & Wood JD (1996) Fatty acids content and composition of English beef, lamb and pork at retail. *J Meat Sci* **42**, 443–456.

Enser M, Scollan ND, Choi NJ, Kurt E, Hallet K & Wood JD (1999*b*) Effect of dietary lipid on the content of conjugated linoleic acid (CLA) in beef muscle. *Anim Sci* **69**, 143–146.

Faichney GJ (1975) The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In *Digestion Metabolism in the Ruminant. Proceedings of the IV International Symposium on Ruminant Physiology*, pp. 277–291 [IW McDonald and ACI Warner, editors]. Armidale, New South Wales: The University of New England Publishing Unit.

Fievez VI, Van Nevel CJ & Demeyer DI (2000) Lipolysis and biohydrogenation of PUFAs from fish oil during *in vitro* incubations with rumen contents. *Proc Nutr Soc* **59**, 193A.

Firkins JL, Weiss WP & Piwonka EJ (1992) Quantification of intra-ruminal recycling of microbial nitrogen using nitrogen-15. *J Anim Sci* **70**, 3223–3233.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV & Bauman DE (2000) Conjugated linoleic acid is synthesised endogenously in lactating dairy cows by Δ9-desaturase. *J Nutr* **130**, 2285–2291.

Harfoot CG & Hazzlewood GP (1997) Lipid metabolism in the rumen. In *The Rumen Microbial Ecosystem*, pp. 383–426 [PN Hobson and CS Stewart, editors]. London: Chapman and Hall.

Harfoot CG, Noble RC & Moore JH (1973) Food particles as a site for biohydrogenation of unsaturated fatty acids in the rumen. *Biochem J* **132**, 829–832.

Hidiroglou N, Cave N, Atwal AS, Farnworth ER & McDowell LR (1992) Comparative vitamin E requirements and metabolism in livestock. *Ann Rech Vet* **23**, 337–359.

Hidoroglou M & Charmley E (1990) Response of plasma and tissue D-α-tocopherol in sheep to graded dietary levels of DL-α-tocopheryl acetate. *Res Vet Sci* **49**, 122–124.

Hidiroglou M & Jenkins KL (1974) Fate of radiotocopherol adminstered into the gastric system of sheep. *Ann Biol Anim Biochim Biophys* **14**, 667–677.

Hino T & Nagatake Y (1993) The effect of grass lipids on fibre digestion by mixed rumen micro-organisms *in vitro. Anim Sci Technol* **64**, 121–128.

Hird FJR, Jackson RB & Weidemann MJ (1966) Transport and metabolism of fatty acids by isolated rumen epithelium. *Biochem J* **98**, 394–400.

Hoover WH (1986) Chemical factors involved in fibre digestion. *J Dairy Sci* **69**, 2755–2766.

Ikwuegbu OA & Sutton JD (1982) The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. *Br J Nutr* **48**, 365–375.

Jenkins TC (1993) Lipid metabolism in the rumen. *J Dairy Sci* **76**, 3851–3863.

Kasapidou E, Wood JD, Sinclair LA, Wilkinson RG & Enser M (2001) Vitamin E supplementation and meat quality in lambs. *Proc Br Soc Anim Sci* **2001**. 56 Abstr.

Leedle RA, Leedle JAZ & Butine MD (1993) Vitamin E is not degraded by ruminal micro-organisms: Assessment with ruminal contents from a steer fed a high concentrate diet. *J Anim Sci* **71**, 3442–3450.

Mathers JC & Miller EL (1980) A simple procedure using ^{35}S incorporation for the measurement of microbial and undegraded food protein in ruminant digesta. *Br J Nutr* **43**, 503–514.

Moore JH & Christie WW (1984) Digestion, absorption and transport of fats in ruminant animals. In *Fats in Animal Nutrition*, pp. 123–149 [J Wiseman, editor]. London: Butterworth.

Mosley EE, Powell GL, Riley MB & Jenkins TC (2002) Microbial biohydrogenation of oleic acid to *trans* isomers *in vitro. J Lipid Res* **43**, 290–296.

Murray CH & Rice DA (1982) Vitamin E and selenium deficiency diseases. *Ir Vet J* **36**, 57–67.

Newbold CJ, Williams AG & Chamberlain DG (1987) The *in vitro* metabolism of D,L-lactic acid by rumen micro-organisms. *J Sci Food Agric* **38**, 9–19.

Nicholson JWG & Sutton JD (1971) Some effects of unsaturated oils given to dairy cows with rations of different roughage content. *J Dairy Res* **38**, 363–372.

Offer NW, Marsden M, Dixon J, Speake BK & Thacker FE (1999) Effect of dietary fat supplements on levels of *n*-3 poly-unsaturated fatty acids, *trans* acids and conjugated linoleic acid in bovine milk. *Anim Sci* **69**, 613–625.

Palmquist DL & Kinsey DJ (1994) Lipolysis and biohydrogenation of fish oil by ruminal micro-organisms. *J Anim Sci* **72**, Suppl. 1, 350 Abstr.

Pocklington WD & Dieffenbacher A (1988) Determination of tocopherols and tocotrienals in vegetable oils and fats by high performance liquid chromatography: results of a collaborative study and the standardised method. *Pure Appl Chem* **60**, 877–892.

Putnam ME & Comben N (1987) Vitamin E. *Vet Rec* **121**, 541–545.

Raes K, Ansorena D, Chow TT, Fievez V, Demeyer D & De Smet S (2001) Introduction of *n*-3 fatty acids and conjugated linoleic acid into the intramuscular fat of Belgian Blue double-muscled bulls. In *Proceedings of the 47th International Congress of Meat Science Technology*, pp. 114–115. Savoy, IL, USA: American Meat Science Association.

Rule DC, Wu WH, Busboom JR, Hinds FC & Kercher CJ (1989) Dietary canola seeds alter fatty acid composition of bovine subcutaneous adipose tissue. *Nutr Rep Int* **39**, 781–786.

Russell JB & Wilson DB (1996) Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J Dairy Sci* **79**, 1503–1509.

Santora JE, Palmquist DL & Roehrig KL (2000) *Trans*-vaccenic acid is desaturated to conjugated linolenic acid in mice. *J Nutr* **130**, 208–215.

Scott TW, Cook LJ & Mills SC (1971) Protection of dietary poly-unsaturated fatty acids against microbial hydrogenation in ruminants. *J Am Oil Chem Soc* **48**, 358–364.

Shi Y & Weimer PJ (1992) Response surface analysis of the effects of pH and dilution rate on *Ruminococcus flavefaciens* FD-1 in cellulose fed continuous culture. *Appl Environ Microbiol* **58**, 2583–2591.

Shin IS & Owens FN (1990) Ruminal and intestinal disappearance of several sources of vitamin E. *J Anim Sci* **68**, Suppl. 1, 544 Abstr.

Siddons RC, Paradine J, Beever DE & Cornell PR (1985) Ytterbium acetate as a particulate-phase digesta-flow marker. *Br J Nutr* **54**, 509–519.

Van Soest PJ, Robertson JB & Lewis BA (1991) Methods for dietary fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**, 3025–3034.

Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD & Fisher AV (2002) Effects of dietary fat source and breed on the carcass composition, *n*-3 polyunsaturated fatty acid and conjugated linoleic acid content of sheep meat and adipose tissue. *Br J Nutr* **88**, 697–709.

Wachira AM, Sinclair LA, Wilkinson RG, Hallett K, Enser M & Wood JD (2000) Rumen biohydrogenation of *n*-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *J Agric Sci Camb* **135**, 419–428.

Weimer PJ (1993) Effects of dilution rate and pH on the ruminal cellulolytic bacterium *Fibrobacter succinogens* S85 in the cellulose fed continuous culture. *Arch Microbiol* **160**, 288–294.

Wonsil BJ, Herbein JH & Watkins BA (1994) Dietary and ruminally derived *trans*-18 : 1 fatty acids alter bovine milk lipids. *J Nutr* **79**, 62–68.

Wood JD & Enser MB (1997) Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Br J Nutr* **78**, Suppl. 1, S49–S60.

Wu Z, Ohajuruka OA & Palmiquist DL (1991) Ruminal synthesis, biohydrogenation and digestibility of fatty acids by dairy cows. *J Dairy Sci* **74**, 3025–3034.

Wulf DM, Morgan JB, Sanders SK, Tatum JD, Smith GC & Williams S (1995) Effects of dietary supplementation of vitamin E on storage and case life properties of lamb retail cuts. *J Anim Sci* **73**, 399–405.

Zinn RA & Owens FN (1986) A rapid procedure for purine measurement and its use for estimating for net ruminal protein synthesis. *Can J Anim Sci* **66**, 157–166.