

The effect of fish oil supplementation of pregnant and lactating ewes on milk production and lamb performance

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Supplementation of pregnant ewes with long-chain n-3 polyunsaturated fatty acids (PUFA) demonstrably improves indicators of neonatal lamb vigour, potentially improving the number of lambs reared per ewe. The present study investigated the effect of supplementing ewes with fish oil and vitamin E (α -tocopherol acetate) throughout both pregnancy and lactation on the performance of lactating ewes and sucking lambs. Forty-eight ewes were supplemented with one of four concentrates containing either Megalac or fish oil plus a basal (50 mg/kg) or supranutritional (500 mg/kg) concentration of vitamin E from 6 weeks pre-partum until 4 weeks post partum in a two-by-two factorial randomised-block design. All concentrates were formulated to contain approximately 60 g/kg supplemental fatty acids. Ewes were housed, penned on sawdust and offered straw ad libitum. Blood samples were taken from ewes and lambs at intervals throughout the experiment and milk samples were obtained at 21 days into lactation. There was no notable effect of dietary vitamin E concentration upon ewe or lamb performance. Ewe dry-matter (DM) intake and yield were unaffected by dietary treatment, although ewes fed fish oil lost less weight during lactation (-1.88 kg compared with -3.97 kg for Megalac-supplemented ewes; $P < 0.01$). Milk fat concentrations (67.3 g/kg compared with 91.8 g/kg; $P < 0.01$) and yields (6.65 g/h v. 9.26 g/h; $P < 0.01$) were reduced in ewes fed fish oil and these decreases were associated with lower litter-growth rates (0.49 g/day compared with 0.54 g/day; $P < 0.05$). Milk protein yield was increased by fish oil supplementation (3.82 g/h) compared with Megalac supplementation (3.28 g/h; $P < 0.05$); moreover, there was an interaction between fat source and vitamin E concentration in that both protein concentration and yield were significantly lower in milk from ewes fed treatment with Megalac + basal vitamin E (MB) compared with the other three treatments. Fish oil supplementation increased the concentrations of $C_{18:1}$ trans-, cis-9, trans-11 conjugated linoleic acid (CLA), $C_{20:5}$ (n-3) and $C_{22:6}$ (n-3) within ewe plasma, milk and lamb plasma. The mechanisms by which fish oil supplementation affects milk composition warrants further investigation.

Keywords: fish oil, growth rate, milk production, sheep, vitamin E

Introduction

The weight of lambs weaned per ewe has a direct influence on the viability of sheep production systems. In order to maintain profitability, it is therefore vital to minimise lamb mortality and maximise lamb growth rate, particularly during the pre-weaning period when lamb growth rate is positively correlated with ewe milk yield and composition. In the UK, approximately four million lambs per year die during the neonatal period on hill and upland farms, representing a loss of £120 million to the sheep industry (Merrell, 1998). A major factor in this high mortality rate is hypothermia due to delayed suckling and exhaustion of

brown fat reserves (Slee, 1981). Lambs that are vigorous at birth with a short latency of standing and suckling may therefore be more likely to survive during the perinatal period.

The long-chain polyunsaturated fatty acids (PUFA), arachidonic acid ($C_{20:4}$ (n-6)) and docosahexaenoic acid ($C_{22:6}$ (n-3)), are essential for the development of neural tissue within the neonatal and sucking animal (Koletzko, 1992) and may therefore influence neonatal viability. The combination of a low dietary supply of long-chain PUFA and the high level of PUFA biohydrogenation within the rumen (Wachira *et al.*, 2000; Chikunya *et al.*, 2004) may limit the quantities of these fatty acids available for absorption at the ovine small intestine. Nevertheless, Cooper *et al.* (2004) demonstrated that feeding diets high in eicosapentaenoic acid ($C_{20:5}$ (n-3)) and $C_{22:6}$ (n-3) to growing lambs

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substantially increased the concentrations of these fatty acids within muscle phosphatidylglycerols and triacylglycerols. Moreover, Capper *et al.* (2006) and Pickard *et al.* (2005) reported that the inclusion of PUFA in the diet of pregnant ewes demonstrably improved neonatal lamb vigour, an effect that may be mediated through increased gestation length or enhanced neural tissue development within the foetus. However, there is little information available on the effects of continuing dietary PUFA supplementation into lactation upon ewe milk composition and lamb growth rate. Studies by Chilliard and Doreau (1997), Keady *et al.* (2000) and Shingfield *et al.* (2003) have focused on the effects of supplementing lactating dairy cattle with PUFA upon milk yield and composition and have produced conflicting results. Despite physiological similarities in terms of milk synthesis, these results may not be applicable to lactating sheep given the differences in management systems. Although Chikunya *et al.* (2002), Kitessa *et al.* (2003) and Cattaneo *et al.* (2006) have investigated the effects of PUFA supplementation of sheep or goats on milk fatty-acid composition, no data are available relating to the effects of such supplementation upon lamb performance.

Supplementation of pregnant ewes with PUFA would be expected to increase the oxidative challenge both to the dam and sucking lamb (Weber *et al.*, 1997), with concurrent effects upon the antioxidant status. Vitamin E primarily acts as a fat-soluble antioxidant essential for the prevention of lipid peroxidation in cell membranes (Wang and Quinn, 1999), particularly during periods of rapid cell growth in the foetal and neonatal animal. If the PUFA concentration of milk is increased via dietary supplementation, it is logical to suggest that the dietary vitamin E supply should also be augmented to maintain oxidative stability and antioxidant status of the lamb. Furthermore, supplemental dietary vitamin E has been suggested to improve growth rate in sucking lambs (Gentry *et al.*, 1992).

Improving neonatal lamb vigour and enhancing the pre-weaning growth rate should improve unit performance with positive effects upon flock profitability. Therefore, the objectives of the current experiment were to evaluate the effects of supplementing pregnant and lactating ewes with fatty acids and vitamin E upon the performance of lactating ewes and sucking lambs.

Materials and methods

Animals

The experiment was carried out at Harper Adams University College in accordance with the Animals (Scientific Procedures) Act 1986. For full details of animal methods, refer to Capper *et al.* (2005). Briefly, 36 twin-bearing and 12 triplet-bearing ewes were allocated to one of four dietary treatments according to litter size, live weight and condition score (Russel *et al.*, 1969).

Table 1 Raw material composition of the treatment concentrates differing in vitamin E concentration and fatty acid source

	Concentrate [†]			
	MB	MS	FB	FH
Ingredients (g/kg fresh weight)				
Barley	457	457	523	523
Sugar-beet pulp	100	100	100	100
Soya-bean meal	100	100	100	100
Rapeseed meal	50	50	50	50
Sopralin [*]	8	8	12	12
Megalac [‡]	52	52	–	–
Straw	68	68	–	–
Straw pellets	70	70	–	–
Fish oil	–	–	45	45
Incrome [¶]	–	–	15	15
Vermiculite	–	–	60	60
Molasses	50	50	50	50
Urea	15	15	15	15
Vitamins/minerals ^{¶¶}	30	30	30	30

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FH = fish oil + 500 mg/kg vitamin E.

^{*}Protected soya-bean supplement; Trouw Nutrition UK, Northwich, Cheshire, UK.

[‡]Ca soap of palm fatty acid distillate; Volac Ltd, Royston, Hertfordshire, UK.

[¶]By-product of n-3 fatty-acid production for the human market, high in docosahexaenoic acid (Trouw Nutrition UK).

^{¶¶}Vitamin/mineral supplement (Roche UK Ltd, Heanor, Derbyshire, UK) supplied per kg of diet: Ca, 7.06 g; Na, 2.67 g; P, 1.65 g; Se, 0.36 mg; vitamin A, 4.32 mg; vitamin D, 0.75 mg; vitamin E, 50 mg (basal); or 500 mg (supranutritional).

A basal concentrate was formulated based on barley and sugar-beet pulp, details of which have been described previously (Capper *et al.*, 2005). To this diet was added 120 g/kg of either a control fat or fish oil pre-mix and a vitamin/mineral supplement containing either a basal (50 mg/kg) or supranutritional (500 mg/kg) concentration of vitamin E (α -tocopherol acetate; Roche UK Ltd, Heanor, Derbyshire, UK; Table 1). The fish oil pre-mix comprised a mixture of crude unrefined Scandinavian fish oil mixed at a ratio of 0.75:0.25 with Incrome (Trouw Nutrition UK, Northwich, UK), a by-product of omega-3 fatty-acid production for the human market, high in docosahexaenoic acid. Both the fish oil and Incrome were combined with a vermiculite carrier (Trouw Nutrition UK) to protect against PUFA biohydrogenation (Sinclair *et al.*, 2005). The control fat source consisted of a calcium soap of palm oil distillate (Megalac; Volac Ltd, Orwell, Royston, Hertfordshire, UK) mixed with straw pellets. The two pre-mixes were formulated to provide equal concentrations of fatty acids (60 g/kg fresh weight) within the concentrate. The treatment diets were therefore: Megalac + basal vitamin E (MB), Megalac + supranutritional vitamin E (MS), fish oil + basal vitamin E (FB) and fish oil + supranutritional vitamin E (FH). The treatment diets were formulated to be isoenergetic (11.4 MJ metabolisable energy (ME) per kg) and isonitrogenous (180 g crude protein (CP) per kg) formulated according to the Agricultural and Food Research Council (1993).

Experimental procedure

Ewes were housed and individually penned on sawdust from 6 weeks *pre-partum* to 4 weeks *post partum*. The concentrate ration was fed in two equal meals during pregnancy at 0800 and 1600 h, stepped from 0.7 kg/day at 103 days of gestation for twin-bearing ewes (0.8 kg/day for triplet-bearing ewes) to 1.2 kg/day at 140 days of gestation (1.3 kg/day for triplet-bearing ewes) and was fed in three equal meals (at 0800, 1200 and 1600 h) at a flat rate of 1.7 kg/day during lactation. Barley straw was offered *ad libitum*, at a rate of $1.25 \times$ daily intake calculated by weighing back refusals three times per week. Ewe live weight was measured weekly (EziWeigh apparatus, Tru-Test Ltd, Auckland, New Zealand) calibrated using standard weights, and ewes were condition-scored weekly. Ewe blood samples were obtained by jugular venipuncture into evacuated plasma tubes containing potassium oxalate at 1100 h on day 103 of gestation and at 12 h, 14 days and 28 days *post partum*. Milk samples were taken from ewes using a method detailed by Capper *et al.* (2005). Milk samples were taken and stored at -20°C before analysis. Lambs were weighed at weekly intervals between birth and 28 days of age (A and D Co., Japan) calibrated using standard weights. Lambs were blood sampled at 1100 h at 14 days of age by jugular venipuncture collected into evacuated tubes containing potassium oxalate.

Chemical analyses

Feed samples were taken weekly and combined to give two samples per diet, which were analysed for dry matter (DM), ash, CP and NDF as detailed in Capper *et al.* (2005). Plasma was separated by centrifugation at $2290 \times g$ for 5 min before storage at -20°C . Ewe plasma was analysed for β -hydroxy-butyrate (βHB ; kit number RB1007, Randox, County Antrim, UK), for urea (kit number TO1-1819-85, Bayer Diagnostics, Newbury, UK) and for non-esterified fatty acids (NEFA; NEFA C Test, Wako Chemicals, USA). Photometric plasma analyses were performed on a Bayer Technicon RA-1000 autoanalyser (Bayer Diagnostics, Berkshire, UK). Fatty acids within plasma and feed samples were saponified in 5 mol/l KOH and aqueous methanol before methylation by diazomethane as detailed in Capper *et al.* (2006). Milk fatty-acid concentration and composition data were provided by extracting fatty acids from a 1 ml sample of milk using methanol and chloroform (2:1 v/v) according to methods modified from Folch *et al.* (1957) and Christie (1982), and methylated using 100 μl of sodium methoxide. Fatty-acid analysis was achieved using a PerkinElmer 8500 gas chromatograph (PerkinElmer Life and Analytical Sciences Ltd, Boston, MA, USA) comprising a 50 m \times 0.22 mm internal diameter fused silica capillary column (model number 50QC2/BPX70 0.25; SGE International Pty Ltd, Ringwood, Victoria, Australia), a flame-ionisation detector and a PerkinElmer AS 8300 autosampler (SGE International Pty Ltd). Fatty-acid separation and identification details have been reported previously (Capper *et al.*, 2005). The

protein and lactose content of milk samples were determined using a DairyLab 2 IR milk analyser (Foss UK Ltd, Cheshire, UK). The analyser was calibrated for the analysis of ewe milk using standards of known concentrations (Quality Management, Lancashire, UK).

Statistical analyses

Data from eight ewes were excluded from the analysis. Two ewes aborted at 131 days of gestation (treatments FB and MS); a further five ewes reared single lambs (one from each of treatments MB, MS and FS, two from treatment FB) and one ewe (treatment MB) suffered from chronic mastitis. Two ewes (treatments MB and MS) bore one live and one stillborn lamb and adopted a lamb of the same breed from six group-housed ewes. The replacement lambs were introduced immediately after expulsion of the dead lamb. Data for these ewes were included; however, records relating to the adopted lambs were not included in the statistical analysis. In total, 12 lambs were excluded from the analysis due to abortion/stillbirth ($n=4$), adoption ($n=2$), supplemental feeding ($n=2$) and being reared singly ($n=4$). Consequently, data from 46 ewes was utilised *pre partum* and from 40 ewes and 84 lambs *post partum*.

Data were analysed as a 2×2 factorial completely randomised-block design with fat source and dietary vitamin E concentration and their interaction as the main effects. The analyses employed the general ANOVA in the statistical package Genstat 6.2 (Lawes Agricultural Trust, 2002). Sex was used as a covariate when analysing lamb birth weight, live weight and growth rate data, and overall lamb growth rate was calculated using linear regression.

Results

Feed analysis

The four concentrates had a similar chemical composition with mean values for DM, organic matter and CP ($N \times 6.25$) of 863 g/kg, 908 g/kg DM and 179 g/kg DM, respectively (Table 2). Concentrations of NDF were lower, and the ash content higher in concentrates of FB and FS as a result of vermiculite inclusion, with mean values of 137 g/kg DM and 111 g/kg DM (fish oil) compared with 211 g/kg DM and 73 g/kg DM (Megalac) for NDF and ash, respectively. Vitamin E concentrations were similar to those predicted, with means of 61 and 522 mg/kg DM for the basal and supranutritional diets, respectively. Total fatty-acid concentrations were higher in concentrates supplemented with Megalac (100 g/kg DM) than those containing fish oil (89.8 g/kg DM). Concentrates supplemented with Megalac had the highest concentrations of $C_{16:0}$, $C_{18:1}$ *cis*-9 and $C_{18:2}$ (n-6), but the long-chain PUFAs $C_{20:5}$ (n-3) and $C_{22:6}$ (n-3) were not detectable. By contrast, the inclusion of fish oil plus Incrome in concentrates FB and FS resulted in mean concentrations of $C_{20:4}$ (n-6), $C_{20:5}$ (n-3) and $C_{22:6}$ (n-3) of 0.72 g/kg DM, 3.15 g/kg DM and 3.32 g/kg DM, respectively.

Table 2 Chemical composition of the treatment concentrates differing in vitamin E concentration and fatty-acid source

	Concentrate [†]			
	MB	MS	FB	FS
Dry matter (DM g/kg)	859	865	865	863
Crude protein (g/kg DM)	186	181	172	176
Ash (g/kg DM)	77.0	68.9	105	116
Neutral-detergent fibre (g/kg DM)	199	223	128	145
Vitamin E (mg/kg DM)	57.3	503	64.4	541
Fatty acids (g/kg DM)				
C _{16:0}	41.0	39.0	18.2	17.3
C _{18:0}	3.39	3.13	2.76	2.82
C _{18:1 cis-9}	30.9	28.8	11.1	10.0
C _{18:2 (n-6)}	19.9	19.7	14.6	13.1
C _{18:3 (n-3)}	1.75	1.83	2.30	1.90
C _{20:4 (n-6)}	0.15	0.14	0.67	0.76
C _{20:5 (n-3)}	ND [‡]	ND	3.75	2.54
C _{22:6 (n-3)}	ND	ND	4.01	2.62
Total fatty acids	102	97.4	94.2	85.4
Total polyunsaturated fatty acids	23.4	23.2	45.5	35.4
Total n-3 fatty acids	1.75	1.83	11.7	7.50
Total n-6 fatty acids	19.5	20.3	15.6	16.2
n-6:n-3 ratio	11.1	11.4	1.3	2.2

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

[‡]ND = not detected.

Ewe performance

The mean daily straw intake declined between 6 weeks *pre partum* (0.68 kg DM per day) and 1 week *pre-partum* (0.55 kg DM per day) across all treatments (Figure 1) before increasing to 1.07 kg DM per day at 3 weeks *post partum*. There was no effect of dietary treatment on daily straw intake ($P > 0.05$), although there was an interaction at 6 weeks *pre-partum* when ewes fed concentrate MB had higher intakes than those offered MS or FB ($P < 0.05$). There was no effect of dietary treatment upon ewe live weight or condition score change *pre-partum* ($P > 0.05$; Table 3). By contrast, *post partum* live weight loss was lower in ewes supplemented with fish oil, and condition score loss was increased by supranutritional vitamin E supplementation.

Plasma β HB concentrations were reduced by the provision of fish oil at both 12 h ($P < 0.001$) and 4 weeks ($P < 0.05$) *post partum* and were increased by supranutritional vitamin E supplementation ($P < 0.01$) at 12 h *post partum*. Plasma NEFA concentrations measured at 12 h *post partum* were lower in ewes supplemented with fish oil ($P < 0.001$) and tended to be reduced by basal vitamin E supplementation at 12 h *post partum* ($P = 0.069$), although no difference was seen at 4 weeks *post partum* ($P > 0.05$). Ewes supplemented with fish oil had higher plasma urea concentrations at 4 weeks *post partum* ($P < 0.01$); however, there was no effect of dietary vitamin E supplementation.

Dietary treatment had no effect upon the total concentration of fatty acids in ewe plasma at 2 weeks *post*

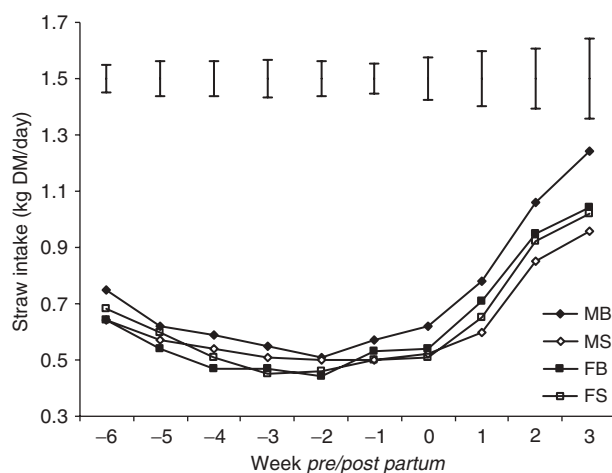


Figure 1 Daily straw intakes of ewes fed diets containing Megalac[®] (M; calcium soap of palm fatty acid, Volac Ltd, Royston, Hertfordshire, UK) or fish oil (F; Trouw UK Ltd, Northwich, Cheshire, UK) supplemented with either 50 (B) or 500 (S) mg/kg α -tocopherol acetate. For details of diets and procedures, see Materials and methods. Values are means with standard errors shown by vertical bars.

partum ($P > 0.05$; Table 4). Ewes supplemented with fish oil had lower plasma concentrations of C_{16:0}, C_{18:1 cis-9} and C_{18:2 (n-6)} ($P < 0.001$), and tended to have lower concentrations of C_{18:0} ($P = 0.067$) and C_{20:4 (n-6)} ($P = 0.051$). By contrast, fish oil supplementation increased the concentrations of C_{18:1 trans; cis-9}, *trans*-11 conjugated linoleic acid (CLA) ($P < 0.001$), C_{18:3 (n-3)} ($P < 0.01$), C_{20:5 (n-3)} and C_{22:6 (n-3)} ($P < 0.001$) in plasma. There was no effect of vitamin E supplementation upon ewe plasma fatty acids ($P > 0.05$).

Ewe milk yield and composition

There was no effect of dietary fat source or vitamin E concentration upon milk secretion rate or yield ($P > 0.05$; Table 5). However, fish oil supplementation reduced milk fat concentration ($P < 0.01$) and yield ($P < 0.01$). There was no main effect of dietary vitamin E concentration upon milk fat concentration or yield ($P > 0.05$), nonetheless, two interactions between dietary fat source and vitamin E concentration was observed. Ewes fed concentrate MB had lower milk protein concentrations than ewes fed concentrates MS, FB or FS ($P < 0.05$), and milk protein yield was reduced in ewes fed concentrate MB compared with those fed concentrate FB ($P < 0.05$). Milk protein yield (g/h) was increased by the provision of concentrates containing fish oil ($P < 0.05$); however, no effects of dietary vitamin E concentration were observed upon milk protein concentration or yield ($P > 0.05$). There was no effect of dietary treatment upon milk lactose concentration or yield ($P > 0.05$).

Fish oil supplementation of ewes reduced the concentrations of C_{16:0}, C_{18:0} and C_{18:1 cis-9} within milk fat ($P < 0.001$; Table 6). By contrast, concentrations of C_{18:1 trans} ($P < 0.01$), C_{18:3 (n-3)} ($P < 0.05$), C_{20:5 (n-3)}

Table 3 Effect of PUFA and vitamin E supplementation of ewes on performance parameters and metabolic profiles

	Diet [†]					Significance		
	MB	MS	FB	FS	s.e.d.	Fat (F)	Vitamin E (V)	F × V
Weight change <i>pre partum</i> (kg)	9.73	7.56	8.88	8.67	1.135			
Weight change <i>post partum</i> (kg)	-3.57	-4.37	-1.94	-1.83	1.059	**		
Condition score change <i>pre partum</i>	-0.17	-0.33	-0.23	-0.33	0.129			
Condition score change <i>post partum</i>	-0.28	-0.46	-0.26	-0.39	0.074		**	
Plasma β HB concentration (mmol/l)								
6 weeks <i>pre partum</i>	0.64	0.65	0.58	0.72	0.053		*	‡
12 h <i>post partum</i>	0.64	0.80	0.40	0.59	0.085	***	**	
4 weeks <i>post partum</i>	0.69	0.85	0.56	0.50	0.137	*		
Plasma NEFA concentration (mmol/l)								
6 weeks <i>pre partum</i>	0.90	0.97	0.90	0.94	0.103			
12 h <i>post partum</i>	0.65	0.73	0.39	0.53	0.081	***	§	
4 weeks <i>post partum</i>	0.60	0.63	0.51	0.52	0.085			
Plasma urea concentration (mmol/l)								
6 weeks <i>pre partum</i>	8.48	8.65	8.90	7.94	0.470			§
12 h <i>post partum</i>	5.90	6.90	5.58	5.76	0.614			
4 weeks <i>post partum</i>	8.55	7.60	8.97	9.29	0.459	**		§

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

[‡]Approaching significance ($P < 0.1$).

Table 4 Effect of PUFA and vitamin E supplementation of ewes on ewe plasma fatty acid composition at 2 weeks post partum

	Diet [†]					Significance		
	MB	MS	FB	FS	s.e.d.	Fat (F)	Vitamin E (V)	F × V
Fatty acid (g/100 g fatty acids)								
C _{16:0}	21.1	21.2	17.1	17.7	0.56	***		
C _{18:0}	19.9	19.8	19.1	18.4	0.85	§		
C _{18:1 trans}	3.06	2.80	4.64	5.01	0.289	***		
C _{18:1 cis-9}	19.4	19.6	15.5	14.0	1.15	***		
C _{18:2 (n-6)}	19.2	20.0	11.6	12.6	0.95	***		
CLA (cis-9, trans-11)	0.12	0.10	1.84	0.78	4.02	***	‡	‡
C _{18:3 (n-3)}	1.78	1.20	2.68	3.40	0.691	**		
C _{20:4 (n-6)}	2.84	2.82	2.34	2.21	0.380	§		
C _{20:5 (n-3)}	1.99	2.11	6.96	7.64	0.445	***		
C _{22:6 (n-3)}	0.26	0.15	3.13	3.82	0.421	***		
Total fatty acids (mg/ml)	1.44	1.30	1.12	1.18	0.187			

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

[‡]Approaching significance ($P < 0.1$).

($P < 0.01$) and C_{22:6} (n-3) ($P < 0.001$) in milk fat were increased by the provision of fish oil in the diet and *cis-9, trans-11* CLA tended to be increased ($P = 0.098$). There was no effect of dietary fat source upon the concentrations of milk C_{18:2} (n-6) or C_{20:4} (n-6) ($P > 0.05$). Vitamin E supplementation level had no effect upon milk fatty-acid composition save for a decrease in C_{20:5} (n-3) conferred by supranutritional supplementation ($P < 0.05$).

Lamb performance

Individual lamb growth rate tended to be reduced by fish oil supplementation of the ewe ($P = 0.054$), an effect that was manifested as a decrease in litter growth rates from birth

until 4 weeks of age ($P < 0.05$; Table 5). Supranutritional vitamin E supplementation of the ewe also tended ($P = 0.074$) to reduce litter growth rates when fed in combination with fish oil (concentrate FS) but not with Megalac (concentrate MS).

The total fatty-acid concentration in sucking lamb plasma was reduced by fish oil supplementation of the ewe ($P < 0.01$; Table 7). Two interactions between maternal dietary fat source and vitamin E concentration were observed: lambs sucking ewes fed concentrate FB had greater plasma C_{20:5} (n-3) concentrations than those from treatment FS ($P < 0.05$); and lambs on the MS treatment had increased plasma C_{22:6} (n-3) concentrations compared

Table 5 Effect of PUFA and vitamin E supplementation of ewes on milk yield, milk composition and lamb growth rate

	Diet [†]				s.e.d.	Significance		
	MB	MS	FB	FS		Fat (F)	Vitamin E (V)	F × V
Secretion rate (ml/h)	96.7	82.9	91.8	102	11.13			
Yield (l/day)	2.32	1.99	2.20	2.44	0.267			
Fat concentration (g/kg)	86.9	96.6	68.0	66.5	9.56	**		
Fat yield (g/h)	9.31	9.20	6.18	7.11	1.072	**		
Protein concentration (g/kg)	31.2	40.1	40.9	38.5	3.59			*
Protein yield (g/h)	3.03	3.52	4.25	3.39	0.585	*		*
Lactose concentration (g/kg)	39.9	47.7	46.6	48.6	4.28			
Lactose yield (g/h)	1.52	1.91	1.89	1.83	0.167			‡
Lamb growth rate (kg/day) [§]	0.26	0.28	0.25	0.25	0.012	‡		
Litter growth rate (kg/day) [§]	0.51	0.56	0.50	0.48	0.026	*		‡

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

*Approaching significance ($P < 0.1$).

[§]Growth rates calculated by linear regression of live weight data from birth to 28 days of age.

Table 6 Effect of PUFA and vitamin E supplementation of ewes on milk fatty-acid composition

	Diet [†]				s.e.d.	Significance		
	MB	MS	FB	FS		Fat (F)	Vitamin E (V)	F × V
Fatty acid (g/100 g fatty acid)								
C _{16:0}	25.9	25.9	21.4	21.5	1.30	***		
C _{18:0}	12.9	14.1	8.78	9.36	0.722	***	‡	
C _{18:1 trans}	3.43	3.28	5.04	5.54	0.680	**		
C _{18:1 cis-9}	26.3	27.5	21.9	22.8	0.946	***		
C _{18:2 (n-6)}	1.49	1.10	1.35	1.69	0.333			
CLA (cis-9, trans-11)	1.09	0.90	1.48	1.16	0.264	‡		
C _{18:3 (n-3)}	0.32	0.31	0.45	0.41	0.076	*		
C _{20:4 (n-6)}	0.08	0.10	0.19	0.19	0.097			
C _{20:5 (n-3)}	0.05	0.00	0.29	0.13	0.073	**	*	
C _{22:6 (n-3)}	0.03	0.00	0.46	0.62	0.162	***		

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

*Approaching significance ($P < 0.1$).

with those on the MB treatment ($P < 0.05$). Maternal fish oil supplementation reduced the plasma concentrations of C_{16:0}, C_{18:0}, C_{18:1 cis-9}, C_{18:2 (n-6)} and C_{20:4 (n-6)} in sucking lambs. By contrast, increases in C_{18:1 trans}; cis-9, trans-11 CLA, C_{18:3 (n-3)}, C_{20:5 (n-3)} and C_{22:6 (n-3)} ($P < 0.001$) were conferred by supplementing the ewe with fish oil. There was no effect of vitamin E supplementation upon plasma fatty-acid composition, save for a reduction in C_{20:5 (n-3)} conferred by supranutritional supplementation ($P < 0.05$).

Discussion

The aim of the current study was to determine the effects of fish oil and vitamin E supplementation of the pregnant and lactating ewe on ewe milk yield and composition and lamb

growth rate. Previously published data (Capper *et al.*, 2005 and 2006) suggested that neonatal lamb vigour may be improved by fish oil and vitamin E supplementation of the pregnant ewe. However, fish oil supplementation of lactating cattle has been reported to reduce both milk yield and milk fat concentration (Shingfield *et al.*, 2003); effects that may potentially reduce lamb growth rate.

Reductions in feed intake have been reported as a result of fish oil supplementation of ewes (Annett *et al.*, 2004) or lactating cattle (Donovan *et al.*, 2000) but dietary fat source had no significant effect upon the daily forage intake of pregnant and lactating ewes within the current study. It should be noted, however, that the aforementioned studies did not include a control fat source in the non-fish oil-supplemented diets, therefore it is possible that the

Table 7 Effect of PUFA and vitamin E supplementation of ewes on sucking lamb plasma fatty-acid composition at 2 weeks post partum

	Diet [†]				s.e.d.	Significance		
	MB	MS	FB	FS		Fat (F)	Vitamin E (V)	F × V
Fatty acid (g/100 g fatty acid)								
C _{16:0}	23.3	21.9	20.3	21.0	0.79	**		§
C _{18:0}	15.6	16.0	13.4	12.3	0.71	***		
C _{18:1 trans}	2.80	2.76	4.33	4.44	0.212	***		
C _{18:1 cis-9}	25.1	25.8	20.4	21.0	1.11	***		
C _{18:2 (n-6)}	12.6	12.6	10.1	9.07	0.856	***		
CLA (cis-9, trans-11)	0.19	0.14	0.78	1.43	0.283	***		‡
C _{18:3 (n-3)}	1.16	1.08	2.05	1.96	0.273	***		
C _{20:4 (n-6)}	3.09	3.50	2.15	1.94	0.299	***		
C _{20:5 (n-3)}	1.06	1.13	5.20	4.16	0.304	***	*	*
C _{22:6 (n-3)}	0.66	1.64	2.57	2.32	0.329	***		*
Total fatty acids (mg/ml)	2.60	2.23	1.84	1.86	0.240	**		

[†]Abbreviations are: MB = Megalac + 50 mg/kg vitamin E, MS = Megalac + 500 mg/kg vitamin E, FB = fish oil + 50 mg/kg vitamin E, FS = fish oil + 500 mg/kg vitamin E.

*Approaching significance ($P < 0.1$).

observed results may have resulted from similar effects of Megalac and fish oil upon DM intake. The depressive effect of long-chain PUFAs upon DM intake has been attributed to toxic effects upon rumen microflora, although the use of protected fish oils appears to negate these effects (Kitessa *et al.*, 2001a). It is therefore postulated that the adsorbent nature of the vermiculite carrier included in the current study may have rendered the long-chain PUFAs less available to ruminal bacteria, thereby negating potential negative effects. Alternatively, PUFA supplementation may have metabolic effects upon DM intake via shifts in ruminal biohydrogenation leading to an increased supply of unsaturated fatty acids at the duodenum as suggested by Shingfield *et al.* (2006). Measures of ruminal biohydrogenation were not made within the current study; however, the increases in C_{18:1 trans} and cis-9, trans-11 CLA within plasma of ewes fed fish oil indicate that biohydrogenation of C_{18:2 (n-6)} and C_{18:3 (n-3)} to C_{18:0} may have been inhibited in favour of intermediaries such as *trans* isomers of C_{18:1}.

Although fish oil supplementation reduced live-weight loss, dietary supranutritional vitamin E was associated with the mobilisation of body fat reserves, with increases in condition score loss and plasma β HB and NEFA concentrations. As vitamin E plays an essential role in preventing oxidation of unsaturated fatty acids, increasing the dietary supply might augment the release of these fatty acids from tissues to compensate for a reduced energy balance during lactation. However, this does not concur with the results of either Kott *et al.* (1998) or Merrell (1998) who reported no effect of vitamin E supplementation on ewe body condition score.

Changes in ewe plasma fatty acids largely reflected the fatty acid composition of the dietary concentrates. The replacement of Megalac by fish oil in the diets of pregnant and lactating ewes conferred a significant reduction in the amount of C_{16:0} within plasma, proportional to differences

in dietary supply. The increased amounts of C_{16:0} observed in plasma of ewes offered Megalac concur with Petit (2002) who reported higher amounts of C_{16:0} in plasma of dairy cows fed Megalac compared with linseed or soya beans. Addition of fish oil to the ruminant diet has been shown to increase the plasma concentrations of both C_{18:1 trans} isomers and CLA produced by incomplete ruminal biohydrogenation of PUFA (Baumgard *et al.*, 2000; Wachira *et al.*, 2000; Chikunya *et al.*, 2004), results that concur with the increase in C_{18:1 trans} fatty acids conferred by fish oil supplementation within the current study. Although concentrations of C_{18:2 (n-6)} were lower in the diets containing fish oil compared with Megalac, proportionally, the reduction in plasma concentrations was higher, suggesting significant ruminal biohydrogenation of C_{18:2 (n-6)}. This concurs with data presented by Chikunya *et al.* (2004) and Sinclair *et al.* (2005) in fish oil-supplemented sheep.

Increases in the n-3 PUFA status conferred by fish oil supplementation are often accompanied by a corresponding increase in plasma C_{20:4 (n-6)} concentration resulting from increased dietary supply (Kitessa *et al.*, 2001a). However, despite increased dietary intakes of C_{20:4 (n-6)}, and in agreement with data presented by Sinclair *et al.* (2005), fat source had no effect upon plasma C_{20:4 (n-6)} concentration within the current study. The significant increase in plasma C_{20:5 (n-3)} and C_{22:6 (n-3)} in ewes offered diets containing fish oil were a direct result of the increased dietary supply of preformed long-chain PUFAs. However, Wachira *et al.* (2002) and Chikunya *et al.* (2004) reported the presence of C_{20:5 (n-3)} and C_{22:6 (n-3)} within muscle and plasma fatty acids of sheep fed diets in which these fatty acids were not detected. The endogenous synthesis of C_{20:5 (n-3)} and C_{22:6 (n-3)} from C_{18:3 (n-3)} may therefore provide an explanation for the presence of long-chain n-3 PUFA within plasma of Megalac-supplemented ewes. Rates of biohydrogenation (Gulati *et al.*, 1999; Sinclair *et al.*, 2005) and transfer into milk (Shingfield *et al.*, 2003) are comparable between C_{20:5}

(n-3) and C_{22:6} (n-3), yet despite significant increases in the amounts of both C_{20:5} (n-3) and C_{22:6} (n-3) in plasma as a response to fish oil supplementation, the increase was proportionally higher for C_{20:5} (n-3).

Lactose serves as the primary determinant of milk osmotic potential and hence of milk yield (Mansbridge and Blake, 1997). Given the lack of significant treatment effects upon milk lactose production between treatments, it is therefore not surprising that milk yields were similarly unaffected by diet within the current study. Within the literature, effects of fish oil on milk yield are equivocal. Cattaneo *et al.* (2006) reported a decrease in milk yield induced by fish oil supplementation of lactating goats, but Shingfield *et al.* (2005) did not observe any significant effect of fish oil supplementation upon bovine milk yield. By contrast, Chilliard and Doreau (1997) and Keady *et al.* (2000) reported increases in milk yield when fish oils were fed to lactating dairy cows.

The phenomenon of milk fat depression (MFD) induced by PUFA supplementation of lactating ruminants has been reviewed in detail (Peterson *et al.*, 2003; Bauman *et al.*, 2006) and it is clear that specific fatty acids produced as intermediates during ruminal biohydrogenation of C_{18:2} (n-6) play a significant role in reducing milk fat synthesis through inhibitory effects on mammary gene expression (Peterson *et al.*, 2003). This mechanism would explain the 27% decrease in milk fat concentration conferred by fish oil supplementation, and, in combination with effects of diet upon milk yield, the 29% decrease in milk fat yield. Although plasma and milk concentrations of C_{18:1} *trans*- and the *cis*-9, *trans*-11 isomer of CLA were increased by fish oil supplementation, it was not possible accurately to detect *trans*-10, *cis*-12 CLA or individual C_{18:1} *trans* isomers within the current study, therefore this hypothesis cannot be verified. However, similar MFD responses to those observed in the current study were reported by Lock *et al.* (2006). CLA supplementation of lactating dairy ewes repartitioned nutrients away from milk fat synthesis towards increased milk yield and milk protein synthesis. Additionally, Keady *et al.* (2000) also reported increases in milk protein yield as a consequence of fish oil supplementation of lactating dairy cows. Diet had no significant effect upon milk yield within the current study; however, milk protein yields were higher in ewes supplemented with fish oil indicating that nutrient partitioning may have been affected by dietary fat source. This effect may also have led to reduced body fat mobilisation, a finding that corresponds with the lower live weight losses observed in ewes supplemented with fish oil. Vitamin E supplementation had no main effect upon milk yield or composition and the metabolic significance of the interaction between fat source and vitamin E concentration resulting in lower milk protein production in ewes fed concentrate MB is not clear, particularly as vitamin E supplementation of lactating cattle did not affect milk protein yield in the studies of Politis *et al.* (2004), Bell *et al.* (2006) or Pottier *et al.* (2006).

Endogenous milk fat synthesis principally results in the secretion of short- and medium-chain fatty acids, with a significant contribution made by C_{16:0} (Bauman and Griinari, 2001). In contrast to results observed by Kitessa *et al.* (2003) and Shingfield *et al.* (2003), the concentration of C_{16:0} within milk fat appeared to be depressed in ewes offered fish oil within the current study. However, rather than a depressive effect of PUFA supplementation *per se*, this may be attributed to the high dietary concentration of this fatty acid in the control (Megalac) diets. By contrast, the lower concentration of C_{18:0} within milk fat conferred by fish oil supplementation concurs with results reported by Donovan *et al.* (2000), Kitessa *et al.* (2001b) and Gulati *et al.* (2003). The methods used to analyse milk fatty-acid composition within the current study did not differentiate between lipid classes, thus both phosphatidylglycerols and triacylglycerols are presented in the results. The major differences observed between plasma and milk concentrations of C_{18:2} (n-6) and C_{18:3} (n-3) may be partly attributable to their location within the phosphatidylglycerols, which contribute a relatively small proportion of milk lipids (Chilliard *et al.*, 2000). Moreover, the requirement for both fatty acids as precursors for endogenous synthesis of long-chain fatty acids and for C_{18:2} (n-6) as a precursor for prostaglandin synthesis may have reduced their deposition into milk fat. Considerable research has been devoted to increasing the concentration of C_{20:5} (n-3) and C_{22:6} (n-3) within milk products. Gulati *et al.* (2003) reported that C_{20:5} (n-3) and C_{22:6} (n-3) were transferred into milk at rates between 6.8% and 8.1% of dietary intake; values lower than those observed within the current study at approximately 10% for C_{20:5} (n-3) and 17% for C_{22:6} (n-3) in ewes fed fish oil. However, these transfer rates do not take into account the possible confounding effects of the incorporation of both fatty acids into adipose tissue prior to parturition, and their subsequent mobilisation. Despite the presence of C_{20:5} (n-3) and C_{22:6} (n-3) within plasma of all ewes, albeit in low concentrations for the control group, the concentrations of C_{20:5} (n-3) and C_{22:6} (n-3) were significantly higher in milk fat from ewes fed fish oil compared with Megalac where they were almost undetectable. These results are in agreement with those observed by Kitessa *et al.* (2003) as a result of fish oil supplementation of lactating ewes. It is interesting to note that although Pottier *et al.* (2006) reported changes in biohydrogenation patterns induced by supernutritional vitamin E supplementation of dairy cows, no such effect was observed in plasma or milk fatty acids within the current study.

Milk was the sole nutrient source available to sucking lambs within the current study and milk component yield was therefore a significant regulator of lamb growth rate. The observed depression in lamb growth rate conferred by fish oil supplementation may be attributed to reductions in energy intake of approximately 21% as a consequence of decreased milk fat supply, despite the increase in protein yield. Nevertheless, it is interesting to note that the decreased growth rate conferred by fish oil may have been

confounded by high growth rates in lambs from treatment MS; particularly as previously published data from this study (Capper *et al.*, 2005) indicated that supranutritional vitamin E supplementation improved lamb birth weight. The growth rate effect concurs with the results of Maiorano *et al.* (2007) although the mechanism by which vitamin E may increase birth weight and growth rate is not known. It may be related to an improvement in immune function as reported in other ruminant species (for review see McDowell *et al.* (1996)), although this was not demonstrated in sheep by Daniels *et al.* (2000) or Hatfield *et al.* (2002) as a result of vitamin E supplementation. The sucking lamb, with no access to long fibre, effectively functions as a monogastric (Valvo *et al.*, 2005); therefore, fatty acids absorbed at the small intestine are not subject to modification by ruminal bacteria. In consequence, the presence of C_{18:1} *trans*-11 and *cis*-9, *trans*-11 CLA within sucking lamb plasma must have resulted either from the secretion of these fatty acids into maternal milk, or the action of Δ^9 -desaturase on *trans*-vaccenic acid as suggested by Valvo *et al.* (2005). Indeed, Knight *et al.* (2004) proposed that the increased concentration of *cis*-9, *trans*-11 CLA in muscle of lambs borne by ewes selected for high *cis*-9, *trans*-11 CLA in milk fat was due to desaturation of *trans*-vaccenic acid rather than due to up-regulation of Δ^9 -desaturase in mammary or muscle tissue. By contrast, although a positive relationship existed between ewe and lamb plasma concentrations of C_{18:2} (n-6), this relationship was not reflected by the proportions of C_{18:2} (n-6) within milk fat. Furthermore, the reduction in plasma C_{20:4} (n-6) exhibited by lambs sucking ewes fed fish oil does not appear to be related to ewe plasma and milk concentrations, but may result from reduced endogenous synthesis within the lamb. Comparison of the concentrations of C_{20:5} (n-3) and C_{22:6} (n-3) in sucking lamb plasma with the amounts supplied by maternal milk suggests that a system for the preferential conservation and deposition of n-3 PUFA exists in animals with a habitually low preformed dietary supply. However, the precise biological mechanisms by which n-3 PUFA status may be maintained in the ruminant is yet to be elucidated.

Conclusion

The results of the current study demonstrate that the previous reported benefits of long-chain n-3 PUFA and vitamin E supplementation upon neonatal lamb vigour and performance are not maintained by extending the supplementation into lactation, whereby milk fat concentration and lamb growth rate are reduced. The mechanisms by which dietary fish oil depresses milk fat synthesis and increases milk protein synthesis warrant further investigation. To exploit fully the potentially beneficial effect of maternal diet upon lamb performance, a strategy that combines PUFA supplementation during pregnancy with a saturated fatty-acid source fed during lactation may improve both neonatal vigour and sucking lamb performance.

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References

- Agricultural and Food Research Council 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC technical committee on responses to nutrients. CAB International, Wallingford.
- Annett RW, Carson AF and Dawson LER. 2004. Effects of concentrate DUP content and fish oil inclusion on colostrum production and lamb output from mature ewes. Proceedings of the Agricultural Research Forum, Tullamore, Ireland on 1–2 March 2004, p. 22.
- Bell JA, Griinari JM and Kennelly JJ 2006. Effect of safflower oil flaxseed oil, monensin, and vitamin E on concentration of conjugated linoleic acid in bovine milk fat. *Journal of Dairy Research Science* 89, 733–748.
- Bauman DE and Griinari JM 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livestock Production Science* 70, 15–19.
- Bauman DE, Mather IH, Wall RJ and Lock AL 2006. Major advances associated with the biosynthesis of milk. *Journal of Dairy Science* 89, 1235–1243.
- Baumgard LH, Corl BA, Dwyer DA, Saebø A and Bauman DE 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *American Journal of Physiology – Regulatory Integrative and Comparative Physiology* 278, R179–R184.
- Capper JL, Wilkinson RG, Kasapidou E, Pattinson SE, Mackenzie AM and Sinclair LA 2005. The effect of dietary vitamin E and long-chain polyunsaturated fatty acid supplementation of pregnant and lactating ewes on placental and mammary transfer of vitamin E to the lamb. *The British Journal of Nutrition* 93, 549–557.
- Capper JL, Wilkinson RG, Mackenzie AM and Sinclair LA 2006. Polyunsaturated fatty acid supplementation during pregnancy alters neonatal behaviour in sheep. *The Journal of Nutrition* 136, 397–403.
- Cattaneo D, Dell'Orto V, Varisco G, Agazzi A and Savoini G 2006. Enrichment in n-3 fatty acids of goat's colostrum and milk by maternal fish oil supplementation. *Small Ruminant Research* 64, 22–29.
- Chikunya S, Sinclair LA and Wilkinson RG. 2002. Influence of dietary n-3 polyunsaturated fatty acids on milk fat composition and performance of lactating Friesian ewes. Proceedings of the British Society of Animal Science Annual Meeting 2002, p. 11.
- Chikunya S, Demirel G, Enser M, Wood JD, Wilkinson RG and Sinclair LA 2004. Biohydrogenation of dietary n-3 PUFA and stability of ingested vitamin E in the rumen and their effects on microbial activity in sheep. *The British Journal of Nutrition* 91, 539–550.
- Chilliard Y and Doreau M 1997. Influence of supplementary fish oil and rumen-protected methionine on milk yield and composition in dairy cows. *The Journal of Dairy Research* 64, 173–179.
- Chilliard Y, Ferlay A, Mansbridge RM and Doreau M 2000. Ruminant milk plasticity: nutritional control of saturated polyunsaturated, trans and conjugated fatty acids. *Annales de Zootechnie* 49, 181–205.
- Christie WW 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. *Journal of Lipid Research* 23, 1072–1075.
- Cooper SL, Sinclair LA, Wilkinson RG, Hallett KG, Enser M and Wood JD 2004. Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *Journal of Animal Science* 82, 1461–1470.
- Daniels JG, Hatfield PG, Burgess DE, Kott RW and Bowman JG 2000. Evaluation of ewe and lamb immune response when ewes were supplemented with vitamin E. *Journal of Animal Science* 78, 2731–2736.
- Donovan DC, Schingoethe DJ, Baer RJ, Ryali J, Hippen AR and Franklin ST 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. *Journal of Dairy Science* 83, 2620–2628.
- Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry* 226, 497–509.

- Gentry PC, Ross TT, Oetting BC and Birch KD 1992. Effects of supplemental α -tocopherol on preweaning lamb performance serum and colostrum tocopherol levels and immunoglobulin G titers. *Sheep Research Journal* 8, 95–100.
- Gulati SK, Ashes JR and Scott TW 1999. Hydrogenation of eicosapentaenoic and docosahexaenoic acids and their incorporation into milk fat. *Animal Feed Science and Technology* 79, 57–64.
- Gulati SK, McGrath S, Wynn PC and Scott TW 2003. Preliminary results on the relative incorporation of docosahexaenoic acid and eicosapentaenoic acids into cows milk from two types of rumen protected fish oil. *International Dairy Journal* 13, 339–343.
- Hatfield PG, Robinson BL, Minikhiem DL, Kott RW, Roth NI, Daniels JT and Swenson CK 2002. Serum α -tocopherol and immune function in yearling ewes supplemented with zinc and vitamin E. *Journal of Animal Science* 80, 1329–1334.
- Keady TWJ, Mayne CS and Fitzpatrick DA 2000. Effects of supplementation of dairy cattle with fish oil on silage intake milk yield and milk composition. *The Journal of Dairy Research* 67, 137–153.
- Kitessa SM, Gulati SK, Ashes JR, Fleck E, Scott TW and Nichols PD 2001a. Utilisation of fish oil in ruminants I. Fish oil metabolism in sheep. *Animal Feed Science and Technology* 89, 189–199.
- Kitessa SM, Gulati SK, Ashes JR, Fleck E, Scott TW and Nichols PD 2001b. Utilisation of fish oil in ruminants II. Transfer of fish oil fatty acids into goats milk. *Animal Feed Science and Technology* 89, 201–208.
- Kitessa SM, Peake D, Bencini R and Williams AJ 2003. Fish oil metabolism in ruminants III. Transfer of n-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. *Animal Feed Science and Technology* 108, 1–14.
- Knight TW, Tavendale MH, Death AF and Agnew M 2004. Conjugated linoleic acid concentration (CLA) in the *m. longissimus thoracis* of the offspring of Romney ewes screened for high and low CLA in their milkfat. *New Zealand Journal of Agricultural Research* 47, 287–297.
- Koletzko B 1992. Fats for brains. *European Journal of Clinical Nutrition* 46, S51–S62.
- Kott RW, Thomas VM, Hatfield PG, Evans T and Davis KC 1998. Effects of dietary vitamin E supplementation during late pregnancy on lamb mortality and ewe productivity. *Journal of the American Veterinary Medicine Association* 212, 997–1000.
- Lawes Agricultural Trust 2002. Genstat 6 release 6.2. Rothamstead Experimental Station. Harpenden, Hertfordshire, UK.
- Lock AL, Teles BM, Perfield JW, Bauman DE and Sinclair LA 2006. A conjugated linoleic acid supplement containing *trans*-10 *cis*-12 reduces milk fat synthesis in lactating sheep. *Journal of Dairy Science* 89, 1525–1532.
- McDowell LR, Williams SN, Hidiogrou N, Njeru CA, Hill GM, Ochoa L and Wilkinson NS 1996. Vitamin E supplementation for the ruminant. *Animal Feed Science and Technology* 60, 273–296.
- Maiorano G, Cavone C, McCormick RJ, Ciarlariello A, Gambacorta M and Manchisi A. 2007. The effect of dietary energy and vitamin E administration on performance and intramuscular collagen properties of lambs. *Meat Science* 76, 182–188.
- Mansbridge RJ and Blake JS 1997. Nutritional factors affecting the fatty acid composition of milk. *The British Journal of Nutrition* 78, Suppl. 1, S37–S47.
- Merrell BG 1998. The effects on lamb survival rate of supplementing ewes with vitamin E during late pregnancy. *Sheep Veterinary Society Proceedings* 22, 57–61.
- Peterson DG, Matishvili EA and Bauman DE 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10 *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. *The Journal of Nutrition* 133, 3098–3102.
- Petit HV 2002. Digestion milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. *Journal of Dairy Science* 85, 1482–1490.
- Pickard RM, Beard AP, Seal CJ and Edwards SA. 2005. Supplementation of ewe diets with algal biomass rich in docosahexaenoic acid for different time periods before lambing affects measures of lamb viability. *Proceedings of the British Society of Animal Science Annual Meeting 2005*, p. 89.
- Politis I, Bizeli I, Tsiaras A and Baldi A 2004. Effect of vitamin E supplementation on neutrophil function milk composition and plasmin activity in dairy cows in a commercial herd. *Journal of Dairy Research* 71, 273–278.
- Pottier J, Focant M, Debier C, De Buysser G, Goffe C, Mignolet E, Froidmont E and Larondelle Y 2006. Effect of dietary vitamin e on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. *Journal of Dairy Science* 89, 685–692.
- Russel AJF, Doney JM and Gunn RG 1969. Subjective assessment of body fat in live sheep. *The Journal of Agricultural Science* 72, 451–454.
- Shingfield KJ, Ahvenjärvi S, Toivonen V, Ärölä A, Nurmela KVV, Huhtanen P and Griinari JM 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. *Animal Science* 77, 165–179.
- Shingfield KJ, Reynolds CK, Lupoli B, Toivonen V, Yurawecz MP, Delmonte P, Griinari JM, Grandison AM and Beever DE 2005. Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower oil and fish oil. *Animal Science* 80, 225–238.
- Shingfield KJ, Reynolds CK, Hervas G, Griinari JM, Grandison AS and Beever DS 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. *Journal of Dairy Science* 89, 714–732.
- Sinclair LA, Cooper SL, Chikunya S, Wilkinson RG, Hallett KG, Enser M and Wood J 2005. Biohydrogenation of n-3 polyunsaturated fatty acids in the rumen and their effects on microbial metabolism and plasma fatty acid concentrations in sheep. *Animal Science* 81, 239–248.
- Slee J 1981. A review of genetic aspects of survival and resistance to cold in newborn lambs. *Livestock Production Science* 8, 419–429.
- Valvo MA, Lanza M, Bella M, Fascone V, Scerra M, Biondi L and Priolo A 2005. Effect of ewe feeding system (grass v concentrate) on intramuscular fatty acids of lambs raised exclusively on maternal milk. *Animal Science* 81, 431–436.
- Wachira AM, Sinclair LA, Wilkinson RG, Enser M, Wood JD and 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. *The British Journal of Nutrition* 88, 1–14.
- Wachira AM, Sinclair LA, Wilkinson RG, Hallett K, Enser M and Wood JD 2000. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. *The Journal of Agricultural Science* 135, 419–428.
- Wang X and Quinn PJ 1999. Vitamin E and its function in membranes. *Progress in Lipid Research* 38, 309–336.
- Weber P, Bendich A and Machlin LJ 1997. Vitamin E and human health: rationale for determining recommended intake levels. *Nutrition (Burbank Los Angeles County, Calif.)* 13, 450–460.