The effect of dietary vitamin E and fatty acid supplementation of pregnant and lactating ewes on placental and mammary transfer of vitamin E to the lamb

Judith L. Capper[1]*, Robert G. Wilkinson[1], Eleni Kasapidou[2], Sandra E. Pattinson[1], Alexander M. Mackenzie[1] and Liam A. Sinclair[1]

[1]*Harper Adams University College, Edgmond, Newport, Shropshire TF10 8NB, UK*
[2]*Division of Food Animal Science, University of Bristol, Langford, Bristol BS40 5DU, UK*

The present study investigated the effect of maternal vitamin E and fatty acid supplementation on lamb antioxidant status. Forty-eight ewes were fed one of four concentrate diets supplemented with a basal (50 mg/kg) or supranutritional (500 mg/kg) level of vitamin E plus a source of either saturated fat (Mega-lac®; Volac Ltd, Royston, Hertfordshire, UK) or long-chain PUFA (fish oil) from 6 weeks prepartum until 4 weeks postpartum. Blood samples were taken from ewes and lambs at intervals throughout the experiment and, at parturition, muscle, brain and blood samples were obtained from twelve lambs (three per treatment). Colostrum and milk samples were obtained at 12 h and 21 d after parturition, respectively. Supranutritional vitamin E supplementation of the ewe significantly increased concentrations of vitamin E in neonatal lamb tissues although plasma concentrations were undetectable. A significant increase in lamb birth weight resulted from increasing the dietary vitamin E supply to the ewe. Furthermore, maternal plasma, colostrum and milk vitamin E concentrations were increased by vitamin E supplementation, as were lamb plasma concentrations at 14 d of age. Neonatal vitamin E status was not significantly affected by fat source although plasma vitamin E concentrations in both ewes and suckling lambs were reduced by fish oil supplementation of the ewe. Fish oil supplementation reduced vitamin E concentrations in colostrum and milk and the activity of glutathione peroxidase in suckling lambs. The data suggest that the vitamin E status of the neonatal and suckling lamb may be manipulated by vitamin E supplementation of the ewe during pregnancy and lactation.

Birth weight: PUFA: Vitamin E: Colostrum

Four million lambs are estimated to die during the neonatal period on hill and upland farms each year in the UK, representing a substantial financial loss to the sheep industry (Merrell, 1998). A major factor contributing to this high mortality rate is hypothermia due to delayed suckling and exhaustion of brown fat reserves (Slee, 1981). Standing and suckling as soon as possible after birth is therefore imperative to facilitate the ingestion of colostrum and ensure maximal lamb survival (O'Connor & Lawrence, 1992). Supplementing ewes with supranutritional dietary vitamin E during pregnancy has been reported to produce lambs that were more vigorous immediately after birth and had higher weight gains prior to weaning (Merrell, 1998), although the mechanism behind this response is unclear. It is well established that the vitamin E concentrations within plasma of neonatal mammals are low compared with maternal concentrations (Malone, 1975). Njeru *et al.* (1994) investigated the effects of maternal vitamin E supplementation upon the transfer of vitamin E to the neonatal lamb and concluded that placental transfer is inefficient in the sheep. Furthermore, Mahan & Vallet (1997) reviewed vitamin transfer across the placenta in pigs and concluded that although a small amount of vitamin E is conveyed to the fetus, neonatal pigs are born deficient in vitamin E and therefore can obtain it in appreciable amounts only from the sow's colostrum. The low efficiency of placental transfer may be attributed to the large molecular size of the vitamin or to the low efficiency of lipid transfer with which vitamin E is associated (Njeru *et al.* 1994). By contrast, there appears to be no barrier to the transfer of vitamin E into colostrum and milk via the mammary gland (Njeru *et al.* 1994); indeed, fat-soluble vitamins are concentrated in colostrum (Hidiroglou *et al.* 2001). Consequently, it is postulated that, prior to the ingestion of colostrum, the vitamin E status of neonatal ruminants cannot be manipulated via the maternal diet (Njeru *et al.* 1994).

The long-chain PUFA docosahexaenoic acid and arachidonic acid have been implicated in the development of fetal brain and nervous tissue (Innis, 1991) and the addition of PUFA to the diet of human infants has been associated with improvements in infant vigour via enhanced neural and visual development (Carlson & Werkman, 1996). Ruminant diets supply negligible amounts of these fatty acids (Givens *et al.* 2000), and it may therefore be argued that pregnant ruminants may be deficient in essential PUFA and benefit from supplementation. Furthermore, despite high levels of ruminal biohydrogenation of dietary PUFA (Wachira *et al.* 2000; Chikunya *et al.* 2004), feeding diets high in docosahexaenoic acid has been demonstrated to substantially increase concentrations of these fatty acids within polar and neutral lipids

in sheep (Cooper *et al.* 2004; Demirel *et al.* 2004). Supplementation of pregnant ewes with PUFA may have positive effects in terms of lamb vigour (Capper *et al.* 2002); however, it may also increase the oxidative challenge to the animal, with concurrent effects upon the antioxidant status. A primary role of vitamin E is as a fat-soluble chain-breaking antioxidant essential for the prevention of lipid per-oxidation in cell membranes (Wang & Quinn, 1999), particularly during periods of rapid cell growth in the fetal and neonatal animal.

Apparent conflicting results have been reported regarding low maternofetal transfer of vitamin E and concurrent effects of maternal supplementation upon lamb vigour and performance. These results may further be complicated by fatty acid supplementation of the dam. Therefore, the objectives of the present experiment were to evaluate the effects of supplementing pregnant and lactating ewes with vitamin E and fatty acids upon the placental and mammary transfer of vitamin E to the fetal and suckling lamb.

Materials and methods

Experimental design and treatments

The present experiment was carried out at Harper Adams University College in accordance with the Animal (Scientific Procedures) Act 1986. Thirty-six twin-bearing and twelve triplet-bearing ewes were chosen from the Harper Adams early-lambing flock and used in a 2×2 factorial design. Pregnant ewes were scanned at 84 d of gestation and weighed and condition-scored at 90 d of gestation according to the method described by Russel *et al.* (1969), before being allocated to treatments according to breed, age, litter size, live weight and condition score. The ewe breeds used in the experiment were the Suffolk $\times$ North Country Mule (n 24), Charollais $\times$ Friesland (n 20) and Friesland $\times$ Lleyn (n 4). Ewes were impregnated by Charollais rams. The majority of the ewes (n 44) were multiparous with a mean age of 3·2 (SD 1·86) years, mean live weight of 76·6 (SD 6·14) kg and mean condition score of 3·32 (SD 0·51).

A basal diet was formulated, containing barley, sugarbeet pulp, soyabean meal, Sopralin® (a source of formaldehyde-protected soyabean meal; Trouw Nutrition UK, Northwich, Cheshire, UK), rapeseed meal, urea, molasses and a vitamin/mineral supplement (30 g/kg) containing either a basal (50 mg/kg) or a supranutritional (500 mg/kg) concentration of vitamin E (α-tocopherol acetate; Roche UK Ltd, Heanor, Derbyshire, UK; Table 1). To this diet was added either a long-chain PUFA or control fat premix, each at 120 g/kg. The PUFA premix comprised a mixture of crude unrefined Scandinavian fish oil mixed at a ratio of 0·75:0·25 with Incromega® (Trouw Nutrition UK), a by-product of *n*-3 fatty acid production for the human market, high in docosahexaenoic acid. Cooper *et al.* (2002) demonstrated that vermiculite was effective in reducing the biohydrogenation of PUFA within the rumen, therefore both the fish oil and Incromega were combined with a vermiculite carrier (Trouw Nutrition UK). The control fat source consisted of a commercial protected fat source (Megalac®; Volac Ltd, Royston, Hertfordshire, UK) mixed with straw pellets. The two premixes were formulated to provide equal concentrations of fatty acids (60 g/kg fresh weight) within the treatment concentrates. The treatment diets were therefore: MB, Megalac plus basal vitamin E; MS, Megalac plus supranutritional vitamin E; FB, fish oil plus basal vitamin E; and FS, fish oil plus supranutritional vitamin E. The treatment diets were formulated to be isoenergetic (11·4 MJ metabolisable energy/kg DM) and isonitrogenous (180 g

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

Fat source	Megalac		Fish oil	
Vitamin E level	Low	High	Low	High
Ingredients (g/kg fresh weight)				
Barley	457	457	523	523
Sugarbeet pulp	100	100	100	100
Soyabean 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
Incromega®‡	–	–	15	15
Vermiculite	–	–	60	60
Molasses	50	50	50	50
Urea	15	15	15	15
Vitamins/minerals§	30	30	30	30
Chemical composition (g/kg DM)				
DM (g/kg)	859	865	865	863
Organic matter	923	931	895	884
Ash	77	69	105	116
Crude protein (N × 6·25)	186	181	172	176
Neutral-detergent fibre	199	223	128	145
Total fatty acids	102	97	93	81
Total PUFA	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
Vitamin E (mg/kg DM)	57	503	64	541

* Protected soyabean supplement (Trouw Nutrition UK, Northwich, Cheshire, UK).
† Ca soap of palm fatty acid distillate (Volac Ltd, Royston, Hertfordshire, UK).
‡ A 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 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).

crude protein/kg DM) according to Agricultural and Food Research Council (1993) guidelines.

Experimental procedure

Ewes were housed and individually penned on sawdust from 6 weeks prepartum to 4 weeks postpartum. The concentrate ration was fed in two equal meals during pregnancy at 08.00 and 16.00 hours, stepped from 0·7 kg/d at 103 d of gestation for twin-bearing ewes (0·8 kg/d for triplet-bearing ewes) to 1·2 kg/d at 140 d of gestation (1·3 kg/d for triplet-bearing ewes), and was fed in three equal meals (at 08.00, 12.00 and 16.00 hours) at a flat rate of 1·7 kg/d during lactation. Barley straw was offered *ad libitum*, fed at a rate of 125 % of daily intake and calculated by weighing back refusals three times per week. Blood samples were obtained from ewes by jugular venepuncture into evacuated plasma tubes containing lithium heparin, potassium oxalate or no additive, at 11.00 hours on days 103, 117 and 131 of gestation and at days 14 and 28 into lactation. Only plasma samples taken at days 103 and 131 of gestation and day 14 of lactation were analysed for vitamin E. At parturition, the second-born lamb from each triplet-bearing ewe was removed immediately after expulsion and the weight and sex recorded. Lambs were then killed by an intrajugular injection of sodium pentobarbitone (200 mg/ml, Pentoject®; AnimalCare Ltd, Dunnington, York, UK) at 0·8 ml/kg live weight. Immediately after cessation of the

heartbeat was confirmed by stethoscope, blood samples were obtained by cardiac puncture and collected in evacuated tubes containing either lithium heparin or potassium oxalate. The brain was removed according to the procedure documented by the US Department of Agriculture Plant and Animal Health Inspection Service (2001) and the *semimembranosis* muscle dissected from the left hind leg. Tissue samples were stored at −20°C before analysis. The weight and sex of all surviving lambs were recorded at 12 h postpartum.

Colostrum and milk samples were taken from ewes using a method adapted from Pattinson & Thomas (2004). At 12 h postpartum, lambs were confined behind a wire mesh barrier within the pen, then 1 ml oxytocin (10 IU/ml, Oxytocin Leo®; LEO Animal Health, Princes Risborough, Buckinghamshire, UK) was administered to the ewe by intramuscular injection, and the udder hand-milked until empty. At 16 h postpartum, 1 ml oxytocin was administered by intramuscular injection, and the udder again hand-milked until empty. The total volume of colostrum produced was recorded and the colostrum secretion rate calculated for the interval between the end of the first and second milking. Subsamples were taken and stored at −20°C before analysis. At 21 d postpartum, the procedure was repeated in order to measure milk yield and obtain samples for compositional analysis. Blood samples were taken by jugular venepuncture from 14-d-old lambs at 11.00 hours and collected into evacuated tubes containing lithium heparin, potassium oxalate or no additive.

Chemical analyses

Feed samples were bulked and analysed for DM and ash according to the method described by the Association of Official Analytical Chemists (1990). Crude protein was determined by the Kjeldahl method (Kjeltec 1035 analyser; Foss UK Ltd, Cheshire, UK) and neutral-detergent fibre by the method of Van Soest *et al.* (1991). Analysis of fatty acids within feed samples was executed by the method reported by Wachira *et al.* (2002) with samples being saponified in a solution of 5 M-KOH in aqueous methanol before extraction of fatty acids into petroleum spirit and methylation of individual fatty acids using diazomethane. Methyl esters of the fatty acids were then injected into a PerkinElmer 8500 gas chromatograph (PerkinElmer Life and Analytical Sciences Ltd, Boston, MA, USA) comprising a 50 m × 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). He gas was employed as the carrier and total fatty acids were quantified with reference to an internal standard of 21:0 (Thames Restek UK Ltd, Windsor, Berkshire, UK). Individual peaks were identified by the retention times of a fatty acid methyl ester standard (Sigma-Aldrich, Poole, Dorset, UK) and the linearity of response confirmed using a reference mixture of fatty acid methyl esters (fatty acid methyl ester 5) containing equivalent amounts of 16:1*n*-9, 18:1*n*-9 *cis*, 20:1, 22:1 and 24:1 (Thames Restek UK Ltd).

Packed cell volume of whole blood was determined by the capillary microhaematocrit method (Hawksley and Sons Ltd, Lancing, West Sussex, UK). Plasma and serum were produced from blood samples by centrifugation at 2290 g for 5 min before storage at −20°C. Whole blood samples were analysed for glutathione peroxidase (GPx) using an RS504 kit (Randox,

Crumlin, Country Antrim, UK) and serum samples analysed for creatine kinase (CK) using a TO1-1882-85 kit (Bayer Diagnostics, Strawberry Hill, Newbury, Berkshire, UK). Both analyses were performed on a Technicon RA-1000 autoanalyser (Bayer Diagnostics).

Quantification of vitamin E within plasma, milk and tissue samples was performed by HPLC following solvent extraction. Vitamin E in plasma samples was determined by a modification of the method reported by McMurray & Blanchflower (1979). Milk and colostrum vitamin E concentrations were determined according to a modification of the method of Burton *et al.* (1985) employing extraction in ethanol and hexane. Lipids within muscle samples were subjected to hydrolysis by a solution of KOH before extraction of vitamin E into hexane (Liu *et al.* 1996), while brain samples were subject to the same procedure with an added saponification stage. Reagent blanks (containing about 0·75 ml distilled water to approximate the water content of the muscle samples) and standards of *rac* 5,7-dimethyltocol were used to quantify results and were prepared using the earlier-mentioned procedure. Elutant from colostrum, milk and tissue samples was passed through a normal phase column packed with silica (250 mm × 4·6 mm Techsphere with 5 μm particle size; HPLC Technology, Macclesfield, Cheshire, UK) with a mobile phase of 96 % n-hexane–methanol and 4 % 1,4-dioxane within an isocratic HPLC apparatus (Gilson Inc., Middleton, WI, USA). The HPLC was calibrated using nine standard solutions containing known concentrations of α-tocopherol ranging from 0·01 to 5 μg. Fluorescence detection of vitamin E was at an excitation of 297 nm and emission of 330 nm. The concentration of α-tocopherol in the sample was calculated according to analysis of the peak height for α-tocopherol compared with internal and external standards. Reported concentrations of vitamin E were adjusted to account for percentage recoveries (94 % for colostrum, 93 % for milk, 86 % for muscle, 95 % for brain). Feed samples were analysed for vitamin E by HPLC according to the method of Manz & Philipp (1981), the analysis being performed by Roche UK Ltd.

Statistical analyses

All data were analysed as a factorial 2 × 2 completely randomised block design with fat source and dietary vitamin E concentration and their interaction as the main effects using a general ANOVA in the statistical package Genstat® 6.2 (Lawes Agricultural Trust, Harpenden, Hertfordshire, UK, 2002).

Results

Feed analysis

The four treatment diets had a similar chemical composition with mean values for DM, organic matter and crude protein (N × 6·25) of 863 g/kg, 908 g/kg DM and 179 g/kg DM, respectively. Concentrations of neutral-detergent fibre were lower, and the ash contents higher in the diets containing fish oil as a result of vermiculite inclusion, with mean values of 137 and 111 g/kg DM (fish oil) *v.* 211 and 73 g/kg DM (Megalac) for neutral-detergent fibre and ash, respectively. Vitamin E concentrations were similar to those predicted by the diet formulation with means of 61 and 522 mg/kg DM for the basal and supranutritional diets, respectively. Total fatty acid concentrations were higher in diets supplemented with
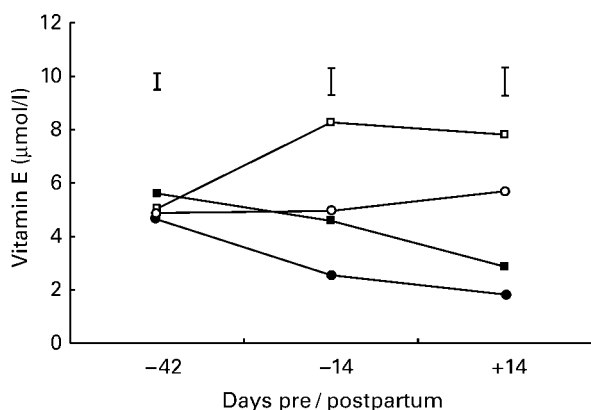
Fig. 1. Plasma vitamin E concentrations in ewes fed diets containing Megalac® (M; Ca soap of palm fatty acid distillate, Volac Ltd, Royston, Hertfordshire, UK) or fish oil (F; Trouw UK Ltd, Northwich, Cheshire, UK) supplemented with either 50 (B) or 500 (S) mg α-tocopherol acetate/kg. ■, MB; □, MS; ●, FB; ○, FS. For details of diets and procedures, see Table 1 and p. 550. Values are means with standard errors shown by vertical bars (twelve ewes per group).

Megalac (100 g/kg DM) than in those containing fish oil (87 g/kg DM). Compared with diets based on Megalac, the addition of fish oil to treatment diets increased the dietary supply of polyunsaturated (40·4 *v.* 23·3 g/kg DM) and *n*-3 (9·6 *v.* 1·8 g/kg DM) fatty acids. Furthermore, the concentration of total *n*-6 fatty acids was reduced in the diets containing fish oil (15·9 g/kg DM), in contrast to the Megalac diets (19·9 g/kg DM).

Ewe antioxidant status

Plasma vitamin E concentrations were similar between treatments at 42 d prepartum (Fig. 1). The vitamin E concentration within plasma at 14 d prepartum was increased by supranutritional dietary supplementation of the ewe ($P < 0.001$); in contrast, addition of PUFA to the diet reduced the concentration of vitamin E within plasma at 14 d prepartum ($P < 0.001$). Supranutritional vitamin E supplementation increased plasma concentrations postpartum compared with basal supplementation ($P < 0.001$), while feeding diets containing fish oil reduced vitamin E concentrations compared with diets based on Megalac ($P < 0.001$).

The provision of treatment diets to pregnant ewes reduced the activity of GPx and the concentration of CK compared with pre-treatment values (Table 2). When contrasted with ewes fed Megalac, supplementation of ewes with long-chain PUFA reduced mean prepartum GPx activity ($P < 0.001$). A similar pattern was seen during lactation ($P < 0.01$). No main or interaction effects of dietary treatment were observed on ewe serum CK concentrations at any time point.

Colostrum yield was not significantly affected by dietary vitamin E supply; however, supplementation with long-chain PUFA reduced colostrum yield compared with feeding Megalac ($P < 0.01$) The vitamin E concentration of colostrum mirrored maternal prepartum plasma vitamin E concentrations, with the highest amounts being recorded in supranutritionally supplemented ewes ($P < 0.001$). An interaction between dietary vitamin E concentration and fat source was also observed on colostrum vitamin E concentration ($P < 0.001$) and yield ($P < 0.001$), with higher amounts of vitamin E recorded in samples from ewes fed diet MS compared with ewes fed any of the other three treatment diets. Furthermore, adding long-chain PUFA to the diets reduced colostrum vitamin E concentration ($P < 0.001$) and yield ($P < 0.001$). Similar effects of dietary vitamin E concentration ($P < 0.001$), fat source ($P < 0.01$) and an interaction between the two ($P < 0.05$) were observed for milk vitamin E concentration. Milk yield was not altered significantly by either dietary vitamin E supply or fat source. Milk vitamin E yield was increased by supranutritional vitamin E supplementation of the ewe ($P < 0.01$) although it was unaffected by dietary fat source.

Table 2. Effect of vitamin E and fatty acid supplementation of ewes on parameters of antioxidant status in ewes†‡

	Diet§					Significance		
	MB	MS	FB	FS	SED	Fat source	Vitamin E	Interaction
Pre-treatment (103 d of gestation)								
Mean erythrocyte GPx activity (U/ml PCV)	191	173	161	186	24·6	NS	NS	NS
Mean CK activity (U/l)	488	337	839	241	441·1	NS	NS	NS
Prepartum								
Mean erythrocyte GPx activity (U/ml PCV)	171	164	109	130	13·8	***	NS	NS
Mean CK activity (U/l)	259	209	393	179	143·1	NS	NS	NS
Postpartum								
Mean erythrocyte GPx activity (U/ml PCV)	185	185	160	147	14·1	**	NS	NS
Mean CK activity (U/l)	135	165	188	261	55·0	NS	NS	NS
Colostrum yield (ml/h)	95	119	77	72	15·2	**	NS	NS
Colostrum vitamin E concentration (μg/g)	8·23	27·40	6·93	9·26	1·814	***	***	***
Colostrum vitamin E yield (μg/h)	947	2846	908	1061	186·5	***	***	***
Milk yield (ml/h)	97	83	92	102	11·1	NS	NS	NS
Milk vitamin E concentration (μg/g)	0·95	3·44	0·65	1·96	0·329	**	***	*
Milk vitamin E yield (μg/h)	106	195	63	217	46·4	NS	**	NS

GPx, glutathione peroxidase; PCV, packed cell volume; CK, creatine kinase.
Mean prepartum activities = mean of values measured at 14 and 28 d prepartum; mean postpartum activities = mean of values measured at 14 and 28 d postpartum.
Significance level: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
† For details of diets and procedures, see Table 1 and p. 550.
‡ MB, *n* 12; MS, *n* 11; FB, *n* 11; FS, *n* 12.
§ MB, Megalac® (Ca soap of palm fatty acid distillate; Volac Ltd, Royston, Hertfordshire, UK) plus 50 mg vitamin E/kg; MS, Megalac plus 500 mg vitamin E/kg; FB, fish oil plus 50 mg vitamin E/kg; FS, fish oil plus 500 mg vitamin E/kg.

Table 3. Effect of vitamin E and fatty acid supplementation of ewes on lamb birth weight and suckling lamb antioxidant status†

	Diet‡					Significance		
	MB	MS	FB	FS	SED	Fat source	Vitamin E	Interaction
Lamb birth weight (kg)§	3·87	4·01	3·85	4·33	0·190	NS	*	NS
Suckling lambs (14 d of age)‖								
Erythrocyte GPx (U/ml PCV)	289	308	273	277	16·3	*	NS	NS
Serum CK (U/l)	194	143	456	421	162·7	*	NS	NS

GPx, glutathione peroxidase; PCV, packed cell volume; CK, creatine kinase.
Significance level: *$P<0.05$.
† For details of diets and procedures, see Table 1 and p. 550.
‡ MB, Megalac® (Ca soap of palm fatty acid distillate; Volac Ltd, Royston, Hertfordshire, UK) plus 50 mg vitamin E/kg; MS, Megalac plus 500 mg vitamin E/kg; FB, fish oil plus 50 mg vitamin E/kg; FS, fish oil plus 500 mg vitamin E/kg.
§ MB, n 23; MS, n 23; FB, n 24; FS, n 24.
‖ MB, n 22; MS, n 23; FB, n 22; FS, n 23.

Neonatal lamb birth weight and antioxidant status

Lambs born to ewes fed supranutritional concentrations of vitamin E during pregnancy were heavier at birth ($P<0.05$) by an average of 0·31 kg (Table 3). This parameter was not affected by maternal dietary fat source. Plasma vitamin E was undetectable in nine out of twelve neonatal plasma samples ($<0.1 \mu$mol/l), being measurable only in lambs from ewes fed diets MS (two samples) and FS (one sample). Vitamin E concentrations in brain tissue were elevated in lambs from treatments MS and FS compared with the basal treatment groups ($P<0.05$), with there being no significant effect of maternal PUFA supplementation on vitamin E concentration (Fig. 2). The vitamin E concentration of neonatal *semimembranosis* muscle was also significantly augmented by maternal supranutritional supplementation ($P<0.01$). Lambs born to ewes fed diets supplemented with long-chain PUFA exhibited decreased muscle vitamin E concentrations, although these differences were not statistically significant ($P>0.05$; Fig. 2).

Suckling lamb antioxidant status

Fig. 3 shows the vitamin E concentrations within lamb plasma at 14 d of age. Main effects of maternal dietary vitamin E concentration ($P<0.001$) and fat source ($P<0.001$) were demonstrated,
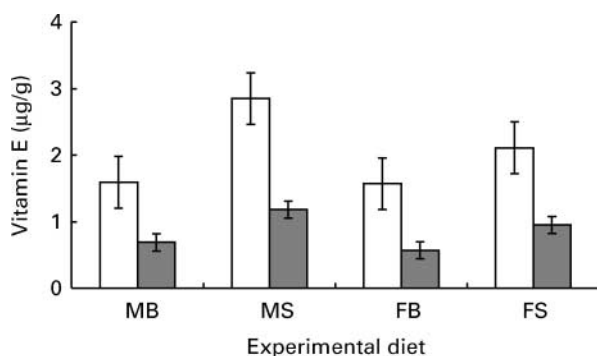
as was an interaction between maternal vitamin E supplementation level and fatty acid source with the vitamin E concentration of plasma increasing in the order FB < MB < FS < MS. Plasma concentrations were similar for ewes and lambs at 14 d postpartum, with a positive correlation between ewe and lamb vitamin E status (r 0·50, $P<0.01$). Vitamin E supplementation of the ewe had no effect upon the activity of GPx in erythrocytes of the 14-d-old lamb (Table 3). By contrast, lambs suckling ewes fed long-chain PUFA had lower GPx activity than those from the Megalac treatments ($P<0.05$). Maternal fish oil supplementation also increased serum CK concentrations in lambs at 14 d of age ($P<0.05$), whilst there was no significant effect of maternal vitamin E supplementation upon this parameter.

Discussion

The aim of the present study was to determine the effects of vitamin E and long-chain PUFA supplementation of the pregnant and lactating ewe upon the vitamin E status of the neonatal and growing lamb. Work published by Mino & Nishino (1973), Njeru *et al.* (1994) and Léger *et al.* (1998) concentrated on the relationship between maternal and neonatal plasma concentrations as the principal indicator of vitamin E status and concluded that low plasma concentrations in the neonate are indicative of negligible placental transfer. However, there are few data available regarding the
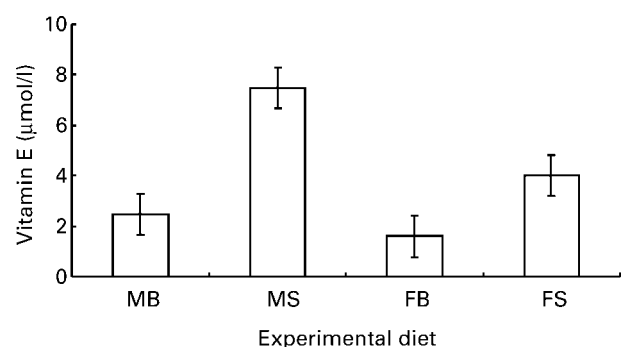

Fig. 2. Brain (□) and *semimembranosis* muscle (▪) vitamin E concentrations in lambs from ewes fed diets containing Megalac® (M; Ca soap of palm fatty acid distillate, Volac Ltd, Royston, Hertfordshire, UK) or fish oil (F; Trouw UK Ltd, Northwich, Cheshire, UK) supplemented with either 50 (B) or 500 (S) mg α-tocopherol/kg acetate. For details of diets and procedures, see Table 1 and p. 550. Values are means with standard errors shown by vertical bars (three lambs per group).

Fig. 3. Plasma vitamin E concentrations in suckling lambs at 14 d of age from ewes fed diets containing Megalac® (M; Ca soap of palm fatty acid distillate, Volac Ltd, Royston, Hertfordshire, UK) or fish oil (F; Trouw UK Ltd, Northwich, Cheshire, UK) supplemented with either 50 (B) or 500 (S) mg α-tocopherol acetate/kg. For details of diets and procedures, see Table 1 and p. 550. Values are means with standard errors shown by vertical bars (twenty-four lambs per group).

vitamin E concentration of neonatal ruminant tissues, specifically brain tissue.

Ewe antioxidant status

A rapid increase in the plasma vitamin E concentration has been demonstrated as a response to dietary supplementation of ewes (Hidiroglou *et al.* 1969; Njeru *et al.* 1994) and pigs (Mahan, 1991; Hidiroglou *et al.* 1993a; Lauridsen *et al.* 2002). Within the present study, supranutritional supplementation of the pregnant and lactating ewe resulted in an increase in plasma vitamin E concentrations, results that concur with the range reported by Gabryszuk & Klewiec (2002). The magnitude of changes in plasma vitamin E concentration in response to dietary supply was demonstrably higher during pregnancy than during lactation. Similar results were reported by Hidiroglou *et al.* (1993a), who supplemented pregnant and lactating sows with dietary vitamin E and attributed the lower plasma vitamin E concentrations observed during lactation to the partitioning of vitamin E into colostrum and milk. The total dietary vitamin E supply (mg/d) was considerably higher during lactation than that fed during pregnancy, and it is probable that the lower concentrations observed in plasma may also have been due to the increased vitamin E requirement during lactation. The animal's requirement for vitamin E in its role as a cellular antioxidant is positively correlated with the oxidative challenge posed to the animal as a result of fatty acid supply. Farnworth *et al.* (1995) and McGuire & Fritsche (1997) have described the abrogating effect of supplemental dietary long-chain PUFA upon the vitamin E status of various animals. The reduced plasma vitamin E concentrations observed in ewes fed fish oil during pregnancy and lactation concur with these results.

High concentrations of vitamin E in colostrum as a result of supplementation of the pregnant ewe have been reported by Gentry *et al.* (1992) and Njeru *et al.* (1994). The colostrum vitamin E concentrations reported herein mirror the values recorded in ewe plasma at 14 d prepartum, with significantly higher concentrations observed as a result of supranutritional supplementation. The increase in milk vitamin E concentrations conferred by supranutritional supplementation within the present study concurs with the results published by Mahan (1991), Charmley *et al.* (1993) and Njeru *et al.* (1994). Furthermore, the lower concentrations of vitamin E in milk compared with colostrum are in agreement with the results of Hidiroglou *et al.* (1993a) and Njeru *et al.* (1994) who observed a threefold increase in colostrum vitamin E compared with that of milk. Mahan (1991) described a similar pattern of vitamin E secretion in lactating sows given supplemental dietary vitamin E and proposed that increased concentrations in colostrum compared with milk may be a result of mobilisation of the labile tissue pool for colostrum production, with a consequent reliance on dietary vitamin E for deposition in milk.

The vitamin E status of the ewe has a complex relationship with the Se status of the animal, specifically with the GPx enzymes, which act to prevent the oxidation of unsaturated fatty acids within the cell (Van Metre & Callan, 2001). Suárez *et al.* (1999) reported that supplementing rats with dietary vitamin E reduced the total amount of GPx present in liver and brain and suggested that this reduction may be due to a sparing effect of vitamin E upon the synthesis of GPx. By contrast, within the present study, no effect of vitamin E supplementation was observed

upon the activity of GPx in ewes, although this activity was significantly reduced in ewes offered fish oil. The reduction in GPx activity in ewes supplemented with fish oil may be attributed to a higher rate of GPx utilisation within the cell and a reduction in active enzyme availability. Indeed, reduced GPx activity in erythrocytes of sheep supplemented with protected PUFA was reported by Smith *et al.* (1994).

Cellular contents are released when tissues are damaged by long-chain PUFA peroxidation (Lefebvre *et al.* 1996), with the serum enzyme CK acting as a reliable indicator of tissue damage (Sacheck & Blumberg, 2001). However, neither PUFA nor vitamin E supplementation had a significant effect upon ewe serum CK within the present study, despite the potential differences in peroxidation challenge and antioxidant supply provided by the four treatment diets. Reference values for CK in sheep serum proposed by Bostedt & Schramel (1990) suggest that the extent of tissue damage induced by long-chain PUFA supplementation within the present study was low, with values of greater than 2000 IU/l suggesting sub-clinical, and above 4000 IU/l acute nutritional myopathy.

Lamb birth weight

Increasing birth weight may improve neonatal survival with an improvement in piglet survival rate as a result of vitamin E supplementation of the sow being reported by Mahan (1991). Reduced pre-weaning mortality rates in lambs produced by ewes supplemented with vitamin E were also reported by Kott *et al.* (1983, 1998) and Gabryszuk & Klewiec (2002). The increase in lamb birth weight conferred by maternal vitamin E supplementation in the present study concurs with previous research published by Gentry *et al.* (1992), who reported that lambs from vitamin E-supplemented ewes tended to have higher birth weights and increased pre-weaning live weight gains. The mechanism by which birth weight may be increased by vitamin E supplementation is unclear; however, it may, in part, be due to the effects of antioxidant vitamins upon the immune system. Supplementary vitamin E has been reported to augment immune status and reduce the incidence and symptoms of disease in ruminant animals (Reddy *et al.* 1986). Consequently, improving the maternal immune status during pregnancy may promote the partitioning of additional nutrients towards the growing fetus, thereby increasing growth *in utero*. Long-chain PUFA supplementation of the dam has been shown to increase birth weight in human and animal neonates (Crawford *et al.* 1997; Rooke *et al.* 2001). In the present study, lamb birth weights were numerically higher as a result of fish oil supplementation of the ewe, although these results did not reach statistical significance.

Neonatal lamb antioxidant status

Concentrations of vitamin E within neonatal lamb plasma were low and unaffected by treatment diet. This result is in agreement with those reported by other authors in neonatal human subjects (Mino & Nishino, 1973; Léger *et al.* 1998), rats (Martin & Hurley, 1977), domestic pigs (Hidiroglou *et al.* 1993a, 1995), cattle (Van Saun *et al.* 1989) and sheep (Hidiroglou *et al.* 1969; Njeru *et al.* 1994) with the consensus view being that it is indicative of low placental transfer, therefore rendering the neonate deficient in vitamin E. Furthermore, Lauridsen *et al.* (2002) concluded that low tissue concentrations of vitamin E in neonatal piglets were evidence

of inefficient placental transfer and suggested that neonatal vitamin E status could not be manipulated by maternal supplementation.

The present data do not support the hypothesis that placental transfer is negligible in the ruminant. Although neonatal plasma vitamin E concentrations were low or undetectable for all treatments, brain and *semimembranosis* concentrations were significantly increased by supranutritional maternal supplementation. Kelly *et al.* (1992) suggested that the fetal liver may act as a labile source of vitamin E and that plasma concentrations may not be a reliable indicator of neonatal vitamin E status. It is debatable whether low vitamin E concentrations in neonatal plasma indicate a deficiency status or an increased rate of uptake from plasma in order to maintain satisfactory tissue concentrations. Furthermore, Vatassery *et al.* (1988) hypothesised that the rodent brain may be resistant to vitamin E depletion even when a vitamin E-deficient diet is supplied for a period of 4 months. It therefore seems logical that organs with an increased requirement for antioxidant vitamins may have an enhanced capacity for uptake in times of diminished availability.

Within the present study, vitamin E concentrations in neonatal tissues were considerably higher than those observed by Hidiroglou *et al.* (1993*b*), suggesting that increasing the maternal vitamin E supply may enhance the amount of this vitamin transferred across the placenta to the fetal lamb. Supranutritional vitamin E supplementation of ewes resulted in concentrations of vitamin E in neonatal lamb muscle similar to those reported for unsupplemented wethers by Ochoa *et al.* (1992). However, Hidiroglou & Batra (1996) observed a mean value of 2·70 µg/g in muscle of lambs with no supplemental vitamin E added to the diet. It would appear that although supplementation increased the concentration of vitamin E in neonatal tissues within the present study, these animals may still have been sub-clinically deficient in vitamin E. The reduction in tissue vitamin E concentration in lambs born to ewes fed diets supplemented with long-chain PUFA concurs with the results of Hidiroglou *et al.* (1970) and Farnworth *et al.* (1995), again suggesting that these diets conferred a greater peroxidation challenge.

Suckling lamb antioxidant status

Concentrations of vitamin E within ewe and lamb plasma were similar at 14 d into lactation, the increases in plasma vitamin E concentration of suckling lambs conferred by maternal supranutritional supplementation reflecting differences observed in maternal milk. Dosing lambs with 1000 IU D-α-tocopherol increased plasma vitamin E concentrations from 0·65 to 1·90 µg/ml in the study of Hidiroglou & Batra (1996), values consistent with the lowest concentrations observed in lambs within the present study. The plasma concentrations observed here were also within the range reported by Gentry *et al.* (1992) and Hatfield *et al.* (2002). The lower vitamin E concentrations observed in lambs suckling ewes supplemented with long-chain PUFA are in agreement with the results found by McGuire & Fritsche (1997) in rats fed additional dietary fish oil and with those of Chikunya *et al.* (2004) and Demirel *et al.* (2004) in sheep.

Activity of GPx within erythrocytes was approximately doubled in lambs at 14 d of age compared with ewe erythrocyte activities. Although placental transfer of Se appears to be limited in the ewe (Bostedt & Schramel, 1990) no barrier to mammary Se transfer is apparent, which may enhance synthesis and activity of GPx in suckling lambs. The activity of this enzyme in lamb erythrocytes was

reduced by maternal long-chain PUFA supplementation, concurring with patterns observed in maternal plasma during lactation. This may be attributed to an increase in cellular oxidative challenge resulting from the addition of PUFA to the maternal diet with consequent transfer of these fatty acids into milk.

Serum CK concentrations observed in lambs at 14 d of age concur with maternal CK results, in that the concentrations of this enzyme were significantly augmented by long-chain PUFA supplementation of the ewe. El-Neweehy *et al.* (2000) reported low concentrations of CK in animals free of nutritional myopathy (37 IU/l), although sub-clinically affected animals had concentrations ranging from 1186 to 3740 IU/l with detectable symptoms being seen at a mean CK concentration of 4291 IU/l. By these criteria, although lambs suckling ewes fed fish oil had an increased susceptibility to nutritional myopathy, clinical disease was unlikely to be present.

Conclusion

Previous studies have suggested that the placental transfer of vitamin E from the ewe to the lamb is negligible. However, the present experiment demonstrates that appreciable placental transfer and subsequent deposition of vitamin E into neonatal lamb tissues may be conferred by vitamin E supplementation of the pregnant ewe. Furthermore, it can be concluded that plasma vitamin E concentrations are not reliable indicators of tissue concentrations in the neonate. The exact mechanism by which vitamin E supplementation increases lamb birth weight warrants further investigation.

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