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Effect of plane of nutrition of 1- and 2-year-old ewes in early and mid-pregnancy on ewe reproduction and offspring performance up to weaning

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The objective of the present study was to determine the effect of plane of nutrition in early pregnancy (EP) and mid-pregnancy (MP), on the productive performance of 1- and 2-year-old ewes and their offspring. Over 2 successive years, between days 0 and 39 after synchronized mating (EP), 1- ($n = 117$*) and 2- (*$n = 52$*) year-old ewes were allowed 60% (low, L-EP), 100% (medium, M-EP) or 200% (high, H-EP) of requirements for maintenance (M). Between days 40 and 90 (MP), 1-year-old ewes were allowed 140% (M-MP) or 200% (H-MP), while 2-year-old ewes were allowed 80% (M-MP) or 140% (H-MP) of their M requirement. After day 90, all ewes were fed to meet requirements for late pregnancy. Increasing the plane of nutrition between days 0 and 39 resulted in increases in live weight (LW) (*$P < 0.001$*) and body condition score (BCS) (*$P < 0.001$*) during the EP period (H-EP > M-EP > L-EP), differences that in 1-year-old ewes were sustained to lambing (*$P < 0.05$*). On day 42 of gestation H-EP ewes had lower plasma progesterone concentrations than L-EP or M-EP ewes in 1- (*$P < 0.01$*) and 2- (*$P < 0.001$*) year olds. This was concomitant with diet H-EP tending to reduce the number of lambs born per ewe in both age groups (*$P = 0.06$ *and 0.07, respectively). Foetuses from 1-year-old L-EP ewes had smaller cranial (*$P < 0.01$*) and abdominal (*$P < 0.05$*) diameters at day 53 of gestation, with H-EP lambs tending to be heaviest at birth (*$P = 0.07$*). Similar findings were recorded for 2-year-old ewes. One-year-old ewes offered diet L-EP presented negative maternal behaviours more frequently (*$P < 0.05$*), while the incidence of lamb mortality at 6 weeks tended to be greater for L-EP lambs (*$P = 0.07$*). In MP, 1-year-old ewes offered diet M-MP were associated with foetuses with bigger abdominal diameters at day 78 (*$P < 0.05$*). However, there were no differences in lamb weight or size at term (*$P > 0.05$*). These ewes exhibited more positive maternal behaviours (e.g. increased grooming frequency and duration; *$P < 0.05$*) than ewes offered diet H-MP, and their offspring were more successful in suckling (*$P < 0.05$*). Results suggest that in young ewes, a temporary nutrient restriction in EP resulted in increased prolificacy. However, ewes and their offspring were lighter at birth and ewe maternal behaviour was poorer, resulting in increased lamb mortality. In MP, a medium plane of nutrition offered to 1-year-old ewes led to improved maternal and offspring behaviour.*

Keywords: foetal development, maternal behaviour, nutrition, pregnancy, sheep

Implications

Poor reproductive performance in young ewes is a major economic and welfare issue facing the sheep industry. Meeting ewe nutritional requirements in pregnancy is critical for placental and foetal development, which has major implications on lamb survival. This paper increases understanding on the effects of plane of nutrition in the early stages of gestation in 1- and 2-year-old ewes and is designed to help develop feeding strategies to improve this situation.

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Introduction

The nutrient requirements of adolescent ewes are a function of age, body weight and condition, and stage and level of production. Because young ewes are still growing and have not yet reached mature body size, their requirements for nutrients are higher than adult ewes (National Research

Council, 2007). Production stages such as gestation and lactation not only determine a higher demand for nutrients but also add complexity in nutrient partitioning and utilization due to numerous pregnancy-induced adaptations in maternal tissue metabolism (reviewed by Robinson *et al.*, 2002). Annett and Carson (2006) demonstrated that mature and adolescent ewes do not respond in equivalent ways to different planes of nutrition during early pregnancy (EP) (day 0 to 30 of gestation). Therefore, meeting the adolescent ewe's requirements during pregnancy is critical for placental and foetal development, and in determining lamb birth weight. This has major implications on lamb survival as extreme birth weights (light and heavy lambs) are associated with higher mortality rates (Sawalha *et al.*, 2007).

Nutrient partitioning in EP influences embryo growth and development. Underfeeding (0.6 M) adolescent dams in EP (day 0 to 30 of gestation) increased the proportion of ewes that successfully conceived, with no effects on lamb birth weight, viability or performance (Annett and Carson, 2006). However, adolescent dams depleted their body reserves and appeared less capable of sustaining an adequate lactational performance. In adult ewes, Muñoz *et al.* (2008a) reported that dams on low-plane diets (0.6 M) for the first 39 days of gestation gave birth to heavier lambs at parturition that had lower mortality rates at weaning than lambs born from control or over-fed ewes, provided nutrition during mid- and late pregnancy was adequate.

During MP, the most sensitive period for nutritional modification of placental growth and consequently birth weight is between days 30 and 80 of gestation, the period of maximal placental weight gain (Schneider, 1996). Several authors have highlighted the inconsistencies between reports that examine the effects of maternal nutrition in MP on foetal and placental growth (Kelly, 1992; Heasman *et al.*, 1999; Redmer *et al.*, 2004). Wallace *et al.* (2006) recently reviewed two adolescent sheep models of nutritional manipulation during pregnancy. In the over-nourished dam model, when adolescent ewes were offered *ad libitum* intakes equivalent to twice maintenance (M) energy requirements, the maternal growth achieved was concomitant with impaired placental and foetal growth, relative to control dams. In contrast, in the under-nourished dam model (adolescent ewes prevented from growing by feeding 0.7 M), limiting maternal energy intake gradually depleted maternal body reserves, leading to reduced foetal growth in late pregnancy, independent of alterations in placental growth.

From the published literature, a series of factors such as level and length of nutrient manipulation, maturity of the dam and period during which nutritional manipulation occurs (early, mid- or late pregnancy) have been identified as having a determining influence in the final outcome of pregnancy. However, there is a paucity of information on the effect of these factors on foetal development, parturition performance and on dam and neonatal behaviours. The objectives of the present study were to investigate the effects of different planes of nutrition during EP and MP and their interactions on maternal and offspring performance of ewes of 1 and 2 years of age.

Material and methods

The work described in this paper was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Animals

Two studies were conducted in parallel over two successive mating seasons. The first study involved a total of 117 ($n = 81$ year 1; $n = 36$ year 2) peri-pubertal crossbred ewes of 7.2 ± 0.3 months of age (Table 1). The second study utilized 52 ($n = 19$ year 1; $n = 33$ year 2) uniparous crossbred ewes of 19.2 ± 0.3 months of age. The breeds of

Table 1 *Number of 1- and 2-year-old ewes involved in the study*

	Early pregnancy nutrition			Mid-pregnancy nutrition		
	L	M	H	M	H	Total
One-year-old ewes						
No. of animals in the study	36	39	42	59	58	117
No. of pregnant ewes	22	20	16	32	26	58
No. of ewes lambed	21	20	13	28	26	54
No. of lambs born	28	28	18	41	33	74
No. of lambs weaned	16	21	14	29	22	51
No. of ewes recorded for behaviour	12	9	6	15	12	27
No. of lambs recorded for behaviour	16	13	8	21	16	37
Two-year-old ewes						
No. of animals in the study	16	15	21	26	26	52
No. of pregnant ewes	15	12	15	21	21	42
No. of ewes lambed	15	11	14	21	19	40
No. of lambs born	28	21	25	36	38	74
No. of lambs weaned	19	16	21	26	30	56

Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.

the ewes were Texel × Greyface (Border Leicester × Scottish Blackface; $n = 63$), Charolais × Greyface ($n = 54$) and Lleyn × Greyface ($n = 52$). Twenty-seven rams (15 and 12 for year 1 and 2, respectively) from three different breeds were used in the study – Charolais, Lleyn and Texel. Prior to the commencement of the study, all animals had *ad libitum* access to fresh pasture. The mean live weight (LW) and body condition score (BCS) of peri-pubertal ewes at the start of the study were 46.7 ± 4.9 kg and 3.5 ± 0.2, respectively. The same parameters for uniparous ewes were 62.6 ± 5.6 kg and 3.6 ± 0.2.

Experimental design

Both experiments were carried out at the Agri-Food and Biosciences Institute, Hillsborough, and had a common experimental design. All ewes were housed in individual pens on wooden slat floors at the start of the study, where they remained throughout pregnancy. In mid-October, the ewes were submitted to a synchronization regime based on intravaginal sponges containing 30 mg flugestone acetate for 12 days (Chronogest®; Intervet UK Ltd, Milton Keynes, UK), followed by an intramuscular injection of 300 IU of pregnant mare serum gonadotrophin (Fostim 6000®; Pharmacia & Upjohn Animal Health Ltd, Kalamazoo, MI, USA). Two days after sponge removal the ewes were naturally mated once and randomly allocated to treatments and subsequently balanced for LW, BCS, ewe breed and sire breed.

Nutritional treatments were applied offering different levels of the same dried grass-based diet that in year 1 contained 897 g dry matter (DM)/kg and supplied the ewes with 149 g crude protein (CP)/kg DM. In year 2, the diet contained 911 g DM/kg and supplied 128 g CP/kg DM. *In vivo* metabolizable energy (ME) values (10.2 MJ/kg DM) determined previously (Annett and Carson, 2006) for grass nuts from the same source were used in the current study. From day 1 to 39 post-mating (EP), ewes were placed on one of three planes of nutrition that were individually estimated to provide either 200% (high, H-EP), 100% (medium, M-EP) or 60% (low, L-EP) of predicted ME requirements for M of a non-pregnant ewe (Agriculture and Food Research Council, 1993). From day 40 to day 90 of gestation (MP) within each EP treatment, primiparous ewes (experiment 1) were allocated to rations providing either 140% (medium, M-MP) or 200% (high, H-MP) of individual ME requirements for M of a non-pregnant ewe. In the same period, uniparous ewes (experiment 2) received either 80% (medium, M-MP) or 140% (high, H-MP) of their ME requirement relative to a non-pregnant ewe.

Within each combination of EP and MP treatments half of the ewes received a selenium supplement (0.5 mg Se/ewe per day), offered from day −14 to day 90 of pregnancy (year 1 = Selplex®; Alltech, Belfast, Northern Ireland, UK; year 2 = Retosel Premix 4.5%®; Trouw Nutrition, Belfast, Northern Ireland, UK) as described in Muñoz *et al*. (2008b). During EP and MP, diets were supplemented daily with 25 g of barley, as a vehicle for the intake of selenium and 25 g of straw as a source of fibre. After 90 days of pregnancy, all ewes were offered increasing levels of the grass-based diet to meet individual energy and protein requirements for late pregnancy calculated in accordance with their LW and expected litter size (Agriculture and Food Research Council, 1993). During this period, diets were supplemented with 25 g of straw, 100 g/day of Sopralin® (Trouw Nutrition) and 25 g/day of a standard vitamin and mineral mix: VMC Sheep® (Trouw Nutrition), which supplied per kg vitamin A, 400 000 IU; vitamin D3, 80 000 IU; vitamin E, 600 IU; Se, 10 mg; Ca, 170 g; P, 40 g; NaCl, 320 g and Mg, 60 g. The total daily feed allocation was offered in a single feed at 0930 h. Pregnancy status of the ewes was determined by ultrasound scanning on day 50 of gestation. All non-pregnant animals were removed from the study and their data (LW and BCS data) were included in the analysis only up to day 55. Following lambing, ewes remained indoors with their lambs for 48 h, after which they were returned to pasture. Twin suckling ewes were kept in a separate field compared to ewes with singles. Creep concentrate feeding (ME 12.3 MJ/kg DM; CP 204 g/kg DM and 30 g/kg VMC Intensive Lamb® (Trouw Nutrition): vitamin A, 400 000 IU; vitamin D3, 66 667 IU; vitamin E, 1000 IU; Se, 17 mg; Ca, 100 g; P, 3.1 g and NaCl, 500 g per kg product) was introduced to lambs from twin suckling ewes in the first year and to all lambs in year 2, from 4 weeks of age and until weaning at 16 weeks.

Measurements

Placental and foetal development was assessed on all animals by trans-abdominal ultrasonography performed by the same operator using a portable Aloka 500 SSD with a 3.5 MHz curvilinear transducer (Aloka, Tokyo, Japan). During the procedure, the sheep were restrained in a crate in a ventral position. All ultrasonographic examinations were performed by the same operator. A continuous 2-min ultrasound recording was obtained for a single foetus from each ewe, at days 57 ± 3, 67 ± 2 and 81 ± 0.5 of gestation (year 1) and on days 41 ± 1, 50 ± 0.4 and 72 ± 0.4 of gestation (year 2). Still images of the foetus were transferred to a PC using DT-Acquire 3.3.0 (Data Translation Inc., Marlboro, NY, USA). Global Lab Image/2 3.6 (Data Translation Inc.) was used for measurements of cranial and abdominal diameters (to the nearest mm), as described by Pharr *et al*. (1994). Placental growth was monitored during the same period (Kelly *et al*., 1987).

In year 1, data on maternal and neonatal behaviours of 1-year-old ewes only were collected *in situ* following parturition. Ewe and lamb behaviours were recorded continuously for the first 30 min after the birth of each lamb, using handheld data recorders and the observer software system for behaviour research (Noldus Information Technology, Wageningen, The Netherlands). The definition of maternal behaviours was based on Dwyer and Lawrence (1998), while neonatal lamb behaviours were based on Dwyer *et al*. (1996), and have been described previously (Muñoz *et al*., 2008b).

Ewe LW and BCS (Russel *et al.*, 1969) were recorded at fortnightly intervals throughout gestation, 48 h after lambing, at 6 weeks post-lambing and at weaning. Jugular blood samples were collected using 8 ml lithium heparin tubes with gel (Vacuette®; Greiner Bio-One Ltd, Gloucestershire, UK) 2 h after feeding from all ewes at mating, at day 42 and at day 83 of gestation. Blood samples were stored at room temperature for 1 h before centrifuging at 4000 r.p.m. for 10 min. Plasma was stored at −20°C until analysed for progesterone concentrations, determined using an enzyme-linked immunosorbent assay (ELISA) kit (Ridgeway Science Ltd, Gloucestershire, UK).

Primiparous ewes lambed at 1.0 ± 0.02 years of age while biparous ewes were 2.0 ± 0.03 years old. At lambing, records were kept of lambing date, litter size, lambing difficulty (0 = no assistance and 1 = assistance), birth presentation (normal or abnormal position), birth weight and sex for all lambs. Within 24 h of lambing, nutritional effects on foetal skeletal development were assessed by measuring head and crown-rump length, thoracic circumference, and right fore limb and right hind limb length, using a flexible measuring tape. Lambs were weighed at 24 h *post partum*, at 3 and 6 weeks of age, and thereafter at fortnightly intervals until weaning. The lambs were blood sampled 24 h after birth by venipuncture using a 5 ml vacuette® gel separation tube (Greiner Bio-One Ltd, Gloucestershire, UK). Samples were kept at room temperature for 1 h after collection and refrigerated at 4°C for a further 24 h. Samples were than centrifuged at 4000 r.p.m. for 10 min. The serum was collected and stored at −20°C until assayed for immunoglobulin concentration estimated by zinc sulphate turbidity analyses (McEwan *et al.*, 1970), free triiodothyronine (free T_3) and thyroxine (T_4) concentrations. Free T_3 was measured in year 1 using a free T_3 kit (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA) on a Centaur Analyzer (Bayer Healthcare LLC, Diagnostics Division, Tarrytown, NY, USA). In year 2, the kit and analyser were Abbott Diagnostics (Berkshire, England, UK) and Abbott Diagnostics Architect Ci 8000, respectively. Thyroxine concentrations were measured using a T_4 Dri kit (Microgenics Corporation, Fremont, CA, USA) on an Olympus AU640 analyser (Olympus UK Ltd, Middlesex, UK). Records of lamb and ewe mortality were kept throughout the study. If an ewe could not raise its own lambs, her data and that of her offspring were included in the analysis only up to parturition.

Statistical analysis

Owing to the unbalanced nature of the experimental design, quantitative data were analysed using the residual maximum likelihood (REML) procedure in GenStat (Genstat for Windows, 11th Edition; VSN International Ltd, Oxford, UK). The model used evaluated the main effects of plane of nutrition in EP, plane of nutrition in MP, selenium supplementation and year of study, in a factorial design. All possible two-way interactions of main effects were examined and then removed from the model if non-significant. Mean comparisons were made with least significant differences (LSD at $P < 0.05$). Maternal data were adjusted for the effect of ram and ewe breed, while lamb sex was included for the analyses of litter weight. For offspring data, the individual dam and sire of the lamb were fitted as random effects while ram breed, ewe breed, lamb sex, litter size, field and age of lamb were included as fixed effects, where appropriate. Categorical data were analysed by binary logistic regression using LogXact (LogXact8; Cytel Inc., Cambridge, MA, USA). The models used for both maternal and offspring traits were as previously described for continuous data. Behaviour data were analysed using Stata survival analysis on the lamb latencies (Stata 9 for Windows; StatCorp LP, TX, USA), and the Tobit method (Amemiya, 1973) for the analysis of the frequency of presentation and duration of behaviours.

Results

There were no interactions between nutritional treatments; therefore, only main effects of plane of nutrition in EP and MP are presented in this paper.

One-year-old ewes

Live weight and body condition score. Increasing the plane of nutrition from day 0 to day 39 post-mating resulted in increases in average LW ($P < 0.001$), BCS ($P < 0.01$) and in LW and BCS changes ($P < 0.001$) during EP (H > M > L; Table 2). Between days 40 and 90, whilst ewes offered diet L-EP had greater LW gains ($P < 0.001$) than ewes offered diet H-EP, the differences in LW between the groups remained, albeit at a lower level, until lambing ($P < 0.05$). At weaning, the differences in LW between treatments L-EP and H-EP were not significant ($P > 0.05$). From MP onwards, EP diets had no residual effects on BCS or on BCS change ($P > 0.05$).

Increasing plane of nutrition between days 40 and 90 resulted in increases in average ewe LW at day 90 ($P < 0.01$) and in LW change for that period ($P < 0.001$). There were no effects of MP diets on BCS ($P > 0.05$). MP nutrition had no significant effects on ewe LW or BCS at either lambing or weaning ($P > 0.05$).

Foetal and placental development. Foetuses carried by dams offered diet L-EP in EP had smaller cranial ($P < 0.01$) and abdominal ($P < 0.05$) diameters on day 53 of gestation compared with H-EP ewes, with M-EP ewes intermediate (Table 3). These differences were not present on day 63 or day 78 ($P > 0.05$). EP nutrition had no effects on cotyledon diameter ($P > 0.05$). MP diets affected foetal development with foetuses from ewes offered diet M-MP having bigger abdominal dimensions than foetuses from H-MP ewes on day 78 of gestation ($P < 0.05$).

Plasma progesterone concentrations. Plasma progesterone concentrations of ewes prior to mating (day 0) were close to zero for all animals (Table 4). Pregnant ewes offered diet

Table 2 *Effect of plane of nutrition of 1-year-old ewes in early and mid-pregnancy on live weight (LW), body condition score (BCS) and on LW and BCS change throughout the study*

	Early pregnancy nutrition					Mid-pregnancy nutrition			
	L	M	H	s.e.d.	Significance	M	H	s.e.d.	Significance[†]
LW (kg)									
Day 0	44.4	43.4	45.1	1.060		44.2	44.4	0.870	
Day 39	42.5^{a}	46.2^{b}	50.3^{c}	0.489	***	46.5	46.2	0.399	
Day 95	50.5^{a}	53.2^{b}	54.7^{c}	0.718	***	51.9	53.7	0.590	**
Day 138	64.5^{a}	67.3^{b}	66.1^{ab}	1.374	*	66.0	66.0	1.084	
Lambing	54.4^{a}	56.1^{ab}	59.5^{b}	2.56	*	56.5	56.8	1.73	
Weaning	53.9	54.4	58.6	3.58		56.0	55.2	2.36	
LW change (g/day)									
Day 0 to 39	-88^{a}	16^{b}	115^{c}	13.5	***	19	10	11.0	
Day 39 to 95	135^{b}	120^{ab}	100^{a}	13.2	***	96	140	13.2	***
BCS									
Day 0	3.70	3.71	3.72	0.022		3.70	3.72	0.019	
Day 39	3.73^{a}	3.78^{a}	3.84^{b}	0.035	**	3.79	3.78	0.029	
Day 95	3.70	3.74	3.78	0.037		3.73	3.75	0.030	
Day 138	3.71	3.69	3.77	0.050		3.70	3.75	0.040	
Lambing	3.62	3.10	3.45	0.267		3.24	3.61	0.181	
Weaning	3.38	2.96	3.50	0.314		3.15	3.41	0.204	
BCS change period									
Day 0 to 39	0.02^{a}	0.03^{a}	0.11^{b}	0.042	**	0.04	0.06	0.034	
Day 39 to 95	−0.06	−0.05	−0.13	0.068		−0.10	−0.06	0.056	

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b,c]Within a row, means without a common superscript letter differ ($P < 0.05$).
*Significant at the 5% level, **significant at the 1% level, ***significant at the 0.1% level.
[†]There were no statistical interactions between plane of nutrition in early and mid-pregnancy.

Table 3 *Effect of plane of nutrition of 1-year-old ewes in early and mid-pregnancy on plasma progesterone concentrations, foetal growth and placental development*

	Early pregnancy nutrition					Mid-pregnancy nutrition			
	L	M	H	s.e.d.	Significance	M	H	s.e.d.	Significance
Cranial diameter (mm)									
Day 53	15.3^{a}	16.3^{ab}	17.1^{b}	0.564	**	16.3	16.1	0.464	
Day 63	22.0	21.7	22.3	0.435		21.9	22.1	0.353	
Day 78	31.5	31.3	30.1	0.895		31.0	31.0	0.720	
Abdominal diameter (mm)									
Day 53	20.8^{a}	22.2^{ab}	22.9^{b}	0.785	*	21.9	22.1	0.651	
Day 63	29.1	29.5	30.1	0.738		29.4	29.8	0.604	
Day 78	43.8	43.9	45.4	1.417		45.7	43.0	1.138	*
Mean cotyledon diameter (mm)									
Day 53	21.5	21.0	20.4	1.575		21.9	20.0	1.288	
Day 63	25.6	24.0	24.0	1.172		25.0	24.1	0.944	
Day 78	36.4	35.1	35.3	0.874		36.2	35.0	0.712	

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
*Significant at the 5% level, **significant at the 1% level.

H-EP in EP had lower plasma progesterone concentrations than L-EP and M-EP ewes on day 42 ($P < 0.01$). There were no differences in plasma progesterone concentrations throughout gestation between ewes offered either of the MP diets ($P > 0.05$).

Ewe reproductive performance and parturition data. The incidence of abortion and of mortality in ewes was not affected by plane of nutrition in EP with average values of 2% and 9%, respectively ($P > 0.05$). There were no significant differences ($P > 0.05$) in conception rates between EP

Table 4 *Effect of plane of nutrition of 1-year-old ewes in early and mid-pregnancy on conception rate, length of gestation and lamb output per ewe mated and lambed*

	Early pregnancy nutrition					Mid-pregnancy nutrition†		
	L	M	H	s.e.d.	Significance	M	H	s.e.d.
Progesterone (ng/ml)								
Day 0	0.06	0.10	0.06	0.049		0.09	0.06	0.040
Day 42	6.18[b]	7.16[b]	3.38[a]	1.055	**	5.01	6.14	0.872
Day 83	13.97	14.42	10.03	3.735		13.8	11.9	3.068
Conception rate (%)	61	51	38	–		54	45	–
Odds ratio	1	0.7	0.4	–		1	0.7	–
95% CI		0.2–1.8	0.1–1.1	–			0.3–1.5	–
Gestation length (day)	146.2	147.0	147.0	0.766		146.6	146.9	0.615
Per ewe mated								
Lambs born (*n*)	0.80	0.71	0.44	0.174	$P = 0.06$	0.74	0.55	0.142
Litter born (kg)	3.26	2.99	2.15	0.775		3.16	2.45	0.633
Lambs weaned (*n*)	0.35	0.38	0.25	0.140		0.37	0.28	0.115
Litter weaned (kg)	9.92	11.9	8.48	5.088		11.5	8.66	4.155
per ewe lambed								
Lambs born (*n*)	1.35	1.54	1.32	0.171		1.50	1.30	0.136
Litter born (kg)	5.91	6.50	6.57	0.641		6.63	6.02	0.527
Lambs weaned (*n*)	0.67	0.92	0.86	0.213		0.87	0.77	0.171
Litter weaned (kg)	19.8	29.9	29.5	7.964		28.2	24.6	6.410

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
†There was no statistical significance between treatments.

treatments (Table 4). The number of lambs born on a per ewe mated basis tended ($P = 0.06$) to be lowest for ewes offered diet H-EP compared with ewes offered diets L-EP or M-EP. Plane of nutrition in EP had no significant effects on either the number or weight of lambs weaned per ewe ($P > 0.05$).

Plane of nutrition in MP had no effects on conception rate, number of lambs born per ewe mated, lamb output (number and weight of lambs weaned) per ewe, ewe mortality or abortion incidence ($P > 0.05$).

Lamb viability and performance. The proportion of normal birth presentations was not affected by EP treatments ($P > 0.05$; Table 5). However, lambs born from ewes offered diet H-EP required more assistance at lambing than lambs born from ewes offered diets L-EP or M-EP ($P < 0.01$). In addition, H-EP lambs tended to be heavier at birth ($P = 0.07$) than L-EP and M-EP lambs. Lamb growth rate from birth to weaning was not significantly affected by plane of nutrition in EP ($P < 0.05$). The incidence of lamb mortality at 6 weeks of age tended to be greater for lambs born from dams offered diet L-EP ($P = 0.07$).

MP nutrition had no significant effects ($P > 0.05$) on birth presentation, birth weight, serum metabolites at birth, skeletal size, lamb growth rate or on mortality, although there was a tendency for ewes offered diet H-MP to require more assistance ($P = 0.06$) at lambing than ewes offered diet M-MP.

Two-year-old ewes

Live weight and body condition score. Increasing the plane of nutrition from day 0 to day 39 resulted in increases in LW ($P < 0.001$) and in LW change ($P < 0.001$) during the treatment period (H > M > L; Table 6). Despite ewes offered diet L-EP gaining more weight than M-EP and H-EP ewes ($P < 0.001$), these differences were sustained during MP ($P < 0.001$). Ewes offered diet M-EP tended to be lighter than L-EP and H-EP ewes at lambing ($P = 0.06$), a trend that was sustained to weaning ($P < 0.05$).

Increasing the plane of nutrition in MP resulted in increases in average LW at day 90 ($P < 0.001$), BCS on day 90 ($P < 0.01$) and on LW ($P < 0.001$) and BCS ($P < 0.01$) change during the treatment period. At lambing, H-MP ewes were heavier ($P < 0.01$) than M-MP ewes, although there were no significant differences between treatments at weaning ($P > 0.05$).

Foetal and placental development. Foetuses carried by ewes offered diet H-EP had bigger cranial diameters than foetuses from ewes offered diet L-EP or M-EP on day 80 ($P < 0.01$) of gestation (Table 7). There were no differences between EP treatments in abdominal diameters ($P > 0.05$). Ewes offered diet L-EP had smaller cotyledon diameters on day 68 than ewes on diet M-EP, with H-EP ewes intermediate ($P < 0.05$). Dietary treatments during MP had no effects on foetal or placental development ($P > 0.05$).

Plasma progesterone concentrations. Plasma progesterone concentrations of ewes prior to mating (day 0) were close to zero for all animals (Table 8). Progesterone concentrations on day 42 of gestation were affected by plane of nutrition during EP, with pregnant ewes offered diet H-EP having lower

Table 5 *Effect of plane of nutrition of 1-year-old ewes in early and mid-pregnancy on lambing ease, skeletal size, concentrations of serum metabolites, live weighs and lamb mortality rates to weaning*

	Early pregnancy nutrition					Mid-pregnancy nutrition			
	L	M	H	s.e.d.	Significance	M	H	s.e.d.	Significance
Normal birth presentation (%)	71	74	46	–		74	59	–	
Odds ratio	1	1.4	0.4	–		1	0.5	–	
95% CI		0.3 to 6.4	0.1 to 2.2	–			0.1 to 1.6	–	
Assistance at lambing (%)	48	52	87	–	*	50	68	–	$P = 0.06$
Odds ratio	1	1.1	8.1	–		1	2.7	–	
95% CI		0.3 to 3.7	1.3 to 95.2	–			0.8 to 9.5	–	
Skeletal size at birth (cm)									
Head length	11.1	11.2	11.6	0.336		11.4	11.2	0.276	
Crown rump	45.1	45.7	47.3	1.179		46.8	45.2	0.968	
Thoracic circumference	36.5	37.8	38.4	1.232		37.9	37.2	1.010	
Fore limb	31.8	31.3	31.7	0.679		32.0	31.2	0.557	
Hind limb	35.0	35.0	35.5	0.938		35.5	34.8	0.770	
Serum metabolites at birth									
ZST units	38.4	33.9	35.7	6.576		35.4	36.7	5.227	
Free T_3 (pmol/l)	19.3	17.6	15.3	2.081		17.1	17.7	1.700	
T_4 (nmol/l)	178.9	176.1	158.6	15.68		168.4	173.9	12.16	
Live weight									
Birth (kg)	4.49	4.64	5.25	0.319	$P = 0.07$	4.87	4.71	0.261	
Weaning (kg)	32.6	33.3	34.0	1.537		33.0	33.6	1.242	
Growth rate to weaning (g/day)	239	240	248	13.42		242	243	11.68	
Lamb mortality (%) –									
At birth	14	7	6	–		7	12		
Odds ratio	1	0.4	0.4	–		1	1.8		
95% CI		0.0 to 3.4	0.0 to 4.6	–			0.3 to 14.1		
At 6 weeks	43	21	17	–	$P = 0.07$	29	27		
Odds ratio	1	0.4	0.2	–		1	0.9		
95% CI		0.1 to 1.3	0.0 to 1.2	–			0.3 to 3.0		
At weaning	43	25	22	–		29	33		
Odds ratio	1	0.4	0.4	–		1	1.2		
95% CI		0.1 to 1.5	0.1 to 1.6	–			0.4 to 3.9		

s.e.d. = standard error of difference; ZST = zinc sulphate turbidity.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
*Significant at the 5% level.

plasma progesterone concentrations than ewes on diet M-EP, with L-EP ewes intermediate ($P < 0.001$). MP treatments had an effect on plasma progesterone concentration on day 42 with ewes offered diet M-MP having lower progesterone concentrations than H-MP ewes ($P < 0.05$).

Ewe reproductive performance and parturition data. Plane of nutrition in EP had no significant effect ($P > 0.05$) on conception rate (Table 8). The number of lambs born per ewe mated tended to be highest ($P = 0.07$) in ewes offered diet L-EP compared with ewes offered diets M-EP or H-EP. Gestation length and litter size per ewe lambed were not affected by EP diets ($P > 0.05$). There were no abortions recorded during the study for 2-year-old ewes. Mortality rates for the ewes were not affected by diets ($P > 0.05$) and had a mean value of 3%. EP nutrition had no effect on either the number or weight of lambs weaned per ewe ($P > 0.05$). Plane of nutrition in MP had no effects on any of the previous parameters ($P > 0.05$).

Lamb viability and performance. There were no differences between treatments ($P > 0.05$) in the proportion of abnormal birth presentations, lambing assistance required or lamb serum concentration of immunoglobulins, free T_3 or T_4 at birth, in either EP or MP (Table 9). Lambs born from ewes offered diet H-EP were heavier ($P < 0.01$) at birth and had longer crown-rump lengths ($P < 0.05$) than L-EP lambs. These lambs also tended to have higher growth rates than L-EP lambs ($P < 0.09$). Lamb mortality rates up to weaning were not affected by treatments ($P > 0.05$).

Maternal and neonatal behaviour
Plane of nutrition in EP affected the frequency and duration of the negative maternal behaviour 'ewe withdraws from lamb' with ewes offered diet L-EP performing this behaviour more frequently ($P < 0.05$) and for longer periods of time ($P = 0.05$) than ewes on diet M-EP or H-EP (Table 10). Lamb neonatal behaviours were not affected by plane of nutrition during EP ($P > 0.05$).

Table 6 *Effect of plane of nutrition of 2-year-old ewes in early and mid-pregnancy on live weight (LW), body condition score (BCS) and on LW and BCS change throughout the study*

	Early pregnancy nutrition					Mid-pregnancy nutrition			
	L	M	H	s.e.d.	Significance	M	H	s.e.d.	Significance
LW (kg)									
Day 0	66.2	62.9	66.5	2.008		64.1	66.3	1.621	
Day 39	56.4[a]	61.1[b]	65.8[c]	0.630	***	60.2	62.1	0.978	
Day 95	59.6[a]	62.3[b]	65.5[c]	0.954	***	60.2	64.7	0.769	***
Day 138	80.3	77.3	82.6	2.422		79.6	80.5	1.691	
Lambing	68.6	65.0	71.0	2.249	$P = 0.06$	65.5	70.9	1.801	**
Weaning	74.2[b]	66.0[a]	74.4[b]	2.917	*	69.6	73.5	2.352	
LW change (g/day)									
Day 0 to 39	−126[a]	−13[b]	100[c]	16.1	***	−32	7	11.6	
Day 39 to 95	70[b]	22[a]	8[a]	15.0	***	6	61	9.9	***
BCS change period									
Day 0 to 39	0.03	0.05	0.10	0.066		0.07	0.05	0.048	
Day 39 to 95	−0.04	−0.02	−0.12	0.075		−0.12	0.00	0.050	**
BCS									
Day 0	3.77	3.79	3.78	0.034		3.77	3.79	0.027	
Day 39	3.83	3.87	3.92	0.052		3.88	3.86	0.042	
Day 95	3.82	3.82	3.80	0.052		3.75	3.87	0.042	**
Day 138	3.70	3.69	3.80	0.046		3.71	3.75	0.037	
Lambing	3.24	3.15	3.24	0.250		3.06	3.36	0.200	
Weaning	3.61	3.66	3.66	0.154		3.62	3.67	0.122	

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b,c]Within a row, means without a common superscript letter differ ($P < 0.05$).
*Significant at the 5% level, **significant at the 1% level, ***significant at the 0.1% level.

Table 7 *Effect of plane of nutrition of 2-year-old ewes in early and mid-pregnancy on plasma progesterone concentrations, foetal growth and placental development*

	Early pregnancy nutrition					Mid-pregnancy nutrition			
	L	M	H	s.e.d.	Significance	M	H	s.e.d.	Significance
Cranial diameter (mm)									
Day 53	14.9	15.2	15.7	0.439		15.1	15.4	0.383	
Day 68	18.8	19.7	18.8	0.458		19.0	19.3	0.368	
Day 80	22.8[a]	22.6[a]	25.9[b]	0.946	**	23.5	23.3	0.765	
Abdominal diameter (mm)									
Day 53	19.2	18.8	19.3	0.850		19.0	19.2	0.692	
Day 68	22.7	22.4	22.6	0.718		22.4	22.8	0.578	
Day 80	36.5	35.7	36.1	1.235		35.7	36.5	1.010	
Mean cotyledon diameter (mm)									
Day 53	19.6	20.3	20.3	1.427		19.1	21.1	1.139	
Day 68	20.8[a]	23.1[b]	22.9[ab]	1.079	*	22.0	22.5	0.869	
Day 80	32.0	32.4	32.1	1.172		32.2	32.1	0.940	

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
*Significant at the 5% level, **significant at the 0.1% level.

Plane of nutrition in MP affected the duration of the maternal behaviour 'grooms lamb(s)', with ewes offered diet M-MP spending more time performing this behaviour than H-MP ewes ($P < 0.05$). This was concomitant with these ewes (M-MP) sniffing/nosing their lamb less frequently ($P < 0.01$) and for shorter periods of time ($P < 0.05$), while the behaviour 'facilitates suckle' was shown more frequently ($P < 0.05$) and for longer periods of time ($P < 0.05$). With regard to lamb neonatal behaviour, lambs born from ewes offered diet M-MP tended to show the behaviour 'to udder' more frequently ($P = 0.10$) than H-MP lambs. Accordingly, these lambs showed a higher frequency ($P < 0.05$) and duration ($P = 0.08$) of the

Table 8 *Effect of plane of nutrition of 2-year-old ewes in early and mid-pregnancy on conception rate, length of gestation and lamb output*

	Early pregnancy nutrition					Mid-pregnancy nutrition		
	L	M	H	s.e.d.	Significance	M	H	s.e.d.
Progesterone (ng/ml)								
Day 0	0.04	0.03	0.01	0.026		0.03	0.03	0.022
Day 42	8.24[b]	11.22[c]	5.33[a]	1.417	***	9.16	7.37	1.126
Day 83	21.2	17.4	16.7	2.706		19.1	17.7	1.982
Conception rate (%)	94	80	71	–		81	81	–
Odds ratio	1	0.3	0.2	–		1	0.9	–
95% CI		0–4.2	0–1.9	–			0.2–4.8	–
Gestation length (days) per ewe mated	146.0	146.1	146.5	1.000		146.7	145.7	0.797
Lambs born (*n*)	1.91	1.69	1.23	0.344	$P = 0.07$	1.59	1.63	0.280
Litter born (kg)	8.67	8.19	6.78	1.515		7.84	7.93	1.230
Lambs weaned (*n*)	1.43	1.39	1.18	0.316		1.23	1.44	0.257
Litter weaned (kg)	54.0	54.4	46.6	9.484		49.3	54.1	7.625
Per ewe lambed								
Lambs born (*n*)	2.17	1.96	1.86	0.308		1.96	2.03	0.227
Litter born (kg)	9.19	9.81	9.55	1.008		9.33	9.70	0.800
Lambs weaned (*n*)	1.50	1.66	1.67	0.290		1.47	1.75	0.231
Litter weaned (kg)	50.0	61.1	60.1	8.572		52.6	61.5	6.799

s.e.d. = standard error of difference.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b,c]Within a row, means without a common superscript letter differ ($P < 0.05$).
***Significant at the 0.1% level.

behaviour 'successful suckle' compared with H-MP lambs. There were no differences between treatment groups in the latency to perform the behaviours by the lambs ($P > 0.05$).

Discussion

Early pregnancy nutrition

In the present study, there was a downward trend, which approached significance, in the number of lambs born per ewe mated as the plane of nutrition increased, in both 1- and 2-year-old ewes. This was concomitant with a non-significant but potentially important effect of EP diets on conception rate and with a smaller non-significant downward trend for number of lambs born per ewe lambing. The mechanism behind the negative relationship between post-mating feed intake and lamb output per ewe may be via peripheral plasma progesterone concentrations. Parr *et al*. (1993) proposed that increased food intake is related to increased metabolic clearance rates of progesterone, due to increased blood flow to the gut and liver. Progesterone has a major role in embryo survival and maintenance of pregnancy, through the control of maternal secretion of nutrients, growth factors and enzymes required for embryo development (reviewed by Ashworth, 1995). In this study, a closer examination of plasma progesterone concentrations at the end of the EP period showed a positive association with conception rate ($P < 0.001$) and with the number of lambs born per ewe lambed ($P < 0.05$), for both 1- and 2-year-old ewes (associations between progesterone and reproductive parameters were tested by regression analysis taking account of dam age). This gives an indication that lower progesterone concentrations (found in treatment H-EP in both age groups) have the potential to affect embryo mortality in the early stages of gestation. Indeed, Parr *et al*. (1987) reported that pregnancy rates of ewes fed a high ration post-mating were reduced compared with ewes fed medium or low rations. However, when exogenous progesterone was given, pregnancy rates significantly increased in ewes fed the high ration, with no effect in medium- or low-ration ewes.

Previous work has indicated that plane of nutrition affects conception rates in primiparous but not multiparous ewes (Annett and Carson, 2006). This is the first study to indicate that sensitivity to plane of nutrition in EP may also extend to 2-year-old biparous ewes. Annett (2004) reported that plasma progesterone levels of primiparous ewes are significantly lower than that of multiparous ewes and that for both age groups there is an inverse relationship between plane of nutrition and peripheral progesterone concentrations. However, this has not been related to decreased conception rates in the multiparous ewes (Annett and Carson, 2006; Muñoz *et al*., 2008a). A closer examination of the data in the present study showed that plasma progesterone levels of 1- and 2-year-old ewes at day 42 differed (average of 5.4 and 8.2 ng/ml, respectively; s.e.d. 0.709, $P < 0.001$). Nevertheless, the increased sensitivity to lower peripheral progesterone concentrations shown by young 1- and 2-year-old ewes may be related to the overall mean level of the hormone. Considering the previously mentioned study of Muñoz *et al*. (2008a), where progesterone concentrations for adult ewes averaged 7.5, 6.4 and 5.7 ng/ml for the three nutritional treatments, L-EP, M-EP and H-EP, respectively, and the results from the present study, in particular average progesterone levels for the H-EP

Table 9 *Effect of plane of nutrition of 2-year-old ewes in early and mid-pregnancy on lambing ease, skeletal size, concentrations of serum metabolites, live weighs and lamb mortality rates to weaning*

	Early pregnancy nutrition					Mid-pregnancy nutrition†		
	L	M	H	s.e.d.	Significance	M	H	s.e.d.
Assistance at lambing (%)	29	19	39	–		34	23	–
Odds ratio	1	0.6	1.6	–		1	0.5	–
95% CI		0.1 to 2.9	0.4 to 6.9	–			0.1 to 1.7	–
Normal birth presentation (%)	82	90	72			91	74	
Odds ratio	1	1.7	0.6			1	0.4	
95% CI		0.2 to 20.8	0.1 to 3.4				0.1 to 2.2	
Skeletal size at birth (cm)								
Head length	10.8	10.5	10.4	0.209		10.5	10.6	0.163
Crown rump	47.5[a]	47.6[a]	49.5[b]	0.903	*	47.8	48.6	0.702
Thoracic circumference	37.5	37.9	38.2	0.695		37.7	38.0	0.541
Fore limb	29.4	29.0	29.5	0.559		29.9	28.8	0.449
Hind limb	34.3	33.6	35.1	0.594		34.6	34.0	0.404
Serum metabolites at birth								
ZST units	46.3	47.8	44.4	5.932		44.1	48.2	4.628
Free T_3 (pmol/l)	19.1	20.3	21.0	2.221		21.3	18.9	1.740
T_4 (nmol/l)	161.9	179.7	180.8	16.04		172.9	175.4	12.56
Live weight								
Birth (kg)	4.77[a]	5.06[ab]	5.40[b]	0.199	**	5.04	5.11	0.154
Weaning (kg)	33.5	37.2	36.6	1.787		35.0	36.6	1.412
Growth rate to weaning (g/day)	248	280	275	15.39	$P = 0.09$	262	274	11.91
Lamb mortality (%)								
At birth	7	0	8			0	11	
Odds ratio	1	–	1.0			1	–	
95% CI		–	0.1 to 16.3				–	
At 6 weeks	25	19	8			17	18	
Odds ratio	1	0.7	0.3			1	1.1	
95% CI		0.1 to 3.2	0.0 to 1.7				0.3 to 4.6	
At weaning	32	24	16			28	21	
Odds ratio	1	0.6	0.4			1	0.7	
95% CI		0.1 to 2.6	0.1 to 1.8			–	0.2 to 2.3	

s.e.d. = standard error of difference; ZST = zinc sulphate turbidity.
Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
*Significant at the 5% level, **significant at the 1% level.
†There was no statistical significance between treatments.

treatments in 1- and 2-year-old ewes (3.4 and 5.3 ng/ml, respectively), there seems to be an indication that there is a minimum progesterone concentration necessary for embryo survival, and that while overfeeding mature ewes depresses progesterone concentration, the level of the hormone remains high compared to younger ewes. Parr *et al*. (1987) proposed a progesterone threshold for satisfactory conception of 2 ng/ml on day 12 of gestation of mature ewes, although the highest conception rates were noted for progesterone levels of 4–5 ng/ml.

The current study is the first to document the effects of EP nutrition on foetal size at various stages of pregnancy and subsequent lamb viability in 1- and 2-year-old ewes. This demonstrates that even in the early stages of embryonic life, when direct nutrient requirements for conceptus growth are negligible (Robinson *et al*., 1999) maternal nutrient intake still exerts direct influences. The present study indicates the long-term nature of foetal growth retardations associated with low planes of nutrition in EP in 1- and 2-year-old ewes, leading to reductions in foetal size measured in MP and continuing through to reductions in lamb birth weight. In contrast, when mature ewes were offered a low plane of nutrition in EP, the foetal growth retardation effects manifested at day 57 of gestation were no longer evident at day 68, showing capacity for compensatory growth (Muñoz *et al*., 2008a). These differences may occur due to a higher sensitivity or to a reduced ability of immature sheep to compensate for nutritional restriction or surplus in a period of specific demand. Nutritional influences during MP that affect placental development and consequently foetal growth rate are well documented (Kelly, 1992, Heasman *et al*., 1999; Redmer *et al*., 2004). EP mechanisms have been less studied. In very early stages of embryo development, maternal nutrition (Walker *et al*., 1996) and hormone levels (Kleemann *et al*., 1994) have been reported to influence the allocation of cells between

Table 10 *Effect of plane of nutrition of 1-year-old ewes in early (EP) and mid-pregnancy (MP) on the frequency and duration of maternal and neonatal behaviour at parturition*

	Frequency per 30 min				Duration (% of observation time)			
	L	M	H	Significance	L	M	H	Significance
EP effects on maternal behaviour								
Withdrawing								
Mean estimates ± e.s.e.	−0.1 ± 0.13	−0.4 ± 0.24	−2.1 ± 0.13	*	−0.3 ± 0.53	−1.6 ± 1.00	−8.9 ± 0.53	$P = 0.05$
Probability	0.53	0.87	1.00		0.58	0.87	1.00	
Expected mean	3	1.5	0.3		1.77	0.92	0.10	
MP effects on maternal behaviour								
Maternal behaviours								
Grooming								
Mean estimates ± e.s.e.	–	0.8 ± 0.11	0.6 ± 0.13		–	8.2 ± 0.86	4.8 ± 0.99	*
Probability†		0.03	0.1			0.01	0.08	
Expected mean‡		27.3	16.8			78.9	36.3	
Sniffing/nosing								
Mean estimates ± e.s.e.	–	−0.0 ± 0.11	0.4 ± 0.10	**	–	0.0 ± 0.35	1.1 ± 0.33	*
Probability		0.52	0.13			0.50	0.17	
Expected mean		3.6	9			1.28	2.84	
Facilitates suckling								
Mean estimates ± e.s.e.	–	−0.6 ± 0.45	−4.4 ± 0.45	*	–	−3.3 ± 2.58	−25 ± 2.58	*
Probability		0.79	1			0.79	1.0	
Expected mean		8.4	0.6			9.04	0.79	
MP effects on neonatal behaviour								
To udder								
Mean estimate ± e.s.e.	–	−0.1 ± 0.19	−0.5 ± 0.27	$P = 0.10$	–	−0.5 ± 0.92	−1.9 ± 1.24	
Probability		0.53	0.78			0.56	0.72	
Expected mean		13.8	8.1			9.36	6.76	
Successful suckle								
Mean estimate ± e.s.e.	–	−0.2 ± 0.18	−0.7 ± 0.33	*	–	−1.4 ± 0.99	−3.7 ± 1.81	$P = 0.08$
Probability		0.69	0.94			0.71	0.93	
Expected mean		4.2	2.1			4.27	2.31	

Nutrition provided: low (L), medium (M) or high (H) as compared to predicted metabolizable energy requirements for maintenance.
Mean estimates and estimated standard errors (e.s.e.) are on the latent scale; *significant at the 5% level, **significant at 1% level.
Probability: probability of not presenting the behaviour; expected mean: expected duration if all ewes present the behaviour.

the inner cell mass (foetus) and the outer cell mass (placenta). Alterations in cell allocation at this stage change the trajectory of foetal growth, with a fast growth trajectory increasing the foetal demand for nutrients (reviewed by Godfrey and Robinson, 1998).

In the present study, 1-year-old ewes offered high-plane diets in EP gave birth to heavier lambs, which required more assistance at lambing. This is the first study to report the effect of EP and MP nutrition on the incidence of dystocia. There is a well-established link between birth weight and the incidence of dystocia in ewes (Grommers *et al*., 1985; Dwyer *et al*., 1996). However, dystocia is not related simply to birth weight. Grommers *et al*. (1985) identified birth presentation as the most significant factor influencing the incidence of parturition difficulty. Normal birth presentations in the current study most certainly influenced the amount of assistance required at lambing, but were not directly affected by plane of nutrition in EP. Also, pelvic dimension of the ewe may also have contributed to the incidence of dystocia (McSporran and Wyburn, 1979), as the differences found in primiparous ewes were not encountered in biparous ewes. However, although long and stressful deliveries have been associated with neonatal death in lambs (Dwyer *et al*., 1996), in the present study, a high plane of nutrition was not associated with increased lamb mortality at birth or at 1 week of age.

In the present study, low-plane diets in EP were associated with a higher frequency and duration of primiparous ewes withdrawing from their lamb. These findings correspond with those of Dwyer *et al*. (2003) who found that primiparous 2-year-old ewes submitted to a moderate nutritional restriction (35%) throughout the whole of pregnancy showed deficits (reduction in total grooming time) in maternal care in comparison to ewes with adequate levels of feeding. To our knowledge, this is the first study to quantify the effects of differential planes of nutrition in EP and MP periods on the expression of maternal and neonatal behaviours at parturition in 1-year-old ewes.

One-year-old ewes offered low-plane diets in early gestation tended to have greater incidences of lamb mortality at 6 weeks of age. Reduced lamb survival following maternal undernutrition during pregnancy has been previously described (Vincent *et al*., 1985; Holst *et al*., 1986; Hinch *et al*., 1996) and it may be related to different factors in the present study. For example, lambs born to ewes offered low-plane diets in EP had the lowest birth weights and therefore could potentially have impaired thermo-regulatory ability (Clarke *et al.*, 1997). From a maternal point of view, primiparous ewes in the present study were lighter during pregnancy and at lambing, as a result of EP treatments. There is a positive correlation between LW of dams in MP and lamb mortality to weaning, associated with heavier ewes having heavier placentas and consequently heavier birth weights (Kelly, 1992). Lighter ewes could also have their milk production potential impaired due to a reduction in udder weight and mammary development, and consequently, reduced colostrum and milk yield (Mellor, 1987). Additionally, there was a small indication of poorer maternal behaviour in ewes on low-plane diets, which potentially could play a role in the reduced survivability of these lambs, as lamb survival is crucially dependent on the adequate expression of maternal care from the ewe (Nowak *et al.*, 2000).

Mid-pregnancy nutrition
In the present study, despite 1- and 2-year-old ewes responding to nutritional regimes imposed during MP, the birth weights of their offspring were not affected. For 2-year-old ewes, this is not surprising as in several studies where maternal nutrient restrictions were imposed between early and mid-gestation and in mid- to late gestation, lamb birth weights at or near term were unaffected (for review see Redmer *et al.*, 2004). However, in 1-year-old ewes, there was a downward trend in foetal size as plane of nutrition in MP increased, although there were no effects on birth weights. It is interesting to compare these findings with the over-nourished adolescent ewe model of Wallace *et al.* (2006) who reported reduced foetal growth and birth weight due to impaired placental development. In the present study there were no significant differences on placental development between MP treatments (no differences in cotyledon diameter), which is consistent with the lack of effect on birth weight at term. The differences between studies may most likely be explained by the level of the moderate and high-plane treatments given to the ewes in each trial. The diets offered in our study provided maternal growth rates of ∼100 and ∼150 g/day during the MP period for the medium- and high-plane diets, respectively, compared with the ∼55 and ∼300 g/day gained by the ewes from Wallace's study (1999). Nevertheless, the planes of nutrition used in the current study are more appropriate to the range likely to be applicable in commercial production systems.

One-year-old ewes offered moderate plane diets in MP were associated with improved maternal behaviour at parturition, with ewes spending more time grooming their lambs and being more receptive to the lamb suckling than ewes offered high-plane diets. Furthermore, ewes offered high-plane diets showed more negative behaviour than moderate plane ewes. These findings could be related with these ewes requiring more assistance with lamb delivery. Ewe grooming behaviour is affected by birth difficulty, with an increase in the amount of intervention required to deliver a lamb resulting in an increase in the time taken for the ewes to start to groom the lamb (Dwyer *et al.*, 2003). Additionally, lambs born from ewes offered high-plane diets had poor offspring behaviour. Lamb neonatal behaviour in this case could be a response to poor maternal behaviour and consequently poor ewe–lamb bonding formation (Nowak, 1996). However, these data are consistent with results described by Muñoz *et al.* (2008a) for adult ewes, who found a positive effect of medium-plane diets on offspring behaviour at parturition, although no differences in maternal behaviour were observed. These differences in maternal behaviour could be explained by parity, which has been reported to affect the expression of maternal behaviour at parturition with adolescent ewes displaying more inappropriate movements when lambs attempt to suckle than mature ewes (O'Connor *et al.*, 1992).

Conclusion

Altering the plane of nutrition in EP, where treatments were more severe than in MP, had greater effects on ewe reproduction and offspring performance. In 1-year-old ewes, a low plane of nutrition in EP was associated with higher prolificacy, although dams were lighter at parturition and presented poor maternal behaviour. Their offspring were lighter at birth and had higher incidences of mortality at 6 weeks. Similar findings were recorded in 2-year-old ewes. A medium plane of nutrition in 1-year-old ewes in MP was associated with larger foetuses in mid-gestation, and improved maternal and lamb behaviour at parturition.

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