

# *Chapter 4* (Experimental)

## **PROTEIN SOURCES FED PREPARTUM AND THEIR EFFECT ON MAMMARY GLAND DEVELOPMENT AND MILK PRODUCTION OF EWES**

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### **4.1 INTRODUCTION**

During the final period of pregnancy, there is a marked change in the endocrine status of the female to accommodate parturition and lactogenesis. These changes influence tissue metabolism and nutrient utilisation. A reduction in feed intake occurs during the prepartum period, at which nutrient demands for support of conceptus growth and initiation of milk synthesis are increasing. Both changes in endocrine status (i.e. prolactin, insulin, growth hormone) and decreases in DMI during late gestation influence metabolism and lead to mobilisation of fat from adipose tissue and glycogen from the liver (e.g. Bell 1995; Grummer 1995).

Supplementing the grazing dairy animal during the prepartum period will provide additional energy and protein to support production without compromising rumen function and within a framework of maintaining moderate body condition loss and optimal reproductive performance. Studying the metabolism of the gravid uterus and individual foetuses has shown some interesting relationships between maternal and foetal metabolism. Sahlu *et al.* (1992) have observed increased prepartum body weight gains with increased prepartum crude protein intake but weight was not influenced by increasing energy intake (Sahlu *et al.* 1995). These observations indicate that protein, not

energy, is the first limiting nutrient for animals during late pregnancy which may have implication for the postpartum period.

Many studies have suggested the need to either increase dietary CP, undegradable protein content, or both during prepartum period to support high production in early lactation (e.g. Clark and Davis 1980; Hook *et al.* 1989; Van Saun *et al.* 1993). Little information is available comparing the influence of feeding degradable and undegradable protein sources during the prepartum period on postpartum production. Therefore, the objectives of this study were to determine whether increased amounts of protein fed prepartum would improve lactation performance in the early postpartum period and whether different sources of crude protein would alter performance of the lactating ewe.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 EXPERIMENTAL ANIMALS**

The experiment was conducted at the University of New England, Armidale, New South Wales, during March to September 1995. One hundred three cross bred ewes (Border Leicester × Merino × Dorset Horn) aged 3-4 years were used. They were oestrus synchronised by using intravaginal progestagen (medroxyprogesterone acetate, MPA). Sponges were inserted on 21st of March and removed after 12 days. All ewes were joined with 10 raddled (Willard *et al.* 1995) crossbred rams. Those returning to oestrus at the following cycle were recorded by a raddle colour change.

In early June 1995, all ewes at an average 110 days of pregnancy were scanned to identify litter size. Sixty pregnant ewes with an average weight of 52 kg ( $\pm$  0.9) were selected based on the results of ultrasound scanning. An equal number of ewes carrying twin and single lambs were randomly allocated into 4 treatment groups.

## 4.2.2 TREATMENTS

Feeding treatments were used from an average 120 days of gestation until expected day of lambing. Treatment groups were grazed through a series of one hectare paddocks. The four treatment groups ( $n = 15$ ) were grazed as separate flocks and paddocks assigned to each group were similar in terms of pasture an offer. Drought conditions occurred during the experimental period and there was far less pasture growth than normal. As a consequence all quantities of lucerne hay ( $200 \text{ g.h}^{-1}.\text{d}^{-1}$ ) were fed to each treatment group when the pasture availability dropped below  $1000 \text{ kg DM/ha}$ .

The four treatments are described below.

Treatment 1 = Control group fed no protein supplement

Treatment 2 = Lupin (*L. angustifolius*) ( $200 \text{ g.h}^{-1}.\text{d}^{-1}$ ) supplement

Treatment 3 = Cottonseed meal (Millmaster feeds, Tamworth, NSW) ( $165 \text{ g.h}^{-1}.\text{d}^{-1}$ ) supplement

Treatment 4 = Formaldehyde treated sunflower meal (Norpro, Norco Co-op. Pty. Ltd., Lismore, NSW) ( $180 \text{ g.h}^{-1}.\text{d}^{-1}$ ) supplement

Treatments 2-4 all provided the equivalent of  $60 \text{g CP.h}^{-1}.\text{d}^{-1}$ .

The levels of feeding in Treatment 2, 3 and 4 were designed to provide  $60 \text{g CP.h}^{-1}.\text{d}^{-1}$ . The supplements were fed each day in long troughs, providing adequate feeding space for each ewe. from day 120 of gestation until all ewes had lambed. After lambing, ewes from all groups were combined together and grazed as a single flock. All ewes grazed pasture and were given supplements of lucerne hay (approximately  $200 \text{ g.h}^{-1}.\text{d}^{-1}$ ) and lupins ( $60 \text{g CP.h}^{-1}.\text{d}^{-1}$ ) during the lactation period of 30 days.

## 4.2.3 MEASUREMENTS MADE DURING THE EXPERIMENT

### 4.2.3.1 Protein degradability measured using nylon bags

The digestibility of three protein supplements (lupins, cottonseed meal, formaldehyde treated sunflower meal) was determined using the nylon bag technique described by Perdok (1987).

Six bags containing three protein supplements were incubated in the rumen of fistulated sheep given a basal diet of oaten chaff, providing a similar low quality roughage to the pasture on offer. Nylon bags with a marble inside were weighed and filled with approximately 3 g of one of the protein supplements and weighed. The tops of the bags were tied with nylon fishing line, then suspended in the rumen of sheep. Nylon bags were removed from the rumen after 24 h incubation and washed thoroughly under a water tap for about 10 min. All bags were dried at 70°C for 24 h in oven, cooled and weighed.

The residues from the bags were finely ground before analysis of N content conducted using the nitrogen analyser (LECC) (%N  $\times$  6.38). The N digestibility is expressed as the percent disappearance of the protein supplement during the 24 hour period of incubation in rumen. The N disappearance of protein supplement was calculated as follows:

$$\text{N disappearance of sample} = [(A-B)/A] \times 100\% \dots \dots \dots (1)$$

where  $A = [(\text{Bag} + \text{Marble} + \text{Sample}) - (\text{Bag} + \text{Marble})] \times \% \text{DM content} \times \% \text{N content of sample before incubation in the rumen}$

$B = [(\text{Bag} + \text{Marble} + \text{Sample}) - (\text{Bag} + \text{Marble})] \times \% \text{N content of sample after incubation in the rumen and drying the sample in the oven}$

### **4.2.3.2 Ewe body condition score and liveweight**

Ewe body condition in each group was scored at the beginning of the experiment (day 80 of gestation; 6th June) and again at the first and fourth week after lambing. The body condition was scored on a scale from 0 (thin) to 5 (fat) (Jeffries 1961).

Changes in body condition score were calculated for “prepartum” and “postpartum” periods. Change in body condition during prepartum was calculated as the difference between the condition score at the first week after lambing and the condition score at the commencement of supplementation. The condition score change during postpartum was also calculated.

Liveweight of the ewes was first recorded at day 80 of gestation and this was used as an “initial weight”. Pre- and postpartum live weights were recorded weekly from approximately day 120 of gestation (1st to 21st August, 1995) to the end of lactation period (4th-25th September, 1996), respectively.

### **4.2.3.3 Mammary gland development**

The volume of the mammary gland was measured for each ewe on 3 consecutive weeks before expected lambing using 2 methods as follows:

#### **4.2.3.3.1 Linear dimensions**

The udder growth was monitored by measuring the circumference of the base of the udder at the same time as the volume measurements were taken. The mammary gland volume was calculated by multiplying the width, length and depth of the gland.

#### 4.2.3.3.2 Displacement of water

During the measurement ewes were held in a standing position. A plastic container was used, since it could be easily bent to an appropriate shape for the base of the udder. The container was filled until the udder was completely submerged and the residual water subtracted from the initial known volume to give the volume of the udder.

#### 4.2.3.4 Dry matter intake

On the day 120 of gestation (1st August 1995), 10 ewes from each treatment groups were dosed with a controlled-release Cr<sub>2</sub>O<sub>3</sub> capsule (Captec, Nufarm, Auckland, New Zealand). The capsule was fitted snugly in the applicator, and placed to the back of the tongue until the animal swallowed. Technical information supplied by the manufacture indicated that between day 8 and 25 after administration of the capsule, faecal concentration of Cr<sub>2</sub>O<sub>3</sub> could be used for determination of faecal excretion.

Eight days after capsule dosing faecal samples were taken, once per week for 3 consecutive weeks. Faecal samples were stored frozen (-20°C) and later oven dried at 55°C for 5 to 6 d. To facilitate mixing and to obtain a representative sample, faecal samples were ground in through a 1 mm screen.

The concentration of Cr<sub>2</sub>O<sub>3</sub> in the faeces samples was determined by using Atomic Absorption Spectrophotometer (Perkin-Elmer 360). The ground faeces was weighted and ashed at 600°C for 4 h. The ash was digested to make up to the concentration of marker free faeces. Faecal output from the Cr<sub>2</sub>O<sub>3</sub> marker was calculated as follows (Smith and Reid 1955):

$$\text{Faecal output (g/d)} = \frac{\text{Cr}_2\text{O}_3 \text{ consumed (g/d)}}{\text{Cr}_2\text{O}_3 \text{ concentration in faeces (g/g DM)}} \dots \dots \dots (2)$$

The manufacturer's specified rate of release of Chromium sesquioxide for the batch of capsules used in this study was 193 mg Cr/day. The result of faecal output and

digestibility of dry matter (96, 74, 58, 70 and 57% for lupin, CSM, Norpro, Lucerne hay and pasture, respectively) allowed estimation of dry matter intake to be calculated (Burns *et al.* 1992):

$$\text{Dry matter intake (g/d)} = \frac{\text{Faecal output (g/d)}}{[1 - \text{Digestibility of dry matter}] \dots \dots \dots (3)}$$

#### 4.2.3.5 Supplement intake

Lithium chloride (22 mg Li/ kgBW) was used to measure intake of the protein supplement intake in the present study. The preparation of lithium-protein sources was done by spraying lithium chloride as an aqueous solution using a hand pump while the feed was mixed in a thin layer in a smooth cement surface. The feed was then dried at 25-30°C for 48-72 h. Feed was turned daily to increase drying efficiency and to prevent pellet aggregation.

The labelled supplements were given to the ewes in the paddocks on 31st July and 21st August 1995. Blood samples (10 ml; sodium heparin vacutainer tubes) were taken by venipuncture from these sheep 18-24 h after the presentation of food coated with lithium chloride. Blood tubes were centrifuged at 3000 rpm for 10 min, and plasma was removed and immediately frozen at -20°C until analysis.

Plasma lithium concentrations were determined using atomic absorption spectrophotometer (Perkin-Elmer 360). To measure the concentration of lithium in plasma, the plasma samples were deproteinised by adding 0.5 ml of 30% W/V sulphosalicylic acid to 5 ml of plasma which, after being mixed vigorously, was centrifuged at 3000 rpm for 10 min. The supernatant fraction was decanted and stored at 4°C and analysed for plasma lithium concentration the following day.

Estimated supplement intake was calculated from the assumptions suggested by Suharyono (1992). To calculate supplement intake, the following information is required: the liveweight of each animal (W; kg), the concentration of Li in blood plasma ([Li];

mg/l) obtained from a single blood sample, the total amount of supplement consumed by all animals in the experimental group (S; g).

Li<sup>+</sup> is known to move into and out of cells (*see* review Suharyono 1992). For convenience, it is assumed that, at the time of blood sampling, the volume of pool in which Li is distributed in the body is probably greater than the extracellular fluid but less than total body water volume. Then, it can be assumed that Li has moved to a hypothetical pool with an effective volume (V; l). If animals are in approximately similar body condition, it is assumed that V is a constant fraction (F) of the liveweight in each animal

$$V \text{ (l)} = W \text{ (kg)} \times F \dots\dots\dots(4)$$

Therefore, the total mass of Li<sub>i</sub> (m<sub>i</sub>; mg) in any individual animal (i), at the time of blood sampling, is given by:

$$\begin{aligned} m_i &= [Li]_i \times V \\ &= [Li]_i \times W_i \times F \dots\dots\dots(5) \end{aligned}$$

The total amount of Li present in the whole flock at the time of blood sample (M; mg) is:

$$M = \Sigma (m_i) \text{ (for } i \text{ individuals in the group)} \dots\dots\dots(6)$$

The fraction (m<sub>i</sub>/M) is then assumed to indicate the fraction of total pellet intake (S) consumed by the i<sup>th</sup> individual in the flock. The fraction (m<sub>i</sub>/M) is independent of the value of F. Thus, the intake of supplement by the i<sup>th</sup> animal (s; g) is as follows:

$$s = S \times m_i / M \dots\dots\dots(7)$$



#### **4.2.3.6 Lamb weight and growth rates**

All ewes were observed daily from the time the first ewe started lambing. Newborn lambs were weighed, tagged, and identified with their mothers. Lambs were weighed weekly postpartum. The average daily gain was calculated by subtraction of the birth weight from later weights (4 weeks old). Lambs which died during the experimental period were recorded and their mothers were removed from the experiment. At the end of the experiment all lambs were weighted, marked and vaccinated.

#### **4.2.3.7 Yield of milk and milk composition**

From each treatment group of ten ewes, which included five bearing single lambs and five bearing twin lambs, were chosen for milking. Milking commenced 1 week after lambing to ensure that ewes lamb bonds had been established. Machine milking continued weekly for 4 weeks.

For milking, the dams were separated from their lambs and were placed in a milking cradle and machine milked by the oxytocin technique (McCance 1959). Initially, a dose of oxytocin (Intervet Oxytocin-S, 10 i.u./ ml) was injected into the jugular vein, and the udder was emptied by milking machine (ALFA-LAVAL VUP-70- Bucket Milker).

A second dose of oxytocin was given at an approximately 4 h after the initial milking, and then the udder was emptied again. Milk was collected directly into the cylinder and the volume was recorded. Milk yield was calculated by conversion the milk volume produced over the 4 h period into a 24 h yield.

At each milking a volume of 20 ml of milk was collected and stored at -20°C until analysed for measurement of milk protein and fat content. Milk protein was measured by using a nitrogen analyser (LECC FP-228 Organic Nitrogen Determinator; using the Dumas Method) (%N x 6.38), and milk fat by FOSS-LET (A/S N Foss Electric, Denmark).

#### 4.2.4 STATISTICAL ANALYSIS

Individual ewes were used as the experimental unit to test the hypothesis that feeding the various protein supplements enhanced mammary gland growth and milk production. Data were analysed by using REG (Gilmour 1990) a generalised linear model program. The model used to analyse ewes body weight change, body condition score, mammary gland growth, dry matter intake, supplement intake, lamb birth weight and growth rate, and milk yield and composition was:

$$Y_{ijk} = \mu + Tr_i + LS_j + TL_{ij} + C_v + E_{ijk}$$

where	$Y_{ijk}$	=	dependent variable for animals
	$\mu$	=	population mean
	$Tr_i$	=	treatment effect
	$LS_j$	=	litter size effect
	$TL_{ij}$	=	treatment and litter size interaction
	$C_v$	=	covariate effect
	$E_{ijk}$	=	error

Initial liveweight of ewes (day 80 of gestation) was used as a covariate for analysis of effects of treatment and litter size on total feed intake. All non significant interactions were removed from subsequent models. Repeated measures (AOV) were used to examine the impact of time on weight and milk yield. Least Significant Difference was used to compare the difference between treatments when tests from analysis of variance were significant (Steel and Torrie 1984).

## 4.3 RESULTS

### 4.3.1 PROTEIN DEGRADABILITY OF SUPPLEMENTS

Rumen degradable protein (RDP) was estimated on the basis of N disappearance ( $\%N \times 6.25$ ) during 24 hour and the undegraded dietary protein (UDP) was calculated as the difference. The composition of protein supplements are shown in Table 4.1.

**Table 4.1:** Chemical composition of lupins, cottonseed meal and formaldehyde treated sunflower meal (Norpro) consumed by ewes during the experimental period.

	Lupins	CSM	Norpro
Proximate composition % (DM basis)			
Crude protein ( $N \times 6.25$ )	29	36	33
<sup>1</sup> RDP	96	75	40
<sup>2</sup> UDP	4	25	60
Crude fibre	15	11	16
Urea	-	2	-
Dry Matter	92	91	91

<sup>1</sup>  $\%N$  disappearance  $\times 6.25$

<sup>2</sup>  $100 - (\%N \text{ disappearance} \times 6.25)$

The manufacturer provided information on values for crude fat and metabolizable energy of cottonseed meal and Norpro were 2% and 11 MJ/kgDM for both of them. The value of crude fat and ME for lupins was assumed to be 6% and 12.5 MJ/kgDM, respectively (Simmons *et al.* 1990).

### 4.3.2. EWE BODY CONDITION SCORE

Mean ewe body condition scores are presented in Table 4.2. No significant effects of treatments or litter size on body condition were found.

**Table 4.2:** Body condition scores ( $\pm$  SE) for ewes on various prepartum protein supplements.

Measurements	Treatment				Average
	Control	Lupin	CSM	Norpro	
n	11	10	9	10	40
Initial condition score	3 $\pm$ (0.6)	3.15 (0.16)	2.88 (0.17)	3.26 (0.17)	3.07 (0.08)
Lambing condition score	1.09 (0.09)	1.30 (0.93)	1.33 (0.10)	1.0 (0.09)	1.18 (0.05)
Lactation condition score	1 (0)	1 (0)	1 (0)	1 (0)	1 (0)
Prepartum BCS* change	-1.91 <sup>ab</sup> (0.14)	-1.85 <sup>a</sup> (0.14)	-1.55 <sup>a</sup> (0.15)	-2.26 <sup>b</sup> (0.14)	-1.89 (0.07)
Postpartum BCS* change	-0.09 <sup>ab</sup> (0.09)	-0.30 <sup>a</sup> (0.09)	-0.33 <sup>a</sup> (0.10)	-0.003 <sup>o</sup> (0.09)	-0.18 (0.05)

a and b in the same row differ significantly ( $P < 0.05$ )

\* Body condition score

Initial body condition scores were similar for animals on the 4 treatment diets with an average of 3.07. Ewes in all treatments lost body condition throughout the periods of pregnancy and lactation and at the end of the experiment all ewes were the same body condition score of 1.

Prepartum body condition score changes were subsequently affected by feeding treatments and the change in condition score of ewes in the Norpro group differed from the other protein feeding groups ( $P < 0.05$ ). The differences between the protein supplements and the control group were not significant ( $P > 0.05$ ).

There was a significant effect of feeding treatments on the condition score change during postpartum period. The Norpro ewes had the smaller reduction in ( $P < 0.05$ ) body condition compared with the other protein supplement groups. There was no significant difference in body condition change between control group and other treatment groups.

### 4.3.3. EWE LIVEWEIGHT PRE- AND POSTPARTUM

Both pre- and postpartum liveweight was similar for sheep in the groups fed supplements, but the ewes supplemented with protein tended ( $P < 0.10$ ) to be heavier than those on the control diet (Table 4.3). The range of liveweight was 61.2-65.9 kg and 48.6-52.2 kg for pre- and post lambing liveweight, respectively.

There was no significant difference between treatments for ewe liveweight during lactation. The range in liveweight of the ewes was 46.5-50.7 kg.

**Table 4.3:** Liveweight and weight changes (Mean  $\pm$  SE) for ewes on various prepartum protein supplements.

Measurements	Treatment				Average
	Control	Lupin	CSM	Norpro	
n	11	10	9	10	40
Initial weight, kg	51.5 $\pm$ (1.7)	52.5 (1.7)	51.2 (1.8)	53.2 (1.7)	52.1 (0.9)
Prepartum weight, kg	61.2 (1.7)	64.9 (1.8)	65.9 (1.9)	65.8 (1.8)	64.4 (0.9)
Postpartum weight, kg	48.6 (1.5)	52.1 (1.5)	50.1 (1.6)	52.2 (1.54)	50.7 (0.8)
Lactation weight, kg	47.2 (1.4)	50.7 (1.4)	46.5 (1.5)	48.4 (1.4)	48.2 (0.7)
Prepartum weight change, g/d	275.4 <sup>a</sup> (21.5)	354.6 <sup>ab</sup> (22.5)	422.6 <sup>b</sup> (23.8)	357.9 <sup>ab</sup> (22.6)	352.6 (11.3)
Lactation weight change, g/d	-67.5 <sup>a</sup> (25.7)	-64.8 <sup>a</sup> (26.9)	-168.2 <sup>b</sup> (28.5)	-193.2 <sup>b</sup> (27.4)	-123.4 (13.6)

a and b in the same row differ significantly ( $P < 0.05$ )

A significant difference was found between feeding treatments for the average daily weight change ( $P < 0.01$ ). Ewes fed the CSM gained more weight prepartum than the controls ( $P < 0.05$ ).

Change in liveweight during lactation differed between feeding treatments ( $P < 0.01$ ). Ewes which consumed control or lupin diets lost less weight than those on CSM or Norpro diets. There were no significant differences in the weight changes between the control and lupin groups, or between CSM and Norpro groups.

A significant interaction between feeding treatment and litter size ( $P < 0.05$ ) occurred for weight change in lactation (Table 4.4). Ewes on the control and lupin treatment rearing twin lambs lost less weight than those rearing the singles. However, the significant differences in weight loss were found only for ewes previously consuming lupins ( $P < 0.05$ ). Differences between litter size was not significant for the cottonseed meal nor the Norpro groups.

**Table 4.4:** Effects of supplements and litter size interaction on live weight change (g/d) during the lactation phase (Mean  $\pm$  SE)

Treatments	Single	Twin
Control	-85.7 $\pm$ (38) <sup>a</sup>	-49.2 (34.7) <sup>a</sup>
Lupin	-106.7 (38) <sup>a</sup>	-22.9 (38) <sup>b</sup>
CSM	-129.8 (42.5) <sup>a</sup>	-206.7 (38) <sup>a</sup>
Norpro	-136.5 (34.7) <sup>a</sup>	-250 (42.5) <sup>a</sup>
Overall Mean	-114.7 (19.2)	-132.2 (19.2)

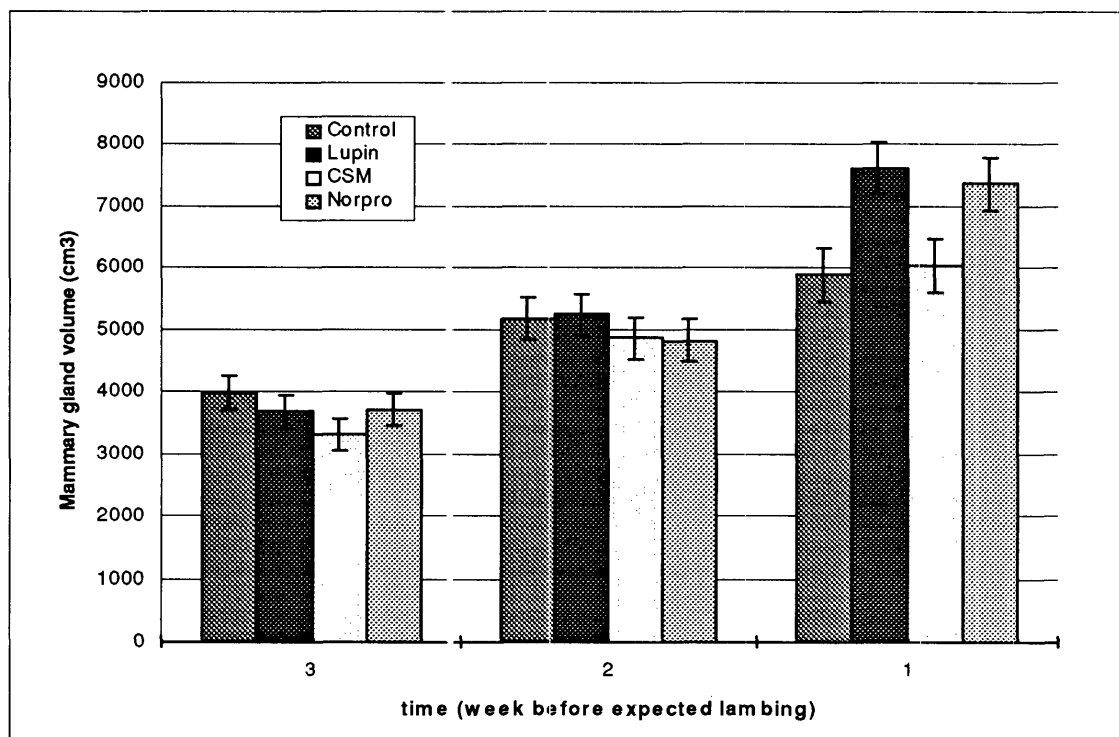
a and b in the same row differ significantly ( $P < 0.05$ )

## 4.3.4 MAMMARY GLAND DEVELOPMENT

### 4.3.4.1 Linear dimension (cm<sup>3</sup>)

The mammary gland development of ewes, as measured by linear dimension for each treatment group is shown in Figure 4.1. The mammary gland volume of ewes in all treatments increased with increasing days of gestation. A significant increase in the mammary volume was found in the last week of pregnancy ( $P < 0.01$ ).

Mammary gland development was affected by feeding treatment ( $P < 0.01$ ). Ewes which were supplemented with lupins showed the greatest increase in volume of the mammary gland compared to the other groups ( $P < 0.01$ ). There was no significant difference in the mammary volume between ewes fed lupins and Norpro or between the control and CSM.



**Figure 4.1:** Mammary gland development of animals in 4 different treatments measured by linear dimension ( $\text{cm}^3$ ) method. (Error bars show SE).

**Table 4.5:** Mean ( $\pm$  SE) of mammary gland volume of ewes bearing single or twin lambs ( $\text{cm}^3$ ) measured by linear dimensions.

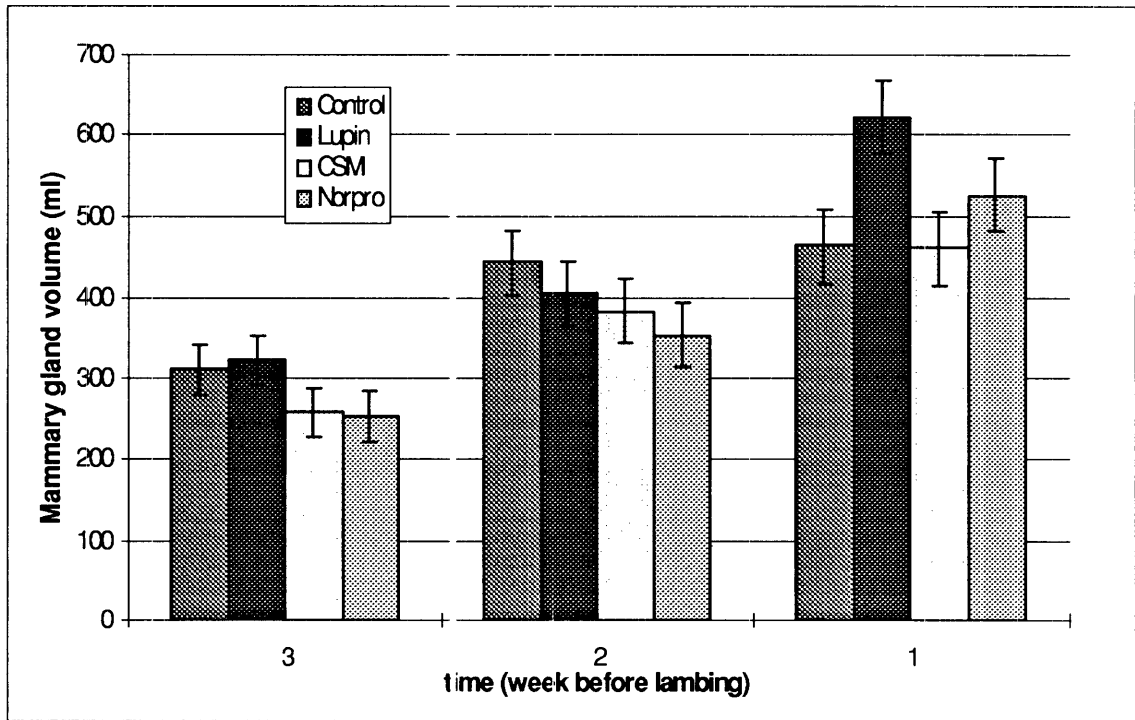
Period (week before lambing)	Single	Twin	Average
n	31	29	
3	3204 <sup>a</sup> (187.6)	4140 <sup>b</sup> (194)	3672 (134.8)
2	4377 <sup>a</sup> (252.4)	5687 <sup>b</sup> (240.3)	5032 (167)
1	6077 <sup>a</sup> (257.7)	7381 <sup>b</sup> (307.8)	6729 (213.9)

a and b in the same row differ significantly ( $P < 0.01$ )

Litter size had a significant effect ( $P < 0.01$ ) on mammary gland development during late gestation (Table 4.5). The mammary gland volume of ewes bearing twin lambs was greater ( $P < 0.01$ ) than those bearing singles. The mammary gland development was not influenced by an interaction between feeding treatment and litter size.

#### 4.3.4.2 Water displacement (ml)

With respect to the linear measurement of the mammary gland volume increased with advancing pregnancy, and the time effect was greatest at the final 3 weeks before lambing ( $P < 0.001$ ) (Figure 4.2).



**Figure 4.2:** Mammary gland development of animals in 4 different treatments measuring by water displacement method (ml). (Error bars show SE).

In the last week of pregnancy, supplementation had a significant effect on the change in the volume of the mammary gland ( $P < 0.05$ ) with ewes in the lupin group having the largest mammary gland volume ( $P < 0.05$ ) compared with the ewes fed CSM, Norpro and unsupplement groups. Differences between the mammary gland volume among ewes in the Norpro, CSM and control diets were not significant.



Litter size had a significant influence on the volume of the mammary gland throughout the periods of measurement (Table 4.6). The mammary volume of ewes having twin lambs was larger than those having the singles ( $P < 0.05$ ).

**Table 4.6:** Mean ( $\pm$ SE) of mammary gland development of ewes bearing single or twin (ml) measured by water displacement.

Period (week before lambing)	Single	Twin	Average
n	31	29	
3	247 <sup>a</sup> (21.8)	326 <sup>b</sup> (22.5)	286 (15.7)
2	320 <sup>A</sup> (27.3)	471 <sup>B</sup> (28.3)	396 (19.6)
1	464 <sup>c</sup> (31.7)	571 <sup>d</sup> (32.7)	518 (22.7)

A and B in the same row differ significantly ( $P < 0.01$ )

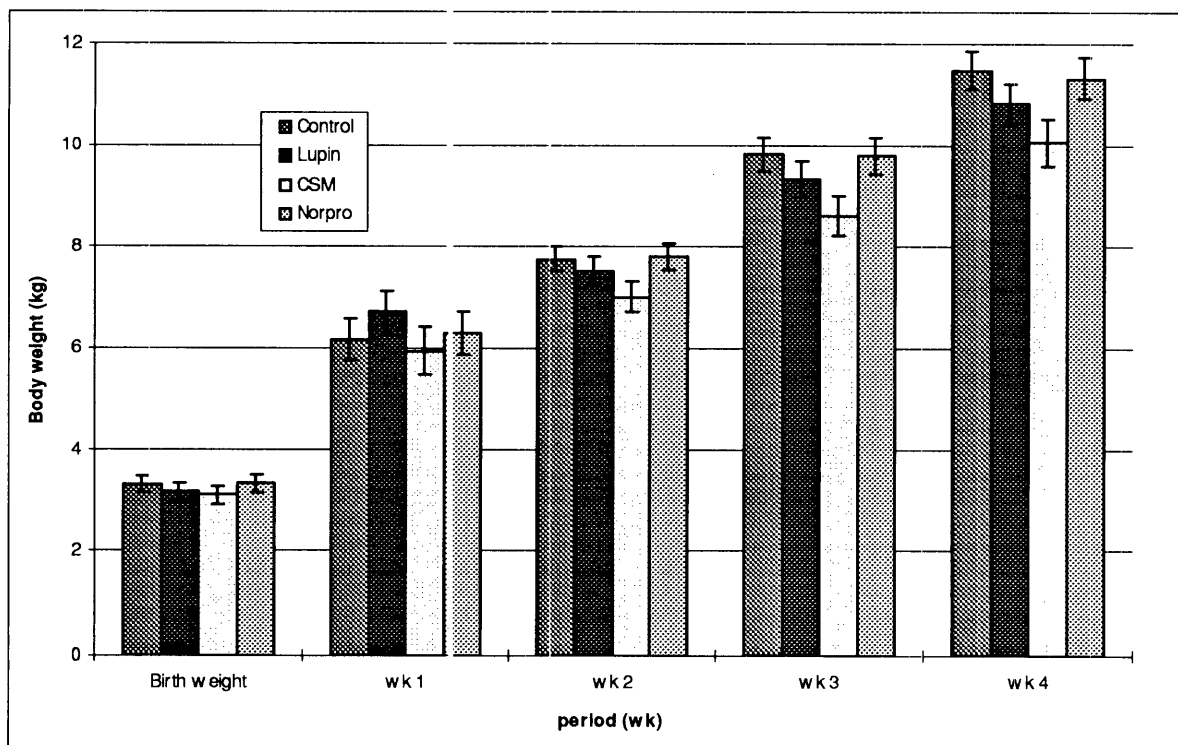
a, b, c and d in the same row differ significantly ( $P < 0.05$ )

#### 4.3.5. LAMB BIRTH WEIGHT AND GROWTH RATES

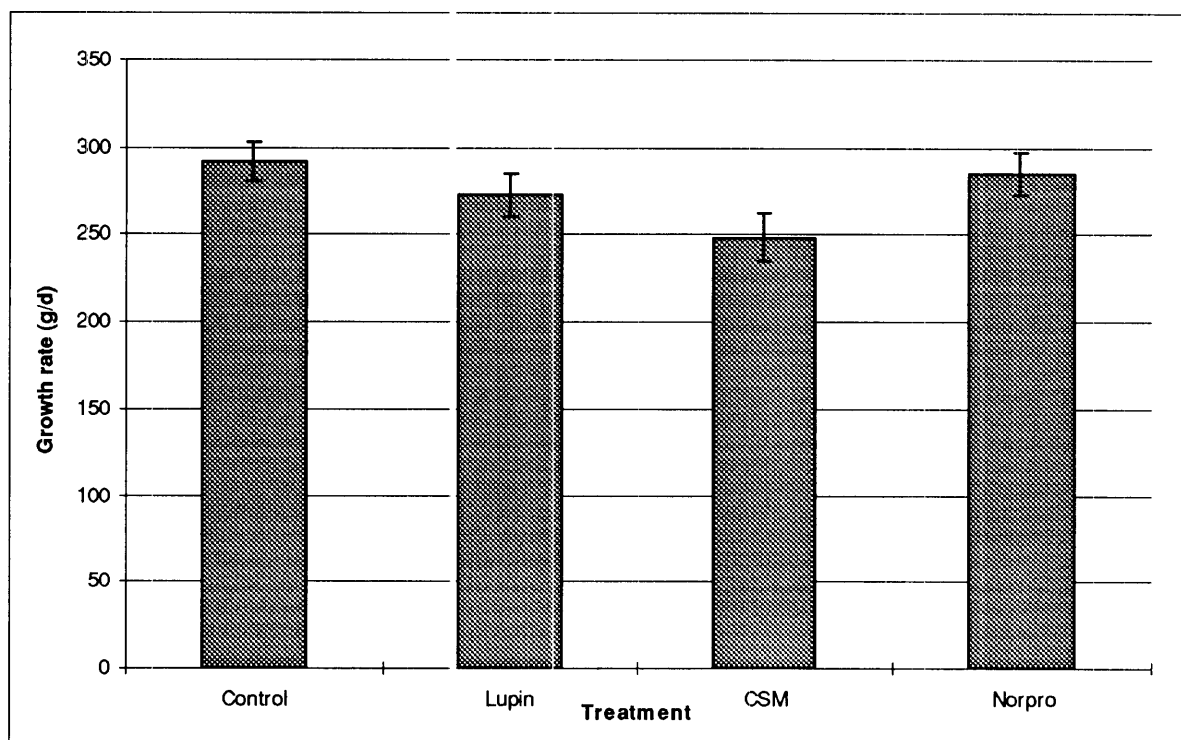
Lamb birth weight was not affected by supplementary feeding of ewes during the prepartum period (Table 4.7). Birth weight of lambs from ewes in the lupin and CSM groups were lighter than the other groups. Treatments and litter size interactions had no significant effect on the birth weight of lambs.

Neither treatments nor an interaction between treatment and litter size influenced body weight of lambs. The range of lamb liveweight gain from birth until 4 weeks of age was 6 to 11.5 kg. At the end of the experiment, lambs from ewes on the control diet were heavier (NS) than those from the groups fed protein supplemented with lambs from the CSM group being the lightest.

No significant difference was found between feeding treatments nor for the interaction between treatment and litter size (Figure 4.3) on lambs growth rate (Figure 4.4). However, lambs from the control ewes tended ( $P < 0.1$ ) to grow faster than those from ewes supplemented with protein.



**Figure 4.3:** Lamb birth weight and liveweight (kg) from ewes in 4 feeding treatments prepartum. (Error bars show SE).



**Figure 4.4:** Mean lamb growth rate during the experimental period (4 weeks) from each ewe treatment group (g/d). (Error bars show SE).

The influence of litter size on lamb birth weight was significant ( $P < 0.01$ ) (Table 4.7) with single lambs being heavier ( $P < 0.05$ ) than twins. Likewise liveweight of lambs was higher for the singles than the twins ( $P < 0.05$ ) at each week after birth with growth rates of lambs from birth to 4 weeks also significantly higher for single lambs ( $P < 0.05$ ).

**Table 4.7:** Lamb data (birth weight and weight during 4 weeks after birth) for lambs from ewes carrying single and twin lambs (Mean  $\pm$  SE).

Measurement	Single	Twin	Average
n	26	22	48
Birth weight, kg	3.5 $\pm$ (0.1) <sup>a</sup>	3.0 (0.1) <sup>b</sup>	3.2 (0.1)
Weight wk 1, kg	6.8 (0.1) <sup>A</sup>	5.7 (0.3) <sup>B</sup>	6.3 (0.2)
Weight wk 2, kg	8.2 (0.1) <sup>c</sup>	6.9 (0.2) <sup>d</sup>	7.5 (0.1)
Weight wk 3, kg	10.2 (0.2) <sup>c</sup>	8.6 (0.3) <sup>d</sup>	9.4 (0.2)
Weight wk 4, kg	12.0 (0.3) <sup>c</sup>	9.8 (0.3) <sup>d</sup>	10.9 (0.2)
Growth rate, g/d	305 (8.3) <sup>c</sup>	245 (9.2) <sup>d</sup>	275 (6.2)

A and B in the same row differ significantly ( $P < 0.05$ )

a and b in the same row differ significantly ( $P < 0.01$ )

c and d in the same row differ significantly ( $P < 0.001$ )

## 4.3.6 FEED INTAKES

### 4.3.6.1 Dry matter intakes

There was no effect of protein supplements, litter size or their interaction on total dry matter intake. Ewes supplemented with the protein sources had a higher total dry matter intake than the control group, but there was no significant difference between feeding treatments (Table 4.8).

After adjustment of intake on the basis of liveweight (52 kg) the difference in total dry matter intake was found to be different between supplement groups in the week before lambing ( $P < 0.05$ ). During this week the total dry matter intake of ewes supplemented with the CSM was higher than the other supplemented groups.

At the first week of measurement, all ewes bearing single lambs tended ( $P < 0.10$ ) to consume more food than those bearing twin lambs (1.8 vs. 1.5).

**Table 4.8:** Total dry matter (kg) after adjustment to the same liveweight of 52 kg and supplementary crude protein intake (g) of the animals during the experimental period (Mean  $\pm$  SE).

Measurements	Treatment			
	Control	Lupin	CSM	Norpro
<b>1. Total DM intake (kg/h/d)</b>				
period (week before lambing)				
n	12	12	13	12
3	1.6 $\pm$ (0.2)	1.5 (0.2)	1.6 (0.2)	1.9 (0.2)
2	1.7 (0.2)	1.7 (0.2)	1.7 (0.2)	1.7 (0.2)
1	1.4 (0.2)	1.4 (0.2)	1.8 (0.2)	1.3 (0.2)
Average (kg/h/d)	1.6 (0.1)	1.5 (0.1)	1.7 (0.1)	1.6 (0.1)
<b>2. Crude protein intake (g/h/d)</b>				
n	-	13	14	14
4	-	62.9 $\pm$ (9.8)	64.7 (9.2)	63.8 (9.2)
2	-	69.9 (9.4)	64 (8.9)	64.6 (8.9)

#### 4.3.6.2 Supplementary crude protein intakes

Mean crude protein intakes are shown in Table 4.8. All ewes fed protein supplements consumed similar amounts of crude protein during the experimental period.

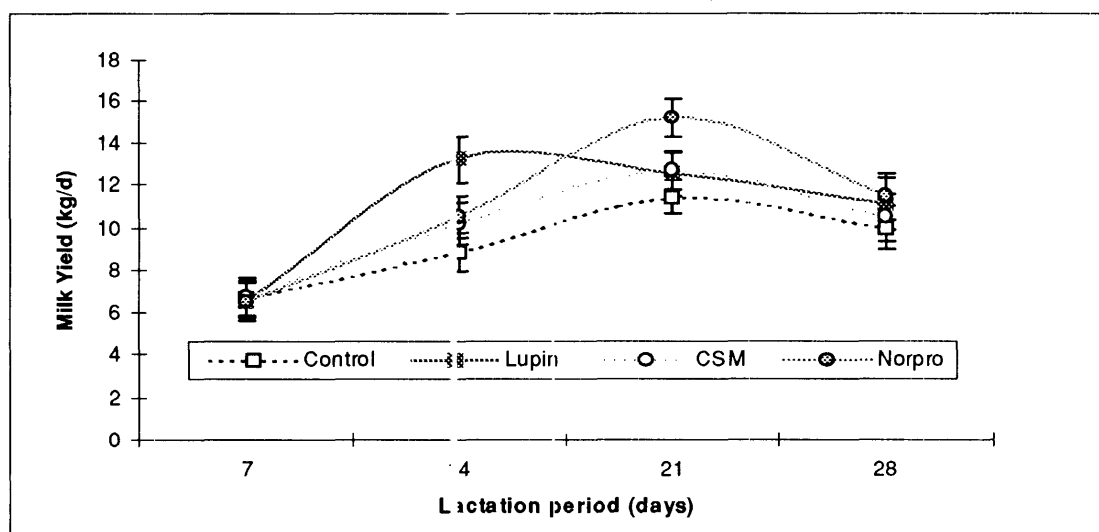
In the last month of pregnancy, crude protein intake was increased for the ewes carrying twin lambs, but was not significantly greater than those carrying single lambs ( $P < 0.10$ ).

### 4.3.7 MILK YIELD AND COMPOSITION

#### 4.3.7.1 Milk yield

Figure 4.5 shows the effect of prepartum feeding of various protein supplements on milk yield. Feeding protein supplements increased milk yield over the experimental period. Differences in yield of milk between feeding treatments were found in weeks 2 and 3 of lactation ( $P < 0.05$ ).

Milk yield from the ewes fed lupin reached an earlier peak in the second week of lactation ( $P < 0.05$ ) than other groups. Overall milk yield of ewes in the lupin treatment was significantly higher ( $P < 0.05$ ) than yield of the control, CSM and Norpro groups. Total estimated milk production over 4 weeks were 64.3, 60.7, 63.3 and 53.4 kg for ewes supplemented with lupins, Norpro, CSM and unsupplemented diet, respectively.



**Figure 4.5:** Mean milk yield (kg/d) for lactating ewes fed various protein supplements. (Error bars show SE).

During the third week of lactation, ewes from the control, CSM and Norpro groups achieved peak yield. There were significant differences between feeding treatments, with greater milk yield ( $P < 0.05$ ) from ewes supplemented with Norpro than ewes supplemented with either lupin or CSM, and ewes from the unsupplemented group.

Yield of milk from ewes rearing twin lambs was higher than those rearing singles throughout the lactation periods (Table 4.9). A significant increase in yield was found during the fourth week of lactation ( $P < 0.05$ ) being 1339 vs. 1740 grams for ewes rearing single lambs and twin lambs, respectively.

There was a significant interaction between treatment and litter size on milk yield ( $P < 0.05$ ). Significant differences between treatments and litter size in milk yield were measured during weeks 2 and 3 of lactation, when the peak yield had been reached.

**Table 4.9:** Average milk yield (g) during the 4 weeks of lactation for ewes rearing single and twin lambs (Mean  $\pm$  SE).

Treatment	Single	Twin
Control	2161 $\pm$ (169.5)	1654 (207.6)
Lupin	2067 (146.8)	2529 (293.5)
CSM	1769 (169.5)	2566 (239.7)
Norpro	2251 (156.9)	2267 (239.7)
Overall Mean	2062 (80.5)	2254 (123.5)

In the second week of lactation ewes suckling twin lambs from both lupin and the CSM treatments produced more milk than those suckling single lambs ( $P < 0.05$ ). The increases in milk yield of ewes rearing twin lambs supplemented with lupins and Norpro were found to be significant from those rearing singles in the third week of lactation ( $P < 0.05$ ).

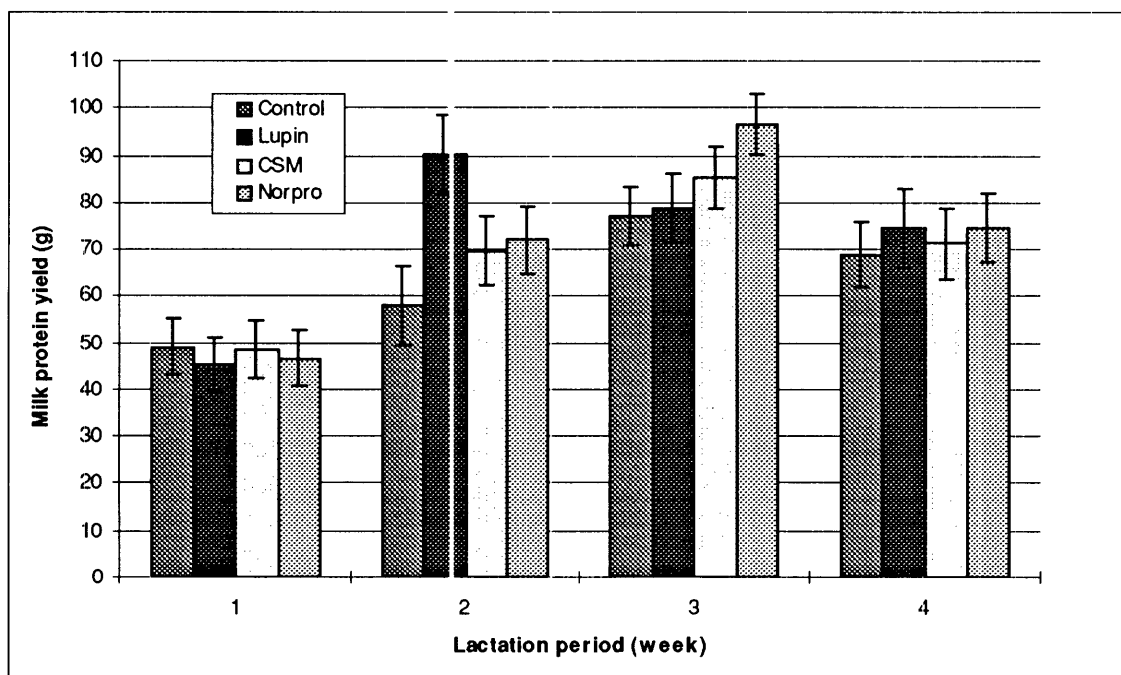
#### 4.3.7.2 Milk protein yield

Protein feeding treatment, litter size and an interaction between treatment and litter size did not influence milk protein percentage over the milking period. An estimated milk protein percentage was 5% and declined to 4% from the first to the fourth week of lactation.

Prepartum protein feeding increased milk protein yield compared to the unsupplemented feeding treatment (Figure 4.6). There were significant effects of treatment ( $P < 0.05$ ),

litter size ( $P<0.05$ ) and an interaction between treatment and litter size ( $P<0.05$ ) for protein yield at various stages of lactation.

The effect of prepartum feeding treatment altered protein yield during the second week of lactation. Among feeding treatments, ewes supplemented with lupins had the greatest protein yield whereas ewes from the unsupplemented treatment produced the least milk protein ( $P<0.05$ ). There were significant differences between control and Norpro ( $P<0.05$ ), but not between control and CSM treatment groups in terms of the protein yield.



**Figure 4.6:** Mean milk protein yield for ewes supplemented during prepartum period (g). (Error bars show SE).

The production of milk protein was increased by litter size (Table 4.10). During the first three weeks of lactation, there was no significant difference in the protein yield between ewes rearing single or twin lambs. However, a significantly greater protein yield was found in twin rearing ewes in the fourth week of lactation ( $P<0.05$ ) when the milk yield of all ewes was declining.

The interaction between treatment and litter size affected protein yield. A significant difference ( $P < 0.05$ ) was apparent at week 2 of lactation. Ewes from the lupin and CSM treatments rearing twin lambs had significantly greater protein yield ( $P < 0.05$ ) than ewes rearing singles. The following week, protein yield was greatest ( $P < 0.10$ ) in the Norpro ewes rearing twin lambs, but the difference was not apparent for those rearing singles.

**Table 4.10:** Effect of litter size on milk protein yield (g) during the 4 weeks of lactation (Mean  $\pm$  SE).

Lactation period	Single	Twin
n	19	20
week 1	46.4 $\pm$ (4.2) <sup>a</sup>	48.4 (4.1) <sup>a</sup>
week 2	67.9 (4.1) <sup>a</sup>	77.0 (6.2) <sup>a</sup>
week 3	81.5 (3.6) <sup>a</sup>	87.2 (5.6) <sup>a</sup>
week 4	62.9 (4.2) <sup>a</sup>	81.6 (6.4) <sup>b</sup>
Overall Mean	99.0 (3.8)	104.8 (5.9)

a and b in the same row differ significantly ( $P < 0.05$ )

#### 4.3.7.3 Milk fat yield

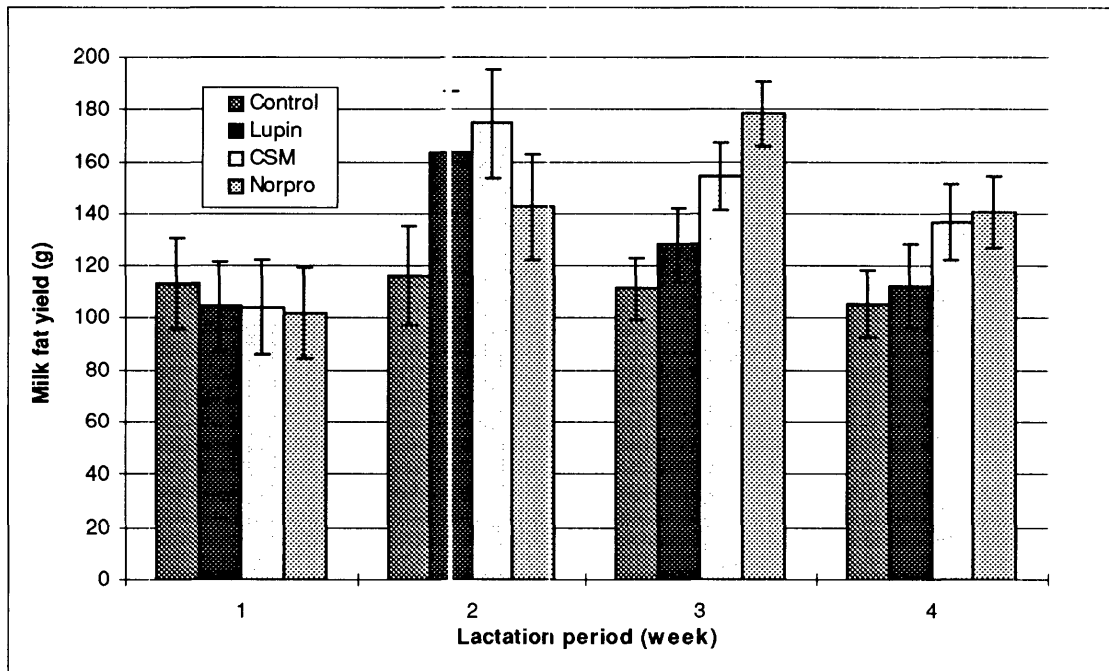
There was no effect of litter size or an interaction between treatment and litter size on milk fat percentage over the milking period. Overall fat percentage was greater for ewes given supplementary protein compared to the unsupplemented control group. A significant difference in fat % ( $P < 0.05$ ) was found in weeks 3 and 4 with ewes supplemented with CSM having the greatest milk fat percentage.

The mean fat yield for the various treatment groups are shown in Figure 4.7. A significant effect of treatment on fat yield was only present in the third week of lactation when the Norpro ewes produced a greater amount of fat ( $P < 0.01$ ) than the control and lupin treatments. Milk fat yield of all ewes declined in the 4th week.

Litter size influenced milk fat yield of ewes at all stages of lactation. Differences between ewes rearing single or twin lambs were only significant in the last week of lactation



( $P < 0.05$ ). Yield of fat was approximately 140 and 107 grams for ewes rearing twin and single lambs, respectively.



**Figure 4.7:** Mean ( $\pm$  SE) milk fat yield for ewes fed various diets (g). (Error bars show SE).

There was a significant interaction between feeding treatment and litter size on milk fat. During week 2 of lactation yield of milk fat increased ( $P < 0.05$ ) in twin rearing ewes supplemented with CSM compared to other treatment groups. In addition, fat yield was influenced by an interaction of factors during the third of week of lactation. Ewes suckling twin lambs from the Norpro treatment produced more fat than those suckling single lambs (206.9 vs. 149.9 grams) ( $P < 0.05$ ). Differences due to the other treatments were not found.

## 4.4 DISCUSSION

The main purpose of the present experiment was to examine the effect of prepartum feeding of protein supplements on mammary gland development and subsequent milk production in early lactation.

### **Milk production and composition**

Milk yield was consistently higher for ewes fed protein supplements compared to those fed no supplement. With a 17, 12 and 16% increase in total yield for ewes fed lupins, cottonseed meal and formaldehyde treated sunflower meal compared to unsupplemented animals. Increases in milk yield as a result of protein supplementation has been reported by other workers. For example, ewes fed lupins, as a protein source, produced significantly more milk than ewes consuming only low quality roughage (Kenney and Roberts 1984). Robinson *et al.* (1979) reported that daily milk yield improved 17% when additional crude protein from fish meal was added to the diet. Chew *et al.* (1984a) reported difference in total 200-d milk production for cows fed 100% of crude protein recommended by NRC (1978) when expressed as a percent of corresponding yield for the low 83% CP group, were 17% for milk and 16% for total milk solids. It would therefore appear to be a consistent finding that more milk is produced from ewes which have been well-fed (supplemented groups) compared with those subjected to a low plane of nutrition (control) in late pregnancy (e.g. Cowan *et al.* 1981b; Hook *et al.* 1989).

Milk production throughout the study is illustrated in Figure 4.5. The ewes on the Norpro diet had the highest peak yield compared to the other treatment groups (15 vs. 13, 12, 11 kg;  $P < 0.05$ ). Sahlu *et al.* (1995) reported that does on the higher CP diets had higher peak milk yield and that the peak occurred later than in unsupplemental animals. It appeared that the “carry over” effect from the plane of feeding in late pregnancy may be associated with the body weight and body condition of the ewes at the start of lactation (Louca *et al.* 1974). Earlier peak yield would probably affect the ability of the ewes to mobilise body reserves during early lactation in order to overcome inadequate intake.

According to Broster (1972), the importance of attaining high peak milk yields in the dairy cow is to maximise lactation performance since total lactation yield is approximately 200 times the peak milk yield. However, this pattern was not observed in the present study, since estimated total 28-d milk yield for all ewes offered protein supplement were similar (64, 60 and 63 kg for lupin, cottonseed meal and Norpro treatments respectively), and the lowest production for the unsupplemented treatment (53 kg).

The level of milk yield also appears to be determined by the number of foetuses carried in pregnancy or the number of lambs suckling. Stern *et al.* (1978) found an 11% increase in milk yield occurred in ewes carrying multiple foetuses compared to those which carried singles. The hormones produced by the placenta are one possible mechanism by which the number of foetuses can affect the potential of milk yield (Cowan *et al.* 1980; Orr and Treacher 1994). Furthermore, most experiments on the yield of ewes suckling different numbers of lambs have shown that ewes rearing multiple lambs have considerably higher yields than ewes under the same management but suckling singles (e.g. Treacher 1983; Snowden and Glimp 1991; Ramsey *et al.* 1994). The present study also observed a similar result in that milk production was higher for ewes rearing twin lambs compared with those with singles.

Milk composition, both fat and protein, were influenced by supplementation prepartum. Milk protein percentage was improved for ewes given the additional prepartum protein. However there were no differences in protein content. Similar result of a minimal increase in milk protein concentration due to protein supplementation have been reported in a number of experiments (e.g. Dhiman and Satter 1993). In general, the proportional increase in the amount of milk components (i.e. fat, protein, lactose) are approximately the same as the proportional increase in milk volume. Sutton (1989) reported that giving a cow more of a particular diet usually results in the production of a greater milk volume, with minimal changes in milk composition.

Supplementation prepartum with extra protein does not appear to have consistent effects on milk protein concentration although it may increase protein yield (Thomas 1984).

Milk protein yield increased significantly ( $P < 0.05$ ) in ewes given a protein supplement compared with the unsupplemented control group. In addition total yield of milk protein was increased due to of increased milk yield of approximately 15% ( $P < 0.05$ ) in ewes fed supplementary lupins compared to those given no supplement.

Undegraded dietary protein supplements can be fed to support conceptus development in the final stage of pregnancy and improve body condition score at parturition and may improve subsequent increased milk protein production (Van Saun *et al.* 1993; Sahlu *et al.* 1995). The observed effect may be due in part to increased body protein mass which can be mobilised to support milking performance (Reid *et al.* 1980). If this is true, ewes on the Norpro supplement (60% UDP) would be expected to produce a more milk protein than ewes supplemented with the more degradable lupin supplement. A review by Boorman (1980) suggested that the quality of the dietary protein is an important determinant of the efficiency with which dietary protein is deposited within the body. The requirement for essential amino acids will depend largely on the amino acid composition of the body reserves (muscle). For example, with lupin feeding the first limiting amino acid will be methionine (Murray *et al.* 1985), on the other hand, lysine is likely to be first limiting amino acid when cottonseed meal and sunflower meal provide the protein (McDonald *et al.* 1995). Therefore, the lack of response in milk protein production for ewes fed highly undegradable protein could be due to these amino acids, essential for milk protein synthesis, being limiting (Lindsay and Buttery 1980; Meijer *et al.* 1995).

The lack of response of undegradable protein on lactation performance in the present study may be explained by the differences in body condition score at lambing. All ewes were in negative energy balance throughout the experimental period as a result of losses of condition. Ørskov *et al.* (1980a) suggested that if animals are in negative energy balance their requirement for UDF increases to support the energy mobilised from body tissue and this implies that animals in positive energy balance may not respond to additional UDP. The results of the current experiment differ from those reported by Van Saun *et al.* (1993) in which heifers fed a UDP supplement started the experiment at a higher body condition and maintained a higher body condition score during the feeding

period. Subsequently, heifers fed the high UDP diet produced milk with a higher protein concentration.

Results from the present study showed that prepartum feeding of supplementary protein increased milk fat percentage compared to the unsupplemented controls. Ewes fed UDP diets had a higher milk fat percentage, and a difference ( $P < 0.05$ ) was found in the cottonseed meal treatment (9.9 vs 8.6, 8.3, 8.8%; for CSM vs. control, lupins, Norpro). Likewise, milk fat production was greater for ewes given a supplementary ration, the highest response being for ewes given cottonseed meal or protected sunflower meal, where production increased about 18-24% compared to the controls. These findings are in agreement with Sklan and Tinsky (1993) who reported that cows fed Doublepro (high UDP) increased production of milk, fat and milk yield over the experimental period. Dhiman *et al.* (1993) reported that post-ruminal infusion of casein resulted in a 17-22% increase in 3.5% FCM. Addition of undegradable protein in the diet of cows has been shown to have no effect on milk yield, however Ørskov *et al.* (1981b) reported that the concentration and yield of milk fat were significantly increased.

The explanation for increases in milk fat with protein supplementation may be related to changes of weight and body condition of animals during the experimental period. The additional protein intake probably affected body reserves and body weight in particular those in the Norpro group. It has been suggested that body fat reserves are utilised more from the day of lambing and in early lactation (e.g. Ørskov *et al.* 1977; Léonard and Block 1988; Dhiman *et al.* 1991), which may partly explain the enhanced fatty acid synthesis (Vernon 1988). This is also in agreement with the report of Annison (1976) that body fat is mobilised as plasma free fatty acids which are taken up by adipose or mammary tissue and incorporated into triglyceride, or are used for lipoprotein synthesis in the liver.

Most of the lipid in milk (50-60%) is synthesised from plasma glycerides, and the remainder are synthesised in the mammary gland from acetate and  $\beta$ -hydroxybutyrate (Annison and McDowell 1980; MacRae *et al.* 1988; Vernon 1988). No information was obtained on blood metabolites in this present study, however, since ewes receiving

cottonseed meal and chemically treated sunflower meal had reduced body condition loss during early lactation, it could be assumed that the plasma FFA concentrations were elevated. Taking this into account, the enhanced milk fat for ewes receiving cottonseed meal and chemically treated sunflower meal during the prepartum period could be explained. This hypothesis is consistent with that reported by Grainger and McGowan (1982), that the larger the difference in liveweight at parturition the larger the differences in milk fat production.

### **Mammary gland development**

Results of the present study showed that the mammary gland developed with advancing gestation in all four treatment groups. Ewes supplemented with additional protein had an additional increase in mammary volume compared to the controls. Significant differences between treatments ( $P < 0.05$ ) occurred in the last week prior to lambing at which time ewes in the lupin and Norpro groups had the greatest mammary volume. This finding agrees with Stephenson and Bird (1992) who reported that supplementation with protein meal was associated with significant increases in udder size. Even though lupin protein is extensively degraded (Table 4.1) it can still produce a lot of microbial protein through provision of fermentable substrate and a good source of nitrogen in the rumen. Improving feeding in late pregnancy could allow extra development of secretory tissue and consequently increased milk production (Greenhalgh and Gardner 1958). Moreover, Flux (1950) visual observation showed that better fed heifers had larger udders at calving, but this may have been due to greater fluid content, greater deposition of fat or greater development of secretory tissue.

The increase in mammary volume in the ewes fed protein (Figure 4.1, 4.2) was possibly due to the greater supply of amino acids being absorbed from the small intestine. Because different protein sources were fed during the prepartum period only, it is possible that differences were the result of changes in metabolism or in specific hormones controlling mammary development (Anderson 1974). For instance, Davis (1972) reported that intravenous infusion of essential amino acids, i.e. arginine, histidine or lysine, stimulate growth hormone (GH) release in sheep. There is an important

interrelationship between the concentration of growth hormone, prepartum dietary crude protein and growth of tissues (Swanson and Poffenbarger 1979; Chew 1984b). Growth hormone is essential for mammary tissue growth and development (e.g. Erb 1977; Pell and Bates 1990). Indirect evidence from Stelwagen *et al.* (1992) reported that administration of growth hormone during the last trimester of gestation resulted in increased milk production, implying an enhanced mammogenesis. Further research studying changes at the mammary tissue level is needed to confirm this hypothesis.

The level of IGF-I concentration generally reflects GH activity (Breier *et al.* 1986). Several papers have suggested the probability of a role of IGF-I and growth hormone in co-ordinating the activity of other hormones necessary for complex processes such as mammary tissue growth and lactation (Winder and Forsyth 1987; Pell and Bates 1990). Hall *et al.* (1992) suggested that prepartum protein feeding of ewes with either lupins or treated sunflower meal resulted in high values of IGF-I, and from that single bearing ewes had higher IGF-I levels than twin bearing ewes. Similarly, Zhang *et al.* (1995) observed that increased dietary protein increased total mammary tissue in ewes. It seems likely that a change in the mammary volume of ewes fed protein supplements prepartum in the present trial may be related to alteration of mammogenic hormones, such as, growth hormone.

In addition to the above hypothesis, hormones could be contributing to mammary development due to an interaction between protein supplements and the number of foetuses may also established the mammary development during gestation. The present study found that ewes carrying twin foetuses had the greatest ( $P < 0.05$ ) mammary gland volume during late gestation. It has been suggested that the developing foetuses may exert considerable control on mammogenesis by producing mammotrophic hormones (i.e. placental lactogens and oestrogens), and thereby directly influence the degree of mammary development (Knight and Peaker 1982a,c). Both placental lactogens and oestrogen concentration in the plasma are associated with the number of foetuses carried and foetus weight which are therefore correlated with placental mass (Bolander *et al.* 1976; Hayden *et al.* 1979; Orr and Treacher 1994) and their secretion plays an important role in the complex of hormones that control development of secretory tissue in the

udder (Treacher 1983). In addition, Hayden *et al.* (1979) demonstrated a positive correlation between mean placental lactogen concentration during the second half of pregnancy and milk yield in the next lactation. Based on this hypothesis, it is possible that ewes carrying multiple foetuses had higher a concentration of these two hormones which in turn increased mammary development and milk production.

### **Feed intakes**

There appears to be a consistent positive effect of increasing CP concentration intake on total dry matter intake and subsequently increases milk yield and composition (Cowan *et al.* 1981a; Sutton *et al.* 1996). Chew *et al.* (1984a) reported that cows fed to 100% of crude protein requirement which is similar to the amount recommended by the NRC (1978) increased DMI compared to cows fed at 80% of CP requirement for 9 weeks prepartum. Dry matter intake increased linearly as gestation progressed, and this increase may be related to changes in the fractional passage rate of digesta from the rumen or increases in digestion of food (Sahlu *et al.* 1992).

In this experiment, total dry matter intake during the prepartum period was increased by the additional CP supplements. There was no significant difference between supplements. Ewes offered cottonseed meal and Norpro had a greater total dry matter intake than those offered lupins or the control diet and the pasture effect of supplementation of UDP supplements on DMI has been observed by others (Dhiman and Satter 1993). Sahlu *et al.* (1995) found no difference in prepartum DMI of cows supplemented with either energy or protein while Dhiman *et al.* (1993) found that abomasal infusion of protein did not alter DMI, but that a negative effect on DMI was evident when energy supplements were given.

The observed depression in DMI of all ewes that occurred in the final week prior to lambing (Table 4.8) was consistent with the other reports. In the study of Sahlu *et al.* (1995), decreases in both CP intake and DMI were observed as a decline in mean retention time of particulate matter in the rumen and were in part due to a decline in appetite of animal in late gestation. This effect was evident for either high or low level of



supplementary protein offered to cows (Zamet *et al.* 1979; Johnson and Otterby 1981), sheep (Robinson and Forbes 1968; Faichney and White 1988) and goats (Van Saun *et al.* 1993). Therefore, it seems that a deficit in CP will reduce DMI in late gestation animals (Chew *et al.* 1984a).

Estimated total dry matter intake when corrected for body weight (52 kg) was significantly increased for ewes supplemented with cottonseed meal in the last week prior to lambing. The question to be considered is whether this intake effect originated in the rumen and is mainly associated with gut fill or whether the effect was derived from the increased uptake of amino acids post ruminally. Studies by Chamberlain *et al.* (1992) using fish and feather meals, providing similar amounts of UDP but differing substantially in amino acid composition, showed an increase in feed intake for both. Evidence from in vitro studies (Maeng and Baldwin 1976) reveal that responses in feed intake due to alteration in amino acids composition are relatively small. Thus, differential response to sources of protein supplements in the present study are most likely to be associated with differences in the post ruminal supply of amino acids (Chamberlain *et al.* 1992). The greater DMI of the cottonseed meal (greater UDP) would support the concept that the supplement works by balancing the nutrient supply to the animal and maintaining efficient rumen fermentation.

### **Ewe liveweight and body condition score**

Pre- and postpartum body weight of ewes groups were unaffected by prepartum protein feeding, but ewes supplemented with protein diets tended to be heavier than the controls. The consequent greater ( $P < 0.05$ ) body weight gains of ewes consuming the higher CP resulted in heavier ewes at parturition, which was similar to results reported by Sahlu *et al.* (1992, 1995) for goats. All ewes were similar in BW at the beginning of lactation, therefore the additional body weight gain during gestation was due to increased weight of amniotic fluid, placental mass or foetal growth which were lost at parturition.

Ewes grazed on pasture only were lighter ( $P < 0.05$ ) at lambing, but body weight changes from parturition to the end of the 4th week of lactation were greater for the control ewes. A similar response to the lower dietary protein intake during late pregnancy was observed in the studies of Robinson and Forbes (1968) and Wiley *et al.* (1991). Unlike those ewes from the control group, ewes fed protein supplements had greater ( $P < 0.05$ ) weight gains during gestation, and also higher weight loss during lactation. This effect was evident for ewes receiving a high concentration of undegradable protein, having the greater liveweight gains (422, 357 vs. 275 g/d; for CSM, Norpro and control, respectively). Similar responses to protein supplement (cottonseed meal) were reported by Stephenson and Bird (1992) who supplemented grazing ewes with cottonseed meal. Differences in weight between early and late lactation showed that ewes consuming cottonseed meal and Norpro lost the greatest amount of body weight, while those fed on pasture only and those supplemented with lupins maintained their weight.

Results from the present study showed that all ewes lost body condition throughout the period of gestation. The reason for this is probably due to the fact that the basal ration was not sufficient to meet the animals maintenance requirement during the pregnancy, indicating that all ewes were in negative energy balance. Ewes supplemented with Norpro had greater ( $P < 0.05$ ) losses in condition score (2.2 units) than the control (1.9), lupin (1.8) and cottonseed meal (1.5) groups. The loss of a large amount of body reserves in early lactation parallels reported responses to prepartal UDP supplementation in sheep (Hoaglund *et al.* 1992), dairy cows (Ørskov *et al.* 1987; Hook *et al.* 1992; Van Saun *et al.* 1993) and beef cows (Laflamme 1991) when a variety of UDP sources (i.e. corn gluten meal, fish meal, blood meal) were given as supplements. It seems that supplementary undegradable protein fed prepartum may improve postpartum performance with improved body reserves being able to support higher level of lactation.

### **Lamb performance**

A high supply of protein in late pregnancy is required for the gain in mass of the foetus (85% of its birth weight), but not for placental growth (Robinson *et al.* 1977). Supplementary feed intake during the last third of pregnancy has been examined by a

number of workers to improve lamb birth weight. For instance, Earl and Male (1988) found that giving lupin seed to ewes for 2 weeks prior to lambing increased lamb birth weights and the survival of twin lambs. Stephenson and Bird (1992) reported a 35% increase in lamb birth weight associated with the addition of bypass protein in the diet during the last 4 weeks before parturition. During late gestation, foetal growth has a large demand for glucose and amino acids (Barry and Manley 1985; Reynolds *et al.* 1986) and the response in foetal weight is greatest when additional protein and glucose are supplied together (Leng 1985; Sahlu *et al.* 1992).

Lamb birth weight observed in the present experiment was not influenced by prepartum protein supplementation. Similar lack of response to prepartum diet on birth weight were reported in calves (Hook *et al.* 1939; Wiley *et al.* 1991; Van Saun *et al.* 1993) and kids (Kayongo *et al.* 1984; Hoaglund *et al.* 1992; Sahlu *et al.* 1995). Similar mean birth weight of lambs across the 4 treatment groups may be attributed to differences in maternal body reserves as suggested by Robinson (1982).

Lamb birth weights were higher for the single bearing ewes, which agrees with many reports e.g. Mellor and Murray (1985). In another study Treacher (1970), showed that nutritional manipulation during the last third of pregnancy in ewes affected the birth weight of lambs, particularly of twins. In this connection Russel *et al.* (1977) and Gunn (1983) concluded that during late pregnancy twin bearing ewes were more likely to be undernourished, with a resultant loss of maternal body weight and lower birth weights than their single counterparts, even when the ewes were fed similar diets. Small twin lambs born as a result of undernutrition of the ewes in late pregnancy were unable to maintain growth rates equal to those of lambs with high birth weight. Therefore, it is possible that the effect of undernourishment during late pregnancy may inhibit the growth potential of lambs and the lambs will not grow as fast if they are given the same milk supply (Peart 1967).

## 4.5 CONCLUSION

Nutrient availability of the nonlactating animal, particularly during the final stage of gestation, is of significance in determining the growth of the conceptus development and to improve postpartum milk production, acting via effects on the depletion of prepartal maternal body reserve. Increasing protein supplementation during late gestation will improve development of mammary tissue possibly by effects on endocrine systems, such as growth hormone. In addition, the number of foetuses carried during gestation can also have an effect on the endocrine status which has a role in the development of the mammary gland, and can enhance milk synthesis.

Prepartum protein supplements have a positive effect on milk production and composition in first 4 weeks of lactation, but not on the growth of offspring. This effect could be associated with condition score and body weight changes during the period prior to lambing.

The prepartum feeding of supplementary protein increased body weight and body condition score and possibly increased the reserve of body protein. These responses were due partly to the animal's use of supplementary nutrients; as well as to an increased forage intake and digestibility associated with the protein supplements. This could be attributed to an effect on microbial activity and consequently a change in the fractional passage rate of digesta from the rumen.

As gestation progressed the requirement for protein was apparently greater than could be supplied by microbial protein synthesis. Several ways of using protected protein to increase milk production have been suggested. Ewes fed the high quality UDP sources had a greater change in body condition score at lambing which is consistent with an increased early milk production compared with the other treatment groups. However, the results from the present experiment clearly showed that protein fed as a high quality UDP source does not improve the lactation performance above those fed high in a RDP source. Three possible explanations for a lack of response to increased UDP sources are: (1) the higher UDP source failed to increase of intestinal supply of limiting essential

amino acids (e.g. methionine, lysine), (2) ewes in this present experiment were in negative energy balance during the experimental period, and consequently, the supply of UDP was used to support the mobilisation of maternal body reserves, or (3) even the supplements with low UDP resulted in improved microbial protein synthesis and more efficient rumen fermentation which improved the nutrient status of the ewes.

A conclusion of the present study was that the addition of a protein supplement in the final month of gestation can improve the energy status of grazing ewes. A positive response in milk production will result from supplementing with either a high or low degradable protein source. Further work is required to understand the relationship between body condition and lactation response throughout the first 4 weeks of lactation and also on the interaction with mammary development.

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