CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Introduction

This chapter gives the materials and methods generally used throughout the research reported in this thesis. Any differences from these procedures are presented in the individual chapters.

3.2 Experimental animals

The sheep used were second cross (Border Leicester x Merino/Dorset Horn) which had been raised on pasture at the University's farm.

3.2.1 General management

In all experiments, the animals were housed indoors in individual pens with access to clean water *ad libitum* at all times. Before commencement of experiments, the sheep were dosed with anthelmintics to control internal parasites according to a routine established at the University in these laboratories (see Bird 1982). In all experiments the feed refusals from the previous day's ration were removed and weighed before feeding. Unless specified, supplements were mixed into the basal diet by hand. Sheep were weighed in the morning at weekly intervals before feeding.

3.3 Treatment of protein meals with formaldehyde

Unless noted, formaldehyde (0.78g HCHO per 16 g N) was applied to both lupins and casein by spraying it as a 10% aqueous solution using a hand held pump while the ground feed materials were being rotated in a cement mixer. The pump was held in the drum of the mixer whilst it was turning. To facilitate even wetting by the formaldehyde solution, the mixture of protein and formaldehyde was allowed to mix for at least 10 min. In order to ensure completion of the reaction between the protein and formaldehyde, the treated lupins and casein were then stored in airtight plastic bags for at least one week before being fed to lambs.

3.4 Sampling of rumen fluid

Rumen fluid samples were collected by a stomach tube. This was done with a soft polythene tube (12 mm internal diameter) which was passed down the sheep's oesophagus into its rumen. The sheep's head was then lowered and the tube manipulated to siphon out rumen fluid. Approximately 40 ml of rumen fluid was collected and strained through a filter of stocking (pore size >200μ) into a beaker and sub-samples taken.

Cannulated animals were sampled via rumen fistulae with a sampling probe. The probe consisted of a thin stainless steel pipe with a small metal cage at one end. A piece of gauze (pore size >200μ) covered the cage. When sampling, the probe was inserted into the rumen with the cage end positioned into the dorsal sac of the rumen. A 50 ml syringe was used to collect the rumen fluid from the probe.

For estimation of protozoal numbers, 4 ml of rumen fluid was placed in a McCartney bottle containing 16 ml of formalised saline (200 ml 37% formaldehyde, 12.2 g NaCl and 1.8 l distilled H_2O). For VFA and ammonia analysis, approximately 15 ml of rumen fluid was placed in a McCartney bottle containing 5 drops of concentrated H_2SO_4 and stored at -20 ^oC until required. Prior to analysis, samples

were thawed and centrifuged for 10 min at 1500 g and the supernatant decanted into clean McCartney bottles.

3.5 Estimation of wool growth

Dyebanding was used to measure wool growth and the procedure given by Chapman and Wheeler (1963) was followed. The fleece was marked with lines of black dye (made by dissolving 0.8% w/w Durafur Black in cold water and adding 0.8% v/v concentrated hydrogen peroxide as an oxidant). Using a Pasteur pipette the dyeband was applied after parting the fleece as a 5 cm line along the skin close to the middle of the back and on the mid side of the sheep. At the beginning and end of each trial period, dyebands were applied along the same area of the sheep. Approximately 7 days were allowed for the final dyeband to grow out from the skin and the wool staple was then removed using 'Oster' small animal clippers model A-5 (Oster Corp. Milwaukee, Wisconsin, USA.) fitted with detachable comb number 80 size 30 (Oster Corp.). The dyeband was then cut and the separate sections of wool weighed, placed in cotton bags and washed as follows: (1) First washing: 20 ml Teepol (a liquid detergent; Shell Chemicals, Australia) in 9 l hot water. (2) Second washing: 24 g Na_2CO_3 in 9 l hot water. (3) Third washing: 12 ml Teepol in 9 l hot water. (4) Fourth washing: 12 g Na_2CO_3 in 9 l hot water. Each washing was for a period of 1.5 h and the bags were gently agitated in the wash solution every 20 min. The samples were rinsed in hot water between each stage. At the end of the washing period, the samples were placed in an oven at 60 ^{o}C for 48 h, cooled in a desiccator and weighed. The total clean fleece weight (TCW) grown during the measurement period was then calculated by using the following formula:

$$TCW = C \times T_g / T_s$$

where C = weight of the clean section of staple grown during the measurement period, T_g = greasy fleece weight and T_s = total weight of the greasy staple. The wool

growth per day was then determined by dividing the TCW by the number of days that the dyeband represented.

3.6 Enumeration of protozoa in rumen fluid

A McCartney bottle containing the sample collected for protozoal counting was thoroughly shaken and a sample pipetted into a counting chamber (Hawksley, Sussex, England) of 0.2 mm depth and covered with a coverslip before it was examined under a light microscope. A minimum of 200 cells were counted and the number of protozoa per ml calculated. The protozoa species were identified by reference to Hungate (1966) and Church (1976).

3.7 Feed conversion ratio

The efficiency of conversion of feed into gain or 'Feed Conversion Ratio' (FCR) was calculated using the following formula:

$$FCR = TDMI/\Delta lwt$$

where TDMI = g total dry matter intake (g/d) and Δlwt = g liveweight gain (g/d). Liveweight gain was calculated by linear regression of liveweight on time using Minitab (Ryan *et al.* 1985).

3.8 *In Sacco* digestibility of feed

The method used to estimate the *in sacco* dry matter disappearance of feed is based on that reported by Ørskov *et al.* (1980).

The bags, prepared from a monofilament polyester with a pore size of 44 μ, measured 7 x 14 cm and had rounded corners. The samples of feed were air dried, ground through a 2 mm sieve. A marble was placed inside each bag to ensure that the bag remained within the rumen contents. The bag and marble were weighed and a 3 g

sample of the feed placed within the bag. The bag was tightly sealed with nylon string, wetted with tepid tap water and placed inside the rumen through the rumen cannula. A maximum of 6 bags were placed in the rumen in the morning before feeding. The bags were withdrawn after incubation and washed under running tap water until the wash water was clear. The bags were dried in a forced air oven at 60°C for 48 h. On removal from the oven the bags were allowed to attain room temperature in a desiccator, weighed and the loss of dry matter determined.

3.9 Analytical methods

3.9.1 Dry matter

Feed and faecal dry matter was determined by drying duplicate samples in an oven at 70 °C for 48-72 h.

3.9.2 Total nitrogen

Duplicate samples were analysed for nitrogen content. These were digested using semi-micro Kjeldahl digestion and distillation method (AOAC 1980). The ammonia was collected in boric acid (2% w/v) and the mixture titrated to pH 5.0 using an autoburette (ABU 12, Radiometer, Copenhagen, Denmark) with 0.05 or 0.0075 N H_2SO_4 depending on the nitrogen content of the sample. A standard $(NH_4)_2SO_4$ solution was used to estimate ammonia nitrogen. A blank (double distilled water) was used as a correction factor and all samples were adjusted to 100% recovery of nitrogen and for dry matter content.

3.9.3 Rumen fluid ammonia nitrogen

Samples of rumen fluid (see section 3.4) were analysed for ammonia nitrogen content according to the method of Crooke and Simpson (1971) modified for small

samples by Bietz (1974) using a Technicon autoanalyser (Technicon Equipment Co., New Jersey, U.S.A.).

3.9.4 Urea nitrogen

Blood plasma and/or urine were analysed for urea nitrogen colorimetrically using the diacetyl monoxime method of Marsh *et al.* (1965) on a Technicon autoanalyzer (Technicon Equipment Co., New Jersey, U.S.A.).

3.9.5 Volatile fatty acids

Gas liquid chromatography (Erwin *et al.* 1961) was used for analysis of the molar concentrations and proportions of acetic, propionic, butyric, isobutyric, iso-valeric and valeric acids. A gas liquid chromatograph (Model 427, Packard Instrument Co., U.S.A.) connected to a data processor (Model 604, Packard Instrument Company, U.S.A.) was used. Iso-caproic acid was used as an internal standard (Geissler *et al.* 1976).

The glucogenic to total VFA energy ratio (G/E ratio) was calculated according to Blaxter (1967) as follows:

$$\text{G/E ratio} = \text{Propionate} / (\text{Propionate} + 0.6\ \text{acetate} + 1.4\ \text{butyrate})$$

where the VFA are expressed as molar percentages.

3.9.6 Glucose

Glucose concentration in plasma was measured enzymatically using a Glucose Rapid Test Kit (La Roche Co. Ltd.) on a Cobas-Bio centrifugal analyser (F. Hoffman-La Roche Co., Ltd., Diagnostica, Basle, Switzerland).

3.9.7 Acetate

Molar concentrations of acetate in plasma were determined using a gas liquid chromatograph (see Section 3.9.5). Iso-butyric acid was used as the internal standard.

3.10 Tracer techniques

Nolan (1971) defined the following terms associated with tracer dilution techniques.

Tracer: a defined substance labelled so that it can be used to study it own movements in a system without affecting the tracee.

Pool (or compartment): a quantity of substance having uniform and distinguishable kinetics of transformation or transport.

Tracer dilution: the change in ratio of tracer and tracee following injection of tracer into a compartment.

Pool size: total mass of tracee distributed through compartments within the biological system.

Turnover: the process of loss and replacement of tracee in a given compartment.

Half life: the time required for half the amount of tracee in a compartment at any given time to be removed by the turnover process.

Irreversible loss rate/entry rate: an amount of tracee that leaves the compartment per unit time and does not return to it during the experimental period.

3.10.1 Estimation of the specific activity of ^{14}C-bicarbonate in blood

The technique described by Leng and Leonard (1965) was followed for isolating ^{14}C-bicarbonate in blood samples.

Blood samples

Blood samples from the jugular vein were taken at 30 min intervals for 3 hours and then at hourly intervals until 7 hours after injection of the tracer. For each sample, approximately 6 ml of blood was immediately transferred into a McCartney bottle containing a small tube containing 1 ml of 1N CO_2-free NaOH. The bottle was then sealed and 1 ml of 1N H_2SO_4 was injected into the blood sample through the cap. After 12 h (for CO_2 absorption) the small tube was removed from the bottle and the NaOH solution was washed into a clean McCartney bottle containing 1 ml NH_4Cl (5% w/v). The carbonate was precipitated as $BaCO_3$ with 0.4 ml $BaCl_2$ (20% w/v). The precipitate was filtered and the filtrate washed three times with distilled water and once with acetone. The precipitate was then dried overnight at 100°C in a preweighed scintillation vial. The scintillation vials were weighed and the precipitate ground to a fine white powder by the use of a vortex mixer and two glass beads.

Injection solution

The radioactivity of injection solutions which contained $NaH^{14}C_{O3}$ was checked after isolation of the carbonate as $BaCO_3$. The injection solution (1 g) was diluted to 100 ml with CO_2 free distilled water containing 1 g Na_2CO_3 and 2 drops of 10N NaOH. Two ml of the diluted tracer solution was then processed in quadruplicate for release of CO_2 in the McCartney bottles and the ^{14}C-bicarbonate was isolated as already described for the blood samples.

Assay of radioactivity

For both the blood samples and the injection solution, the solid was suspended in 10 ml of scintillation fluid containing 3.5% (w/v) Cab-o-sil Thixotropic powder (G.L. Cabot Inc., U.S.A.), 0.4% (w/v) PPO (1,2,5-diphenyloxazole) and 0.02% (w/v) POPOP (1,4 bis-2-5 phenyloxazolyl-benzene).

3.10.2 Estimation of the specific activity of ^{3}H-glucose

Blood samples

After collection into heparinized tubes the blood was centrifuged (1000 g for 10 min) and the plasma fraction stored at -20^{o}C until analysed. When analysed the samples were defrosted and the plasma was deproteinised using barium hydroxide and zinc sulphate and the labelled glucose isolated as the penta-acetate derivative according to the method of Jones (1965).

The glucose penta-acetate crystals were filtered, washed, dried and weighed into scintillation vials.

Injection solution

The injection solution (1g) was diluted to one litre with distilled water. One ml of the diluted solution was processed in quadruplicate using the method given above for the blood samples.

Assay of radioactivity

Five ml of scintillation cocktail (4.0 g PPO and 0.2 g POPOP in one litre toluene) were added to the vial containing the weighed glucose penta-acetate crystals and the radioactivity determined in a Packard Tri-Carb scintillation spectrometer (Tri-Carb, Model 3320, Packard, U.S.A.).

3.10.3 Estimation of the specific activity of ^{14}C-urea in urine

Urine samples (0.20 ml) were placed in scintillation vials and dried in a desiccator under vacuum. They were reconstituted with 1 ml distilled water and mixed with 10 ml Tritonex (Teric X10, I.C.I., Australia) containing 0.4% (w/v) PPO, 0.02% (w/v) POPOP and 69% toluene. The injection solution (0.1 g) was diluted to 100 ml with distilled water and 1.0 g was placed into scintillation vials with 10 ml of scintillation fluid. Blanks were prepared by adding 1 ml distilled water and 10 ml of the scintillation fluid.

3.10.4 Counting of radioactivity

All samples, injection solutions and blanks were counted in a Packard Tri-Carb automatic liquid scintillation spectrometer (Tri-Carb, Model 3320, Packard, U.S.A.) using the automatic external standard to allow for quenching. The counting efficiencies were corrected using known standards.

3.10.5 Calculations for tracer methods

Isotope tracers were used in the studies reported in this thesis to make quantitative estimates of rumen metabolism.

The basic assumptions of tracer studies is to regard the function of metabolism under investigation as a pool or compartment within a system, both of which are in a steady state and have a constant flow of material through the pool. The entry rate of glucose or urea, estimated with ^{3}H-glucose and ^{14}C-urea respectively, was determined by assuming (see Judson and Leng 1972) that tracer recycling is neglibible during the sampling period and that the rate of dilution of tracer in a single compartment follows the form of an exponential curve which can be described by the following equation:

$$SR_t = A_0 e^{-mt}$$

where SR_t = Specific radioactivity of tracer at time t, A_0 = zero time intercept (i.e. tracer concentration at t_0), m = rate constant described by the slope of the regression of $\log_e$ (SR) versus time (min^{-1}), t = time (min).

From the tracer concentration at zero time (A_0), the pool size (P) is calculated from:

$$P = I/A_0$$

where I is the injected dose of tracer.

The entry rate (or irreversible loss) is that part of the pool which leaves and does not return to the primary compartment during the measurement period, and this is deduced from:

$$\text{Entry Rate} = P \times m$$

The entry rate of carbon dioxide, estimated by using ^{14}C-glucose, because some recycling of the tracer does occur, is best estimated using an equation with two exponential components:

$$SR_t = A_1e^{-m_1t} + A_2e^{-m_2t}$$

and the entry rate can be calculated as follows:

$$\text{Entry rate} = \text{Dose}/(\text{area under SR curve})$$
$$= \text{Dose}/\{(A_1/m_1) + (A_2/m_2)\}$$

A computer programme (BMDP-3V, Dixon *et al.* 1983) was used to obtain the line of best fit to the data. The computer programme minimised variance about the curves by an iterative process involving sequential alteration of the intercept (A) and rate constant (m) values until the residual variance was altered by less than 0.1% of its preceding value.

3.11 Acetate clearance rate

Clearance rate of an exogenous load of acetate was determined in sheep using the procedures of Weston (1966) as modified by Cronjé (1987).

3.11.1 Injection solution

The sodium acetate was prepared by dissolving 8.2 g of sodium acetate in 50 ml of sterile distilled water. The dose (4 mmol/kg liveweight), warmed to 37^{o}C, was injected into the jugular vein of sheep over a period of 2 to 3 min and was followed by 3 to 5 ml of sterile physiological saline solution.

3.11.2 Blood samples

Blood samples (6 ml) were taken via the jugular catheter at the following times: pre-injection, 10, 20, 30, 40 and 50 min post injection.

The samples were taken into a 10 ml heparinised centrifuge tube which were stored on ice until they were centrifuged for 10 min at 1000 g. The plasma sample was deproteinised by adding 3 ml plasma with 0.3 ml of 50% w/v sulphosalicylic acid which, after being mixed vigorously, was recentrifuged for 10 min. The supernatant fraction was decanted and stored at -20^{o}C until analysed for acetate concentration (see Section 3.9.7).

3.11.3 Calculation of acetate clearance

The blood acetate concentrations (mmole/l) determined for each sheep were transformed to the natural logarith and regressed against time after injection. The time required for half the dose of acetate to clear from the blood of the sheep ($t^{1/2}$) was calculated using the equation:

$$t^{1/2}=\ln 2/k \text{ or } 0.693/k$$

where k is the calculated fractional rate constant (slope) of the regression line.

3.12 Statistical procedures

The Gould computer of the University of New England was used to run the programs Minitab (Ryan *et al.* 1985) for linear regression analysis and data processing, NEVA (Burr 1980) for analysis of variance, BMDP-2V (Dixon *et al.* 1983) for repeated measurements of feed intake and liveweight and BMDP-3V (Dixon *et al.* 1983) for fitting points to a curve. Statistical significance of data was determined by the programs NEVA and BMDP-2V.

The levels of significance in the tables were indicated by the following symbols:

ns = non significant, † = $P<0.10$, * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

CHAPTER 4

USE OF PROTEIN MEALS TO BALANCE NUTRITION FOR PRODUCTION IN LAMBS FED LOW QUALITY DIETS

4.1 Introduction

It has often been assumed that the poor productivity of sheep fed low quality forages is due to a low metabolisable energy intake (MEI) as a result of an upper limit on appetite of these forages. However research from several laboratories in Australia is providing evidence that contests this traditional view. Data have been accumulated which support the concept that the cause of low productivity is an inefficient utilisation of the nutrients absorbed because the diets are not balanced for essential nutrients required for efficient digestion (by microbes) and efficient anabolic synthesis (by the animal) (see Section 2.4.2).

Low quality forages are low in nitrogen and are composed mostly of cell wall components with little soluble cell content. Two steps are required in the effective use of low quality forages for livestock production (Nolan *et al.* 1986). The first is to establish an efficient rumen ecosystem for fermentative digestion of fibre in the rumen which can be achieved by supplementation of dietary deficient nutrients, such as ammonia, which can be provided by supplementation with urea. This then leads to

enhanced ruminal fermentation which will increase the digestibility of fibre and also the amount of microbial protein flowing into the small intestine relative to VFA produced. The second step is to balance the products of fermentative digestion with dietary nutrients that escape degradation in the rumen (see Section 2.4.2.1). Supply of additional glucogenic nutrients and lipids may also be critical in growing and fattening ruminants fed fibrous diets. The processed protein meals (meat, fish and oilseed meals) have been shown to be effective in supplementing low quality roughage diets (see Preston and Leng 1987) with dietary intestinally digestible protein to enhance the P/E ratio in the nutrients absorbed.

With the majority of ruminants kept entirely on pasture in Australia, the use of meat, fish or oilseed meals for supplementing forage fed ruminants is only economic in areas close to the production of these materials. There are vast areas of Australia which have no obvious sources of local bypass protein. During the last decade lupins have been developed as a major crop in southern and western Australia and is recognised as an important high protein stockfeed (Holmes *et al.* 1991). Lupins are low in starch, of high digestibility (80-90%), have a moderate oil (5-8%) and a high crude protein (28-34%) content . They are therefore suitable as a supplement for grazing sheep because they are complementary to the low protein, high fibre forages and provide a number of essential nutrients potentially deficient in such diets. In addition, the advantages of supplementation with lupins are that they are palatable to sheep, easy and safe to feed as well as being convenient to store (Cottle 1988a). However, values for rumen degradation of lupin meal of between 65% and 86% have been reported (Hume 1975, Neutze and Forbes 1990) and this indicates that lupins may only satisfy the first requirement stated above, i.e. in enhancing rumen fermentation by providing a source of nitrogen (and possibly minerals) for use by the microorganisms.

Several research groups have investigated the use of lupins as a supplement to low quality forage diets. For example, lupins were compared with oat or wheat grain as

a supplement (270 g/d) to a basal diet of poor quality straw (1.7% N) for lambing ewes (Kenney and Roberts 1984). Lupins were slightly superior to both oats and wheat grain in increasing wool production, milk production and growth of lambs. In addition, lupins were comparable to green pasture in improving the subsequent reproductive rate of the ewes. Kenney and Smith (1985), in another experiment, showed that inclusion of 15% lupins in cereal grain rations fed to lambing ewes held under simulated drought conditions substantially improved survival and liveweight of ewes and lambs and wool production by ewes.

In both of these experiments, it appeared that lupins behaved only as a soluble nitrogen source which enhanced microbial growth efficiency in the rumen. The lupins produced only slightly better liveweight gain responses than did the oats in the first experiment even though the lupins' crude protein content (31%) was far higher than that of the oats (16%). In the second series of experiments the level of lupins included in the diet varied from 15 to 60% with the liveweight gain responses of the sheep greater at higher levels. This indicated that some rumen escape (bypass) of the protein may have occurred at the higher levels of lupin inclusion. Hence, the sheep may have been responding to two factors; the enhanced rumen fermentation due to soluble nitrogen, and/or the enhanced efficiency of feed utilisation through the increase in P/E ratio from increased microbial protein entering the intestines and increased amino acid absorption from lupin protein.

In an experiment comparing urea and lupins as a source of nitrogen for weaner sheep on a basal diet of oats, Butler and McDonald (1986) found no significant differences in liveweight gain and wool growth when either urea or lupins were included in the diet to obtain a crude protein (CP) level of 12%. When CP was increased to 15%, lupins produced significantly higher liveweight gains by the weaner sheep but had no effect on wool growth.

Formaldehyde treatment of protein meals has been successful in protecting protein from rumen fermentation and use of these protected proteins has led to increased liveweight gains, feed conversion efficiency and reproductive potential in cattle and sheep and wool growth in sheep (for review see Chalupa 1975, Ferguson 1975). There has been only a limited number of reports in the literature of the use of formaldehyde to protect lupin protein from rumen degradation (Ferguson 1975).

The research reported in this Chapter investigated the production responses of lambs to supplementing low protein diets with lupins and aminogenic and/or glucogenic substrates. Experiment 1 investigated the responses of lambs fed a basal diet of oaten chaff to supplementation with formaldehyde treated casein. Experiment 2 examined whether formaldehyde treatment could protect lupins from rumen degradation. In Experiment 3, a lamb growth study investigated whether treated lupins produced greater liveweight gains than urea or untreated lupins in growing lambs fed a basal diet of oaten chaff. Experiments 4 and 5 investigated the use of a basal diet of cottonseed hulls with supplements of propionate and formaldehyde treated casein as a test diet to attempt to establish whether the responses in efficiency due to supplementation could be attributed to glucogenic or aminogenic substrates.

4.2 Production of lambs fed a basal diet of oaten chaff supplemented with formaldehyde treated casein

4.2.1 Experimental

4.2.1.1 Animals and housing

Second-cross ewe lambs (approximately 7 months of age and with a mean liveweight of 30 kg) were used. They were housed in individual pens on a slatted floor inside a well ventilated shed.

4.2.1.2 Experimental design

The experiment used 48 lambs in a 2 x 4 factorial design with two levels of feeding and four diets (6 lambs/group). The experimental period was 105 days.

4.2.1.3 Diets and feeding

The lambs were fed a basal diet of oaten chaff. A vitamin/mineral mix (17.5 g/d) was added to the basal diet and consisted of 40% dicalcium phosphate, 20% sodium sulphate, 20% sodium chloride and 20% vitamin/mineral premix (Pfizer Q422).

The four diets fed were: (1) basal, (2) basal plus 50 g casein, (3) basal plus 50 g formaldehyde treated casein, (4) basal plus 50 g formaldehyde treated casein plus 10 g urea. The basal diet was fed at either *ad libitum* or 80% *ad libitum*. The amount of oaten chaff for the 80% *ad libitum* fed sheep was determined by calculating 80% of the previous weeks' intake of the *ad libitum* fed sheep in the appropriate treatment group.

Formaldehyde (0.78 g HCHO per 16 g N) was applied to the casein as a 1:1 aqueous solution (see Section 3.3). The supplements were mixed by hand with the oaten chaff (88% DM and 0.5% N) and fed as one meal.

4.2.1.4 Procedure

Prior to supplementation the sheep were allowed a 2 week period during which they were fed oaten chaff *ad libitum* plus 50 g/d lucerne chaff. At the commencement of the experiment the sheep were weighed, ranked according to weight and allocated to groups using stratified random sampling. The sheep were weighed every 2 weeks prior to daily feeding. Wool growth during the experimental period was measured using the dyeband technique (see Section 3.5).

4.2.1.5 Statistical analysis

Results were analysed using the computer program, NEVA, see Section 3.12.

4.2.2 Results

The results and statistical analysis of dry matter intake, liveweight gain, feed conversion ratio and wool growth by lambs are given in Table 4.1 and Appendix 4.1.

4.2.2.1 Intake

Oaten Chaff Intake Supplementation with treated casein significantly ($P<0.01$) increased intake of oaten chaff by lambs. However, there was no significant difference in intake by lambs given treated casein due to the addition of urea. Addition of untreated casein to the basal diet did not increase intake. The oaten chaff intake by the basal, untreated casein, treated casein (TC) and TC plus urea (TCU) supplemented sheep were 734, 709, 766 and 772 g/d respectively.

Total Dry Matter Intake (TDMI) The TDMI by the sheep given treated casein was greater ($P<0.001$) than the TDMI of sheep fed either the basal ration or the basal ration supplemented with untreated casein. Addition of urea and treated casein did not increase TDMI above that produced by treated casein alone. The TDMI of the basal, untreated casein, TC and TCU supplemented sheep were 751, 782, 839 and 855 g/d respectively.

4.2.2.2 Liveweight gain

Sheep fed *ad libitum* had greater ($P<0.001$) liveweight gains than did sheep fed 80% *ad libitum* (90 g/d compared with 57 g/d). Supplementation of the basal diet with treated casein increased ($P<0.001$) liveweight gain of the sheep compared to those fed the basal diet alone or with untreated casein. Addition of urea to the treated casein did not produce a significant effect on liveweight gain of the sheep. The liveweight gain

Table 4.1 Dry matter intake, liveweight gain, feed conversion ratio and wool growth of lambs fed a basal diet of oaten chaff and supplemented with casein, formaldehyde-treated casein (FC) or FC plus urea (FCU). The oaten chaff was fed *ad libitum* (AL) or 80% AL (R).

Measurements	Supplement											Significance$^{\Phi}$		
	Nil		Casein		FC		FCU		Means		SEM	L	D	LxD
	R	AL	R	AL	R	AL	R	AL	R	AL				
Intake (g DM/d)														
Chaff	655	813	635	783	689	842	689	856	824	667	19	***	**	ns
TDMI	672	830	672	856	762	915	772	939	885	728	19	***	***	ns
Liveweight gain (g/d)	38.0	72.1	38.0	89.7	71.3	98.8	73.8	99.8	90.1	56.8	6.3	***	***	ns
Feed Conversion Ratio (g/g)	19.2	12.3	19.2	9.8	11.0	9.3	10.8	9.6	10.2	15.2	2.1	**	*	ns
Wool Growth (g/d)	4.2	4.8	4.9	6.1	5.8	6.3	5.5	6.7	6.0	5.1	0.64	**	**	ns

$^{\Phi}$ L - level, D - diet

by the basal, untreated casein, TC and TCU supplemented sheep were 55, 67,85 and 87 g/d respectively.

4.2.2.3 Feed conversion ratio (FCR)

Restricting the intake of the basal diet to 80% *ad libitum* increased the feed conversion ratio by 49% ($P<0.01$). Supplementation of the basal diet with treated casein or treated casein plus urea decreased the FCR ($P<0.05$). Supplementation with untreated casein had no significant effect on FCR. The FCR by the basal, untreated casein, TC and TCU supplemented sheep were 15.7, 14.8, 10.2 and 10.2 g/g respectively.

4.2.2.4 Wool growth

Wool growth by the sheep was decreased ($P<0.001$) by restricting intake of the basal diet to 80% of the *ad libitum* intake, however, supplementation of the diet with treated casein increased ($P<0.001$) wool growth. Addition of urea and treated casein did not increase wool growth above that produced by treated casein alone. The wool by the basal, untreated casein, TC and TCU supplemented sheep were 4.5, 5.5, 6.0 and 6.1 g/d respectively.

4.2.3 Discussion

The results from this experiment showed that supplementation of an oaten chaff diet with formaldehyde treated casein (treated casein) significantly improved liveweight gain, feed conversion efficiency and wool growth in growing lambs.

The 27% and 31% improvement in liveweight gain and feed conversion ratio of the *ad libitum* fed lambs in response to supplementation with treated casein as compared with that obtained with supplementation of untreated casein was higher than the 9% and 8% improvements obtained by Faichney (1971). The diet used by Faichney

(1971) consisted of 45% lucerne hay, 9% wheaten hay, 9% starch, 27% maize and 10% casein and the crude protein content of the dry matter was 22%. The casein provided approximately 40% of the total nitrogen as compared with the present experiment in which casein contributed over 90% of the total nitrogen. In the experiment reported by Faichney (1971), wool growth did not respond to treatment of casein, indicating that absorption of amino acids by the lambs receiving the untreated casein was probably sufficient for maximum wool growth. In the experiment reported here, the lambs supplemented with treated casein had higher, but not significantly, wool growth rates than those supplemented with untreated casein.

The differences in FCR and liveweight gain between the lambs on the control diet and those supplemented with treated casein were greater at the restricted feed intake. This indicated that the animals were more efficient at the restricted intake when the diet provided more protein for intestinal absorption. Under drought conditions where the amount of roughage is limited, supplementation with a source of bypass protein could be critical to animal survival.

The increases in liveweight gain and wool growth in the untreated casein supplemented sheep when intake of the oaten chaff was not restricted indicated that more protein may be flowing into the small intestine. This would be composed of microbial protein and/or feed protein. In the control sheep (no supplements), the increase in liveweight gain when intake of the oaten chaff was changed from restricted to *ad libitum* was 34 g/d with a 0.6 g/d increase in wool growth. In comparison, the TDMI of the casein supplemented sheep was only 26 g/d higher and the equivalent increases in liveweight gain and wool growth were 52 g/d and 1.2 g/d respectively. The large increase in wool growth on the diet containing untreated casein indicated that at the higher intakes a greater proportion of the protein may be escaping to the intestinal system.

Kempton and Leng (1979) reported that sheep when given free access to a diet containing oat hulls (700 g/kg), Solka-Floc (300 g/kg) and vitamins/minerals changed

from a liveweight loss of 40 g/d to a liveweight gain of 56 g/d when supplemented with either urea, treated casein or casein plus urea. Urea plus treated casein further increased lamb growth to 112 g/d. However, in the present experiment, the addition of urea and treated casein gave no additional benefit. Two factors may be involved in this difference. The first one is that the diet used by Kempton and Leng (1979) was deficient in soluble nitrogen and hence supplementation with urea may have stimulated rumen fermentation. Secondly, the casein used in the present experiment may not have been completely protected by the formaldehyde and thus would have provided a source of nitrogen and amino acids for the rumen microbial population.

The total dry matter intake of the *ad libitum* fed sheep supplemented with treated casein was greater (10%) than that of *ad libitum* fed sheep on the basal ration. This is much less than the increase in TDMI by sheep as found by Kempton and Leng (1979) in response to supplementation with 75 g treated casein. The diet used by these workers had a low ME and nitrogen content and the intake of the roughage was constrained because of these factors. When post-ruminal absorption of protein was increased due to supplementation, there would be some recycling of urea to the rumen which could stimulate the microbial production, particularly later in the feeding period, leading to greater production of microbial protein and VFA. In sheep fed low quality roughage with low N intakes, a considerable amount of recycling of urea back to the rumen has been shown (Kennedy and Milligan 1978, Nolan and Stachiw 1979). These factors could further stimulate feed intake. In the current experiment the dry matter intake of 830 g/d by sheep on the control diet was high and reflected the superior quality of the basal roughage compared with that used by Kempton and Leng (1979).

From the experiment reported here, liveweight gain, FCR and wool growth of lambs were shown to increase in response to supplementation of an oaten chaff diet with formaldehyde treated casein. The next two experiments examined the use of lupin meal as a protein source and determined whether treatment of this protein meal with

formaldehyde could protect it from rumen degradation. A growth study was used to ascertain whether supplementation with formaldehyde treated lupin meal would lead to greater liveweight gains by lambs than when a non-protein source of nitrogen was fed.

4.3 *In Sacco* digestibility of treated or untreated lupin meal

4.3.1 Experimental animals

Four mature crossbred wethers weighing about 32 kg with permanent rumen fistulae were used for this experiment. They were housed in single pens with slatted floors in an open-sided shed.

4.3.2 Diets and feeding

The sheep were offered a basal diet of oaten chaff *ad libitum* and 100 g/d lupins (89.5% DM, 5.6% ether extract and 4.8% N), for a period of 4 weeks before the digestibility study commenced and during the period of digestibility measurements.

4.3.3 Procedure

Lupins were ground and passed through a 2 mm sieve and sprayed with formaldehyde (see Section 3.3) at the following rates: 0.6, 1.0, 1.4, 1.6, 1.8, 6.0 g HCHO/16 g N of lupin meal.

The *in sacco* digestibility of heat treated lupin meal was also measured. Heat treatment involved placing 100 g of lupin meal in a forced draught oven at 200^{o}C for 35 min.

As a reference the *in sacco* digestibility of formaldehyde treated casein (0.78 g HCHO per 16 g N) was measured.

The *in sacco* digestibility of lupin meal was estimated after 6, 24 and 48 h incubation in the rumen and follows the procedure detailed in Section 3.8. Samples from the different treatments were incubated in duplicate in each of the 4 sheep.

4.3.4 Results

Treatment of casein with formaldehyde rendered it insoluble in the rumen and at 24h the amount of casein that had left the nylon bags was only 2% (Table 4.2). Treatment of lupin meal with formaldehyde did not alter the amount leaving the nylon bags in comparison with untreated lupin meal. Untreated lupin meal was very soluble in the rumen and 90% had disappeared from the nylon bags after 24h incubation. Heat treated lupin meal had similar *in sacco* digestibility to that of untreated lupin meal.

Table 4.2 ***The in sacco dry matter digestibilities of lupin meal in the rumen of mature crossbred wethers fed a basal diet of oaten chaff and 100 g/d lupins***

Lupin Treatment	*in sacco* digestibility (%)		
	6 h	24 h	48 h
nil	65	90	91
0.6 gHCHO/16 gN	ndΦ	88	97
1.0 gHCHO/16 gN	nd	80	90
1.4 gHCHO/16 gN	57	71	88
1.6 gHCHO/16 gN	64	66	83
1.8 gHCHO/16 gN	55	66	82
6.0 gHCHO/16 gN	nd	29	95
heat treatment	nd	82	nd
Treated Casein	nd	2	0

Φ- not done

4.3.5 Discussion

The reasons for the lack of protection of lupin meal by formaldehyde may be due to a combination of factors: (1) the lupins contained almost 6% fat and this may have prevented the formation of the cross linkages between the amino and amide groups with the formaldehyde, (2) the grinding of the lupins resulted in a fairly coarse meal unlike the fine texture of the casein thus the formaldehyde may not have come into

sufficient contact with the lupin protein, and (3) the protein content of the lupins (30%) was much lower than that of the casein (88%).

Chalupa (1975) considers that protection of plant protein with formaldehyde may be difficult to achieve without affecting microbial metabolism, microbial protein production or intestinal digestibility and absorption.

Ferguson (1975) attributed the increase in rumen escape of heat treated protein meals to the reduction of rumen solubility of the protein. In this experiment, heat treatment only had a small affect on reducing the solubility of the lupin meal with 18% of the heat treated lupin meal left in the nylon bags after 24 h incubation as compared with 10% left in the untreated lupins. Kung *et al.* (1990) used commercially roasted whole lupins as a supplement for sheep fed diets of 90% concentrate (corn based) and 15% CP. The protein source being either raw lupin or roasted lupins. They found that roasting tended to improve nitrogen retention in sheep which indicated only a slight decrease in lupin solubility in the rumen.

The results obtained in this experiment showed that formaldehyde treatment or heat treatment of ground lupins did not protect the lupin meal from rumen degradation to any significant extent. At a high levels of formaldehyde treatment (6 g HCHO/16 g N), the 24 h digestibility of lupin meal was reduced from 90% to 29% but after 48 h *in sacco* there were digestibility of the lupin meal was 95%.

4.4 Production of lambs fed a basal diet of oaten chaff and supplemented with urea, lupins and formaldehyde treated lupins

4.4.1 Experimental

4.4.1.1 Animals and housing

Crossbred 4-month-old ewe lambs with a mean liveweight of 20 kg were placed in single pens with slatted floors in a well ventilated shed. To control internal parasites, the lambs were drenched with Seponver (Smith Kline Animal Health Products) and Nilverm (ICI Australia) according to the manufacturers' recommendations before the trial commenced.

4.4.1.2 Experimental design

The experiment involved 72 lambs in a 2 x 4 factorial design with two levels of feeding and four diets (9 lambs/group). Data were collected over a period of 60 days.

4.4.1.3 Diets and feeding

The lambs were fed a basal diet of oaten chaff with an added vitamin/mineral mixture (17.5 g/d). The vitamin/mineral mixture consisted of 40% dicalcium phosphate, 20% sodium sulphate, 20% sodium chloride and 20% premix (Pfizer Q422). The four diets were: (1) basal plus 10 g urea, (2) basal plus 100 g lupins, (3) basal plus 100 g formaldehyde treated lupins, and (4) basal plus 100 g formaldehyde treated lupins plus 10 g urea.

The basal diet was fed either *ad libitum* or 75% *ad libitum*. The amount of oaten chaff for the 75% *ad libitum* fed sheep was determined by calculating 75% of the previous weeks' intake of the *ad libitum* fed sheep in the appropriate treatment group.

Lupins, from the same batch as used in the digestibility study, were ground through a 2 mm sieve. Formaldehyde (1.4 g HCHO per 16 g N) was applied to the lupin meal as a 25% aqueous solution (see Section 3.3). The treated lupins were stored in airtight plastic bags for at least one week prior to use in order to ensure completion of the reaction between the protein and formaldehyde.

The supplements and the vitamin/mineral mix were placed on top of the oaten chaff and mixed in by hand.

4.4.1.4 Procedure

Prior to the growth study, all lambs were given 2 weeks to adapt to the pen conditions. During this time, they were fed a mixture of oaten and lucerne chaff *ad libitum*. At the commencement of the experiment the sheep were weighed, ranked according to weight and allocated to groups using stratified random sampling. The sheep were weighed every 2 weeks on the morning prior to feeding. During the final week of the study, rumen fluid samples were collected through stomach tubes (see Section 3.4) for measurement of VFA and ammonia before and 4 h after feed was offered. The samples before and 4 h after feeding were taken on the same day. Wool growth during the experimental period was measured using the dyeband technique (see Section 3.5).

4.4.1.5 Statistical analysis

The data were analysed (using the computer program, NEVA, see Section 3.12) as a two-way analysis of variance with supplements and level of feeding as the factors. The residuals were examined and in no case was a transformation of data necessary in order to stabilise the variance. The SNR (studentised range) multiple comparison procedure (Steel and Torrie 1981) was used to test for significant differences between treatment means.

4.4.2 Results

The results and statistical analysis for dry matter intake, liveweight gain, feed conversion ratio and wool growth are given in Table 4.3 and Appendix 4.2.

4.4.2.1 Intake

Oaten Chaff Intake The analysis of variance indicated a supplement by treatment effect that approached significance ($P<0.10$). Examination of the treatment means showed two overlapping groups of three treatment means so that, although intake (596 g/d) by the sheep supplemented with lupins was significantly less ($P<0.05$) than that by the sheep supplemented with treated lupins and urea (656 g/d), there was no difference in intake between the groups of any practical importance. The mean intakes by the sheep supplemented with urea or treated lupins were 610 g/d and 612 g/d respectively.

Total Dry Matter Intake (TDMI) TDMI by sheep fed different supplements were significantly different ($P<0.001$). Sheep supplemented with urea had significantly lower intakes than either the lupin or treated lupin supplemented sheep and that these in turn had significantly lower intakes than the treated lupin plus urea supplemented sheep (637 g/d, 703 g/d, 718 g/d and 773 g/d respectively). TDMI of the lupin and treated lupin supplemented sheep were not significantly different.

4.4.2.2 Liveweight gain

There were no significant differences in mean liveweight gain between the sheep supplemented with nil, urea, untreated lupins, treated lupins (FL) or FL plus urea (FLU) (71, 84, 81 and 90 g/d respectively. However, as might be expected, the 75% *ad libitum* fed sheep had significantly lower ($P<0.001$) mean liveweight gains than did the sheep fed *ad libitum* (58 g/d compared with 105 g/d).

Table 4.3 Dry matter intake, liveweight gain, feed conversion ratio and wool growth of lambs given a basal diet of oaten chaff and supplemented with 10 g urea, 100g lupins, 100g formaldehyde-treated lupins (FL) or FL plus urea (FLU). Oaten chaff was fed either *ad libitum* (AL) or 75% AL (R).

Measurements	Supplement											Significance$^{\Phi}$		
	Urea		Lupins		FL		FLU		Means		SEM	L	D	LxD
	R	AL	R	AL	R	AL	R	AL	R	AL				
Intake (g DM/d)														
Chaff	509	711	510	682	531	692	564	749	529	709	22	***	†	ns
Total	537	738	617	789	638	799	680	865	618	798	22	***	***	ns
Liveweight gain (g/d)	44	99	61	106	56	107	72	109	58	105	8.1	***	ns	ns
Feed Conversion Ratio (g/g)	14.2	8.1	10.9	7.9	13.5	8.1	10.2	8.0	12.2	8.0	1.5	***	ns	ns
Wool Growth (g/d)	4.6	4.6	4.6	5.2	4.4	5.2	5.2	6.7	4.7	5.4	0.4	**	**	ns

$^{\Phi}$ L= level of feeding; D= diet

4.4.2.3 Feed conversion ratio (FCR)

The FCR was lower (P<0.001) for the sheep fed *ad libitum* compared with the sheep fed 75% *ad libitum* (8 g/g and 12 g/g respectively). No significant differences were noted in FCR between sheep fed with different supplements. FCR for the nil, untreated lupin, FL or FLU supplemented sheep were 11.2, 9.4, 10.8 and 9.1 g/g respectively.

4.4.2.4 Wool growth

Wool growth of sheep fed *ad libitum* was higher than that of sheep fed 75% *ad libitum* (5.6 compared with 5.0 g/d). No significant differences were detected in wool growth for the sheep supplemented with either urea, lupins or treated lupins; however supplementation with treated lupins plus urea increased wool growth (P<0.001). Wool growth by lambs given nil, untreated lupins, FL or FLU was 4.7, 5.2, 5.1 and 6.3 g/d respectively.

4.4.2.5 Rumen fermentation

The results and statistical analysis of VFA concentration in the rumen and molar proportions of individual VFA before and 4 h after feeding are given in Table 4.4 and Appendices 4.3 and 4.4.

Restricting feed intake increased the total concentration of VFA in the rumen before feeding (P<0.10) and 4 h after feeding (P<0.05). Before feeding, the acetate levels were higher (P<0.05) in the rumen of sheep on restricted intake. In the samples taken 4 hours after feeding, the levels of butyrate and valerate were higher (P<0.05) in the rumen of sheep on restricted intake. Supplementation with 100 g lupins increased (P<0.05) valerate levels 4 h after feeding. No other significant differences were noted

Table 4.4 Concentrations and proportions of VFA in the rumen fluid of lambs fed a basal diet of oaten chaff supplemented with urea, lupins, formaldehyde-treated lupins (FL) or FL plus urea (FLU). The oaten chaff was fed either *ad libitum* (AL) or 75% AL (R).

Measurements	Supplement											Significance$^{\Phi}$		
	Urea		Lupins		FL		FLU		Means		SEM	L	D	LxD
	R	AL	R	AL	R	AL	R	AL	R	AL				
Before Feeding														
Total VFA (mmol/l)	83.3	53.4	51.3	59.2	71.8	68.8	78.0	57.3	71.1	59.7	9.2	†	ns	ns
VFA Proportions (%)														
Acetate	74.1	76.9	71.9	75.2	72.7	74.2	69.1	75.1	72.0	75.4	2.1	*	ns	ns
Propionate	17.9	15.0	17.2	16.2	18.3	18.1	22.0	16.0	18.9	16.3	2.4	ns	ns	ns
Butyrate	5.9	5.9	7.2	6.1	6.4	5.8	6.3	6.3	6.4	6.0	0.6	ns	ns	ns
Isobutyrate	0.8	0.7	1.1	0.8	0.8	0.6	0.8	0.8	0.9	0.7	0.1	†	ns	ns
Isovalerate	1.0	1.1	2.1	1.3	1.3	0.9	1.3	1.4	1.4	1.2	0.3	ns	ns	ns
Valerate	0.3	0.5	0.5	0.4	0.6	0.4	0.4	0.5	0.4	0.4	0.1	ns	ns	ns
Ammonia (mgN/l)	91	102	71	81	81	86	67	83	78	88	13.0	ns	ns	ns
G/E	0.25	0.22	0.24	0.23	0.26	0.25	0.29	0.23	0.26	0.23	0.03	ns	ns	ns
4h after feeding														
Total VFA (mmol/l)	109.8	90.8	92.8	96.3	98.3	91.5	100.7	97.5	100.4	94.0	4.3	*	ns	†
VFA Proportions (%)														
Acetate	67.9	68.9	64.8	67.1	67.0	67.4	66.9	66.9	66.7	67.7	1.2	ns	ns	ns
Propionate	21.9	19.9	21.6	22.5	21.3	22.9	22.1	22.7	21.7	22.0	1.3	ns	ns	ns
Butyrate	9.2	9.9	12.3	8.9	10.5	8.6	9.9	9.0	10.5	9.1	1.0	ns	ns	ns
Isobutyrate	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.03	ns	ns	ns
Isovalerate	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.5	0.1	ns	ns	ns
Valerate	0.5	0.7	0.7	0.8	0.6	0.5	0.6	0.6	0.6	0.6	0.05	*	**	*
Ammonia (mgN/l)	145	97	105	90	126	130	155	108	133	106	26	ns	ns	ns
G/E	0.29	0.27	0.28	0.30	0.28	0.30	0.30	0.30	0.28	0.29	0.02	ns	ns	ns

$^{\Phi}$ L= level of feeding; D= diet

in either proportions of VFA, ammonia concentration or the glucogenic to total VFA ratio.

4.4.3 Discussion

The primary aim of the two experiments with lupins was to find a readily available, palatable protein source that could be protected from rumen degradation. However the results from both the *in sacco* digestibility study and growth study showed that treatment of lupin meal with formaldehyde protected it only slightly from rumen degradation, and, that no significant improvements in liveweight gain, feed conversion efficiency or wool growth were obtained above that by feeding untreated lupins or urea.

Liveweight gain, feed conversion efficiency and wool growth were decreased by restricting intake of the oaten chaff. The differences in liveweight gain and feed conversion ratio were greater between treatments at the 75% *ad libitum* feeding than at *ad libitum* feeding. The supplement (treated lupin plus urea) which produced the greatest liveweight gains and most efficient feed conversions under *ad libitum* feeding was also the most efficient when restricted feeding was imposed. Under drought conditions where intake of the basal roughage will be restricted the use of supplements that increase efficiency of utilisation of the basal diet will be of primary importance in the survival of ruminants.

These data suggest that at *ad libitum* intakes of the basal roughage the higher rumen dilution rate allow escape of some protein from the rumen. This is evidenced by the higher wool growth in the untreated lupin supplemented sheep at *ad libitum* intake compared with wool growth of sheep on restricted feed intake.

Ferguson (1975) reported that, in a diet of 65% lupins, 25% lucerne hay and 10% barley, the treatment of lupins with formaldehyde gave an 18% increase in wool growth above that obtained by feeding untreated lupins. However, the proportion of

lupins used in the experiment by Ferguson was considerably higher than that which was used in the present study and so the small decrease in *in sacco* digestibility due to formaldehyde treatment found may have had more of an effect at the higher level of feeding. In addition the high concentrate diet fed by Ferguson would decrease the rumen retention time, a factor which by itself has been shown to be associated with higher levels of rumen bypass (Broderick *et al.* 1991).

Yilala and Bryant (1985) treated rapeseed meal (25% CP) with 1.47 g formaldehyde per 100 g CP. When fed at 120 g/kg diet DM in a basal diet of *ad libitum* grass silage supplemented with minerals, both treated and untreated rapeseed meal increased dry matter intake, daily gains, carcass weights and nitrogen retention. However the treatment of the rapeseed had no effect. In contrast, Grobbelaar *et al.* (1973) found that formaldehyde treatment of sunflower oil cake meal resulted in a higher N retention and wool growth when Merino wethers were fed a basal diet of wheaten chaff, lucerne hay and maize meal and supplemented with approximately 50 g/d of treated or untreated sunflower meal.

Clean wool growth was increased by 10% when 50 g/d of either lupins or extruded lupins was included in a basal ration of 500 g oats, 50 g wheat straw and 10.5 g vitamin/mineral mix fed to 2-year-old Merino wethers in an experiment reported by Cottle (1988b). The increase in wool growth was unaffected by the form of lupins and Cottle suggested that the lupins were either already naturally protected and that heat extrusion over-protected the lupins. Examination of the diet that Cottle used suggests that the lupins acted as a rumen stimulant as work by Maeng *et al.* (1989) has show that the provision of a nitrogen source of 75% urea and 25% casein to a continuous culture media in which the energy source was soluble starch increased microbial dry matter yield by 37% above that when the nitrogen source was 100% urea.

Supplementing a basal diet of barley and hay with urea, meatmeal or lupins gave similar improvements compared with the basal diet in intake, growth rate and efficiency

of feed conversion in yearling steers (Barker *et al.* 1985). The basal diet of 80% barley may provide a lot of bypass starch and some bypass protein and hence be adequate for glucogenic precursors and the protein supplements would provide nitrogen for the rumen microorganisms.

It was shown in that the use of either untreated or treated lupin meal did not improve the productivity of growing lambs beyond that attained with urea supplementation. This indicated that (1) there was no response to protection of lupin protein, or (2) that the treatment failed to protect the lupin protein, or (3) that under the conditions of the experiment the animals were not able to respond to a bypass protein. Lack of protection was indicated by the high solubility of the treated lupins in rumen fluid and because similar animals fed an oaten chaff diet were shown to respond to supplementation with treated casein by increasing liveweight gain, wool growth and feed conversion efficiency (see Section 4.2).

The aim of the research that follows in this thesis was to study the effects of supplements of glucogenic and/or aminogenic substrates on the productivity and metabolism of lambs fed a low protein roughage.

4.5 Effect of protein, propionate or acetate supplementation on production, glucose metabolism and acetate clearance in lambs fed a basal diet of cottonseed hulls

4.5.1 Introduction

Using starch as the energy source, Maeng *et al.* (1989) have shown that the yield of microbial dry matter from a continuous culture system increased by 37% with the substitution of a small amount of casein for the urea nitrogen source. A similar substitution when the energy source was cellulose increased yield by only 6%. This indicated that the requirement by cellulolytic organisms for amino acids is low and that response to soluble protein will only be due to the nitrogen content. For this reason,

oaten chaff (which has variable starch content) was replaced by cottonseed hulls (which had a negligible starch content) as the basal roughage in the next experiment. The response in terms of liveweight gain, wool growth and increased efficiency of feed conversion should be attributable to amino acids from the casein being digested post-ruminally. This contrasts to the experiment reported in Section 4.2 in which the basal diet contained a reasonable amount of starch and the lamb responses may have been due to increased microbial production from the rumen in addition to that provided post ruminally by the treated casein.

As discussed in Section 2.5, ruminants require glucose for many essential functions. Whether they obtain sufficient glucose from a diet of low quality roughage is controversial (Preston and Leng 1987, Leng 1990b, Ørskov and Macleod 1990). The following experiment was designed to test whether supplying additional propionate to a diet of low quality roughage would, by providing glucogenic precursors, increase the liveweight gain and feed conversion ratio of lambs. It was hypothesised that the supply of bypass protein in addition to propionate would further stimulate productivity by providing protein to be digested post-ruminally. Conversely if acetate was added to the basal diet, it was hypothesised that the animals already on a diet producing a high molar proportion of acetate in the rumen would be metabolically imbalanced by the excess acetate and have to resort to unproductive metabolism to dispose of it, or they would need to reduce feed intake and, as a consequence, liveweight gain would be lowered.

In the following experiment, a standard bypass protein (formaldehyde treated casein) was used as a supplement to a diet of cottonseed hulls. In addition, two levels of propionate (20 and 40 g) were included to determine the effect of these levels of propionate on lamb performance. A supplement of 58 g sodium acetate, calculated to be isocaloric to 40 g sodium propionate, was included to determine if loading the animal with acetate would have any deleterious effects on animal performance.

The primary aim of this experiment was to determine animal performance in response to aminogenic and/or glucogenic supplements to a basal diet of low quality roughage.

4.5.2 Experimental

4.5.2.1 Animals and housing

Forty-two second-cross shorn ewe lambs (approximately 11 months of age and weighing between 25 kg to 34 kg) from the previous experiment were housed in individual pens on a slatted floor inside a well ventilated shed.

4.5.2.2 Experimental design

The experiment involved forty-two lambs and 6 experimental diets (7 lambs/group). Data were collected for 71 days for the growth trial and for 10 days for the metabolic study.

4.5.2.3 Diets and feeding

The basal diet consisted of *ad libitum* cottonseed hulls (94% DM and 0.63% N). Lucerne chaff (50 g) plus a vitamin/mineral/urea premix were also added to the basal diet. The cottonseed hulls were from a single batch reserved for the experiment to minimise variation. The premix consisted of 33.3% urea, 33.3% dicalcium phosphate, 16.7% sodium sulphate and 16.7% vitamin/mineral premix (Pfizer Q422) and was fed to achieve 1% of urea in the total ration. Equivalent sodium content of all diets was achieved by adding appropriate amounts of sodium chloride to the ration.

The six diets were: (1) basal, (2) basal plus 20 g sodium propionate (P20), (3) basal plus 50 g formaldehyde treated casein (TC), (4) basal plus P20 plus TC, (5) basal plus 40 g sodium propionate (P40), and (6) basal plus 58 g sodium acetate (Acet).

Formaldehyde was applied to the casein following the procedure detailed in Section 3.3. The supplements, lucerne and premix were mixed in with the cottonseed hulls and fed as one meal at 0900h daily.

4.5.2.4 Procedure

The lambs were adapted to the basal diet for a 2 week period prior to the start of the experiment. At the commencement of the experiment the lambs were weighed before feeding, ranked according to weight and allocated to groups using stratified random sampling.

Feed intake (g DM) was measured daily and liveweight of the lambs was measured each week prior to daily feeding. Wool growth was measured using dyebands (see Section 3.5). Rumen fluid samples for analysis of VFA and ammonia were taken by stomach tube from all lambs before and 4 h after feeding during the 5th and 10th weeks of the experiment. An additional sample for enumeration of protozoa was taken whilst rumen sampling in the 10th week of the experiment. The samples, before feeding and 4 h after feeding were taken on consecutive days. Rumen fluid was processed as previously described (Section 3.4).

Glucose Entry and Acetate Clearance Rate After the growth study, the total entry rate of glucose and the clearance of an exogenous load of acetate introduced into the blood was measured as described below. For 1 week before and during the metabolism study the lambs were offered 90% of their previous week's intake in 3 equal amounts at 8 h intervals. To synchronise injection times, the morning feeding on the days of injection was staggered so as to ensure that all animals received their tracer doses at the same time after feeding. This feeding regime and dosing technique had been found to give reasonable steady state conditions for tracer dilution methods in previous studies reported from these laboratories (Habib 1988, Forster 1989).

Glucose Entry Rate A catheter was inserted into one of the jugular veins of each lamb the afternoon before the 2-^{3}H-glucose isotope injection. The injection solution was prepared by adding 2.5 mCi of 2-^{3}H-glucose (Lot 2187-141, New England Nuclear) with 50 mg glucose (as carrier) to 350 ml sterile physiological saline (Travenol). Each animal received approximately 7.5 ml of this dose thus receiving approximately 54 μCi. All animals were injected simultaneously with the isotope and blood samples were taken from each lamb via the jugular vein at 30 min intervals for 3.5 hours. Blood samples and injection solutions were assayed for radioactivity according to the procedures given in Section 3.10.

Acetate Clearance Rate This was measured the day following the glucose study using the method is given in Section 3.11.

4.5.2.5 Statistical analysis

Data from the basal, basal plus treated casein (TC), basal plus 20 g propionate (P20) and basal plus TC plus P20 groups were analysed as a 2 x 2 factorial. Data from the 2 groups, 58 g acetate and 40 g propionate, were then included with the above 4 treatment combination in a one-way analysis of variance and between treatment means examined. The standard errors of means given in the tables are from the one way analysis of variance. Results of the statistical analyses for the factorial experiment are given in the appropriate Appendix.

4.5.3 Results

The results and statistical analysis for dry matter intake, liveweight gain, feed conversion ratio and wool growth are given in Table 4.5 and Appendix 4.5.

Table 4.5 Dry matter intake, liveweight gain, feed conversion ratio and wool growth of lambs fed a basal diet of cottonseed hulls (CSH) and supplemented with 20 or 40 g sodium propionate (P20, P40), 50g formaldehyde-treated casein (FC), FC + P20 or 58 g sodium acetate (Acet).

Measurements	Supplement							
	Nil	P20	P40	FC	FC/P20	Acet	SEM	Significance
Intake (g DM/d)								
CSH	898	1001	932	946	1060	976	47	*
Total	966	1077	1016	1050	1172	1061	47	*
Liveweight gain (g/d)	75	99	84	138	141	95	11	***
Feed Conversion Ratio (g/g)	15.5	11.2	12.4	7.6	8.3	12.9	1.6	**
Wool Growth (g/d)	6.1	5.7	5.9	9.1	8.8	5.8	0.4	***

4.5.3.1 Intake

Cottonseed Hull Intake (CSH) Lambs given 20 g propionate had significantly higher ($P<0.05$) intakes of CSH than lambs in other supplement groups. No other supplements affected significant differences in intake. CSH intake by the nil, P20, P40, FC, FC/P20 and Acet supplemented sheep was 898, 1001, 932, 946, 1060 and 976 g/d respectively.

Total Dry Matter Intake (TDMI) The addition of 20 g sodium propionate to the basal diet significantly ($P<0.05$) increased total dry matter intake of lambs. There was a trend ($P<0.10$) for sheep given treated casein to have a higher TDMI than control sheep. The TDMI by the nil, P20, P40, FC, FC/P20 and Acet supplemented sheep was 966, 1077, 1016, 1050, 1172 and 1061 g/d respectively.

4.5.3.2 Liveweight

Liveweight (kg) Analysis of weekly liveweights for the casein treatments showed a significant ($P<0.001$) linear trend. The increase in liveweight due to treated casein was significantly different ($P<0.05$) after week 2.

Liveweight Change (g/d) Liveweight gain was increased ($P<0.001$) in lambs given treated casein. No other treatment differences were significant. Liveweight gain by the nil, P20, P40, FC, FC/P20 and Acet supplemented sheep was 75, 99, 84, 138, 141 and 95 g/d respectively.

4.5.3.3 Feed conversion ratio

Feed conversion ratio was significantly ($P<0.001$) improved in lambs given treated casein. No other treatments differences were significant and no interaction was observed between the treatments treated casein and 20 g sodium propionate on their

effect on FCR. FCR by the nil, P20, P40, FC, FC/P20 and Acet supplemented sheep was 15.5, 11.2, 12.4, 7.6, 8.3 and 12.9 g/d respectively.

4.5.3.4 Wool growth

Wool growth was increased ($P<0.001$) in lambs given treated casein. No other treatments differences were significant and no interaction was observed between the treatments treated casein and 20 g sodium propionate. Wool growth by the nil, P20, P40, FC, FC/P20 and Acet supplemented sheep was 6.1, 5.7, 5.9, 9.1, 8.8, and 5.8 g/d respectively.

4.5.3.5 Protozoa numbers

No protozoa were found in any of the rumen fluid samples taken before or four hours after feeding in the last week of the trial after 12 weeks of cottonseed hull feeding.

4.5.3.6 Glucose metabolism

The results and statistical analysis of glucose entry rate, glucose pool size, plasma glucose concentration, acetate clearance rate and $t^{1/2}$ of acetate in the blood are given in Table 4.6 and Appendix 4.6.

There were no significant differences in glucose entry rates between treatments. The addition of treated casein to the basal diet significantly ($P<0.05$) increased glucose pool size from 4.9 g/d to 5.5 g/d. No other treatments produced significant effects.

4.5.3.7 Acetate clearance rate

There were no significant differences between lambs given different supplements in $t^{1/2}$ of acetate in the blood.

Table 4.6 Mean plasma glucose, glucose entry rate, glucose pool size and the half time ($t^{1/2}$) for acetate in the blood of lambs fed a basal diet of cottonseed hulls (CSH) and supplemented with 20 or 40 g sodium propionate (P20, P40), 50g formaldehyde-treated casein (FC), FC + P20 or 58 g sodium acetate (Acet).

Measurements	Supplement							
	Nil	P20	P40	FC	FC/P20	Acet	SEM	Significance
Mean Plasma Glucose (mg/100ml)	62	62.9	62.4	62.4	65.8	65.3	1.6	ns
Glucose Entry Rate (g/d)	88.6	91.0	99.0	96.2	103.0	92.1	5.5	ns
Glucose Pool Size (g)	4.9	5.0	5.1	5.3	5.6	5.0	0.2	*
Blood Acetate $t^{1/2}$ (min)	27	25	23	25	23	30	2.0	ns

Table 4.7 Total concentration of VFA and molar proportions of individual VFA, ammonia levels and glucogenic energy ratio in the rumen fluid of lambs fed a basal diet of cottonseed hulls (CSH) and supplemented with 20 or 40 g sodium propionate (P20, P40), 50g formaldehyde-treated casein (FC), FC + P20 or 58 g sodium acetate (Acet).

Measurements	Supplement							
	Nil	P20	P40	FC	FC/P20	Acet	SEM	Significance
Before feeding								
Total VFA (mmol/l)	61.7	56.8	53.4	50.0	52.0	63.3	6.1	ns
Molar Proportions of VFA (%)								
Acetate	79.8	80.6	82.0	68.7	80.2	79.3	4.9	ns
Propionate	14.7	14.1	13.2	12.5	14.1	14.7	1.8	ns
Isobutyrate	0.24	0.27	0.20	0.27	0.36	0.20	0.06	ns
Butyrate	4.4	4.2	3.8	3.5	4.0	5.0	0.4	ns
Isovalerate	0.32	0.23	0.21	0.24	0.30	0.15	0.08	ns
Valerate	0.55	0.59	0.54	0.54	0.79	0.61	0.06	ns
Ammonia (mg N/l)	18	19	19	34	34	23	4	ns
G/E	0.21	0.21	0.19	0.21	0.21	0.21	0.02	ns
4 h after feeding								
Total VFA (mmol/l)	60.9	69.3	75.1	55.8	67.2	84.7	5.3	*
Molar Proportions of VFA (%)								
Acetate	75.8	66.9	58.9	64.6	65.9	82.2	4.5	*
Propionate	19.1	28.6	37.3	16.9	29.7	13.7	1.6	ns
Isobutyrate	0.08	0.05	0.06	0.12	0.10	0.05	0.02	†
Butyrate	4.3	3.7	3.0	3.5	3.3	3.7	0.3	*
Isovalerate	0.16	0.07	0.08	0.11	0.08	0.05	0.06	ns
Valerate	0.57	0.69	0.66	0.57	0.85	0.39	0.06	**
Ammonia (mg N/l)	250	187	187	157	182	229	24	†
G/E	0.27	0.39	0.49	0.28	0.40	0.20	0.01	***

4.5.3.8 Rumen fermentation

The results and statistical analysis of total VFA concentration and molar proportions of VFA for before and 4 h after feeding are given in Table 4.7 and Appendices 4.7 and 4.8. No significant differences between the samples taken during the 5th or the 10th weeks of the experiment were noted and hence only the means are presented.

Addition of 20 g sodium propionate to the basal diet increased ($P<0.05$) the molar proportion of valerate but no other significant effects were noted in the sample taken before feeding. Propionate proportions were higher ($P<0.001$) 4 h after feeding in lambs given 20 g or 40 g sodium propionate. Valerate molar proportions were increased ($P<0.01$) 4 h after feeding in lambs given 20 g sodium propionate. The molar proportions of acetate and butyrate were lower ($P<0.01$) 4 h after feeding in lambs given 40 g sodium propionate. Acetate and valerate proportions were higher ($P<0.05$) 4 h after feeding in the rumen fluid of lambs given sodium acetate.

Rumen ammonia levels were higher before ($P<0.001$), and after feeding ($P<0.10$) in lambs given treated casein. No other treatment differences were significant.

Sodium propionate increased ($P<0.001$) the glucogenic potential of the diet. No other treatment differences were significant.

4.6 *In vivo* digestibility of a basal diet of *ad libitum* cottonseed hulls with or without formaldehyde treated casein

When it was found that protozoa had been eliminated from the rumens of the sheep in the experiment reported above, a small trial was conducted to monitor the effect of feeding cottonseed hulls on the protozoa numbers in the rumen. The digestibility of the cottonseed hulls used in the experiment reported above was also determined.

4.6.1 Experimental

4.6.1.1 Animals and housing

Eight mature crossbred wether sheep with permanent rumen fistulae and a mean liveweight of 39 kg were used for this experiment. They were housed in metabolism crates in a well ventilated animal house. Feed was offered at 0900 h daily with water being available at all times.

4.6.1.2 Experimental design

The experiment involved 8 sheep with 2 dietary treatments (4 sheep/group).

4.6.1.3 Diets and feeding

The basal diet was cottonseed hulls (CSH) fed *ad libitum* from the same source as that used in the previous experiment. Lucerne chaff (50 g) and a urea/vitamin/mineral mix was added to the basal diet. The premix has been described previously (Section 4.5).

The two diets were (1) basal, (2) basal plus 50 g treated casein (see Section 3.3 for details of formaldehyde treatment).

4.6.1.4 Procedure

The sheep were gradually introduced to the CSH diet for a period of 1 week before the start of the experiment. The experimental diets were fed for a period of 6 weeks. For the last 7 days of this period the feed intake was restricted to 90% of the previous week's intake and a total collection of faeces was made from each sheep. The daily production of faeces during this 7 day period was weighed and a subsample taken for dry matter analysis. These were bulked and stored at -20^{o}C until analysed. Feed intake was measured daily and samples of feedstuffs and feed refusals were taken daily

for dry matter analysis. Crude protein content of the CSH was determined using the method given in Section 3.9.

The dry matter disappearance of CSH was measured using the *in sacco* technique (see Section 3.8) using the 4 sheep fed the basal diet.

Rumen fluid samples were taken from the rumen fistula using a sampling probe (see Section 3.4) for enumeration of protozoa numbers on days 1, 5, 8, 10, 17, 24 and 36 using the procedure described in Section 3.6.

4.6.2 Results

4.6.2.1 Digestibility study

There was no significant differences in digestibility of the diet due to supplementation with treated casein (Table 4.8).

Table 4.8 ***Cottonseed hull intake and dry matter digestibility of a diet of cottonseed hulls supplemented with 0 or 50 g/d formaldehyde treated casein and fed to mature crossbred wethers***

Supplement	Mean Lwt (kg)	CSH Intake (g/d)	Digestibility *In vivo*	*In sacco* 24h	48h
Nil	37.5	880	45.6 (±1.9)	16%	31%
Treated casein	40.1	1090	45.3 (±1.9)		

The disappearance of CSH from the nylon bags was slow with only 31% digestion of the CSH occurring after 48 h.

4.6.2.2 Protozoa numbers

Protozoa numbers in the rumen fluid, estimated immediately before feeding are given in Table 4.9.

Table 4.9 ***Changes in the protozoa populations in the rumen fluid of sheep fed a basal diet of cottonseed hulls with 0 or 50 g/d treated casein.***

Supplement	Protozoa Numbers ($x10^{-5}$)						
Days	1	5	8	10	17	24	36
nil	1.5	0.70	1.09	0.69	0.80	0.70	0.67
nil	0.99	0.30	0.04	0.04	0.03	0.16	0.34
nil	1.77	1.04	1.74	1.78	1.19	1.06	0.90
nil	0.79	0.23	0.15	0.13	0.49	0.15	0.11
treated casein	2.50	1.66	1.35	2.10	1.90	1.06	1.23
treated casein	0.67	0.81	0.24	0.29	0.49	0.13	0.48
treated casein	2.10	0.63	0.38	0.65	0.19	1.16	1.77
treated casein	0.58	0.38	0.41	0.47	0.16	0.43	0.16

These results are represented graphically in Figure 4.1. Although the total elimination of protozoa did not occur in this study, it can be seen (Figure 4.1) that a significant reduction in protozoa numbers occurred when the sheep were fed CSH.

4.6.3 Discussion

The results obtained from the digestibility study indicated that CSH were a low quality roughage being 45% digestible with a crude protein content of 3.9%. This was similar to the results reported by Hsu *et al.* (1987) of an *in vivo* digestibility of CSH of 42%. The observation that CSH were highly palatable to sheep was also noted by Hsu *et al.* (1987).

From the *in sacco* study, it was shown that CSH, when held in the rumen, were very slowly digested. However, the small size of the CSH may lead to a rapid passage through the rumen allowing the animal to maintain a high intake.

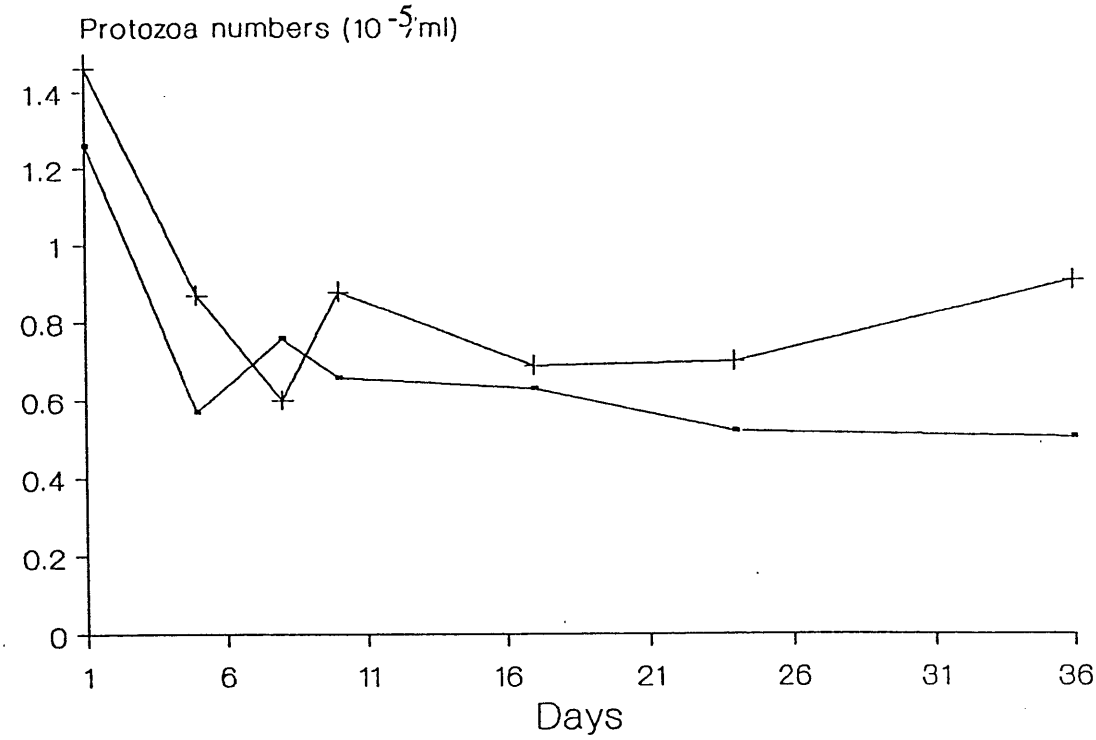

Figure 4.1 *Changes in protozoa numbers over time in the rumen fluid of lambs fed a basal diet of cottonseed hulls.*

The relatively high liveweight gain and feed conversion efficiency of the sheep in the growth study may be due in part to the elimination of protozoa from the rumen. Bird (1989) has reported significant increases in liveweight gain and wool growth in response to defaunation of sheep. He suggested that these responses are most likely due to an improvement in the protein economy of the animal. It is now generally recognised that the absence of ciliates in the rumen is associated with enhancement of microbial growth in the rumen and more microbial and dietary protein flowing from the rumen (Veira *et al.* 1983, Ushida *et al.* 1986).

The differences in results of protozoa numbers between the growth study and the trial noted above is probably due to a combination of the following factors: (1) the growth study was continued for a much longer period (14 days pre trial and 71 day growth study) as compared with the 35 day period for the above experiment, (2) fistulated sheep were used for the protozoa experiment and these are known to be more

resistant to defaunation (S.H. Bird, *pers. comm.*), (3) the level of intake of CSH was much higher for sheep in the growth study than in the protozoa study (the growth study sheep ate an average of 3.6% of their bodyweight in CSH as compared with only 2.8% intake of the sheep in the protozoa study).

Glucose entry rates (GER) were high in these lambs compared with that of similar sheep fed a basal diet of wheat straw (Habib 1988). However, unlike the results obtained by Habib (1988), supplementation with a source of bypass protein did not increase glucose entry rates. This may be due to the high GERs produced by feeding CSH rendering the need for additional gluconeogenesis superfluous. Leng (1970) has suggested that the synthesis of glucose in excess of an animals requirements would be energetically wasteful.

The data from this experiment did not clarify the roles of propionate or acetate. The high GER may have ensured adequate precursors for the utilisation of acetate excessive to the requirements of the animal for oxidative energy. Similarly, the additional propionate appeared not to be utilised for gluconeogenesis. Addition of 40 g propionate did not produce any significant differences in intake, liveweight gain, feed conversion efficiency or wool growth above that attained by 20 g propionate. Indeed, all these parameters were lower in the sheep given the higher level of propionate.

Acetate clearance is used as a measure of glucose sufficiency (Weston 1966, Preston and Leng 1987). The clearance of an acetate load from the blood of lambs in this experiment was not significantly affected by supplementation with treated casein, propionate or acetate, this may indicate that the diet was adequate in glucose.

From the studies reported in this Chapter it became obvious that feed intake responses by lambs to supplementation of a low protein diet with bypass protein could not be predicted. A possible interaction between supplementation and environmental temperature was investigated in the following Chapter.