CHAPTER 5

THE EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURE ON RUMEN FERMENTATION, DIGESTION AND PRODUCTION IN LAMBS

5.1 Introduction

In the previous experiments reported in this thesis, formaldehyde-treated casein, when added as a supplement to basal diets of oaten chaff or cottonseed hulls (CSH), induced highly significant increases in liveweight gain, wool growth and feed conversion efficiency in lambs. However, responses in intake by lambs to supplementation were variable. When formaldehyde-treated casein was added to an oaten chaff diet (Experiment 4.1) intake of the oaten chaff by lambs was significantly increased. In contrast, no increase in CSH intake by lambs was observed in Experiment 4.5 in response to the addition of a similar supplement. Leng (1990b), in reviewing the literature in this area, hypothesised that increased feed intake of low protein forages in response to supplementation with bypass protein was greatest where the animals' intake had been lowered by heat stress. He postulated that in the tropics balancing the nutrients from low quality forages with a supplement of bypass protein allowed the animal to utilise excess energy as VFA for lipogenesis rather than wasting C2 energy in futile cycles with the commensurate production of heat. Metabolic heat production would be minimised and may enable an animal to increase its intake of a basal roughage to that attained under thermoneutral conditions. Protein supplementation then allows the utilisation of C2-energy for production and in the absence of the need for oxidation

of substrate to maintain body temperature there is more substrate available for production purposes.

For ruminants on roughage diets in cooler areas, Leng (1990b) suggested that the intake of low protein roughage was higher as metabolic heat production from acetate is advantageous in maintaining body temperature. However, this meant that there was less substrate available for production purposes. The reduction in demand for glucose which would have been required for synthesis of acetate into fat (see Section 2.5) decreased the demand for amino acid utilisation for gluconeogenesis. This increased the amino acids available relative to energy in the nutrients that are surplus to requirements for anabolic processes in ruminants kept under temperate to cool conditions.

The hypothesised interaction in the animal, between balancing nutrient supply to demand and environmental temperature, led to the examination of the environmental conditions prevailing when the previous experiments were carried out. It was observed that the experiment using oaten chaff as the basal roughage (Experiment 4.3) was done during summer and mean intake of the unsupplemented lambs was 60 g DM/$W^{0.75}$/d. In contrast, the experiment using CSH (Experiment 4.5) was carried out during winter and the intake of the unsupplemented lambs was 73 g DM/$W^{0.75}$/d. In Armidale the temperature in summer generally ranges from 20 to 30 ^{o}C during the day falling to 15 to 20 ^{o}C at night, whilst winter temperatures range from 5 to 15 ^{o}C during the day with night temperatures frequently dropping to -5 ^{o}C.

These observations suggested that environmental temperature may be one factor influencing the unsupplemented sheep's intake. However, there are many other considerations involved in the intake of two disparate feeds that could also explain the differences in intake. The experiments which follow were designed to test the concept that environmental temperature may be influencing intake.

A series of three experiments were undertaken with lambs kept at medium or high environmental temperatures and given a basal diet of either CSH or ammoniated barley straw and supplemented with treated casein or sodium propionate. The major objective of these studies was to examine the possible interactions between high environmental temperature and supplementary feeding on productive performance of lambs. The underlying metabolic responses were also studied at the end of the second feeding trial.

5.2 Liveweight gain, feed intake and wool growth of lambs fed a basal diet of cottonseed hulls supplemented with bypass protein and kept at high temperatures

5.2.1 Experimental

5.2.1.1 Animals and housing

Twenty-four crossbred wether lambs with a mean liveweight of 25 kg and approximately 3 months of age were obtained from a flock at pasture. They were kept in metabolism crates in rooms that were ventilated by forced-draught fans and continuously illuminated with fluorescent lighting. Before commencement of the study the lambs were drenched with Seponver (Smith Kline Animal Health Products) and Nilvem (ICI Australia) against gastro-intestinal parasites.

5.2.1.2 Experimental design

The experiment involved 24 lambs in a 3 x 2 factorial design with three different environmental temperatures and two diets (4 lambs/group).

5.2.1.3 Diets and feeding

The basal diet was CSH fed *ad libitum*, lucerne chaff (50 g), urea (1% w/w) and 17.5 g mineral/vitamin mixture. The mineral/vitamin mixture was previously described (Section 4.2).

The two diets fed were (i) basal diet, and (ii) basal diet plus 50 g formaldehyde-treated casein (treated casein). The casein was treated with formaldehyde to achieve 0.78 g HCHO/16 g N following the procedure detailed in Section 3.3.

The supplement, lucerne chaff, urea and vitamin/mineral mixtures were mixed with the CSH and fed as one meal at 0900 h.

5.2.1.4 Temperatures

The animals were housed in three rooms maintained at either 25±4 ^{O}C (room 1), 27±3 ^{O}C (room 2) and 37±2 ^{O}C (room 3). The relative humidity varied between 40-70% for rooms 1 and 2 and from 35-55% for room 3.

5.2.1.5 Procedure

After selection, the lambs were housed in individual pens with slatted wooden floors in a well-ventilated shed for 2 weeks and fed a mixture of oaten and lucerne chaff to accustom them to pen conditions. They were then put in metabolism crates in one of the three rooms and given a further 2 weeks acclimatization. During this time they were fed a mixture of CSH and oaten chaff and rooms were maintained at ambient temperature.

At the commencement of the experiment the room temperatures were changed to the experimental temperatures. On this day the lambs were weighed, ranked according to weight and allocated to groups using stratified random sampling. The experiment lasted for 49 days. Feed intake (g DM) was recorded daily throughout the

experimental period. The lambs were weighed before feeding on days 1, 14, 29 and 49. During the last week of the experiment, rumen fluid samples were taken from all lambs through stomach tubes on two consecutive days. The first sample was taken before feeding and the second sample was taken 4 h after feeding. These samples were analysed for VFA and ammonia following the procedures described in Section 3.9. Rumen fluid samples for enumeration of protozoa were taken from all lambs before feeding on two occasions. The first sample was taken at the time the lambs were selected for the trial (before they had started consuming CSH). The second sample was taken on the last day of the experimental period (day 49).

Wool growth during the experimental period was estimated using the dyeband technique described in Section 3.5.

5.2.1.6 Statistical analysis

The data were analysed using the computer program, NEVA (see Section 3.12). Due to the temperatures in rooms 1 and 2 being similar the temperature sum of squares was partitioned into components of temperature 1 (T1) versus temperature 2 (T2) at 1 degree of freedom and the pooled temperature (T1 and T2) versus temperature 3 (T3) at 1 degree of freedom. This partitioning was performed for the parameters liveweight gain, feed conversion ratio, wool growth and dry matter intake of the lambs.

5.2.2 Results

In all instances there were no significant differences between T1 and T2 in the parameters measured. The partitioned AOV is given in Appendix 5.1. Lambs kept in rooms 1 and 2 are designated as being kept at 26^{o}C. The results given below are for the combined data of lambs in rooms 1 and 2 compared with that of lambs housed at 37 ^{o}C.

Table 5.1 Dry matter intake, liveweight gain, feed conversion ratio and wool growth of lambs fed a basal diet of cottonseed hulls (CSH) and supplemented with 0 or 50g formaldehyde treated casein (FC). Lambs were housed at 26 or 37 °C.

Measurements	Supplement							Significance		
	Nil		50 g FC		Means		SEM	Temp	Diet	TxD
	26°C	37°C	26°C	37°C	26°C	37°C				
Intake										
CSH (g DM/d)	960	899	961	955	961	927	20	ns	ns	ns
(g DM/$Wt^{0.75}$/d)	81	73	79	75	80	74	2	*	ns	ns
Total (g DM/d)	1026	964	1074	1067	1050	1015	34	ns	ns	ns
(g DM/$Wt^{0.75}$/d)	86	79	88	84	87	81	2	**	ns	ns
Liveweight Change (g/d)	103	93	141	114	122	104	10	*	***	ns
Feed Conversion ratio (g/g)	10.1	10.4	7.8	9.6	9.0	10.0	0.6	*	***	*
Wool Growth (g/d)	3.1	3.3	4.5	3.8	3.8	3.6	0.3	ns	***	*

5.2.2.1 Thermal humidity index

The calculated thermal humidity indices (THI, see Section 2.6.1) for rooms at 26 ^{o}C or 37 ^{o}C were between 71-74 and 85-87 respectively.

5.2.2.2 Intake

The results and statistical analysis of dry matter intake, liveweight gain, feed conversion ratio and wool growth are given in Table 5.1 and Appendix 5.2.

Lambs housed at 37 ^{o}C had a significantly lower intake (g DM/$W^{0.75}$/d) of CSH ($P<0.05$) and total dry matter ($P<0.01$) than lambs housed at 26 ^{o}C. Supplementation with treated casein had no significant effects on intake by lambs at any temperature.

5.2.2.3 Liveweight gain

Lambs housed at 37 ^{o}C had a lower ($P<0.05$) liveweight gain than lambs housed at 26 ^{o}C whilst liveweight gain was increased ($P<0.001$) in lambs given treated casein.

5.2.2.4 Feed conversion ratio (FCR)

FCR by the lambs improved ($P<0.001$) in response to supplementation of the basal diet with treated casein and declined ($P<0.05$) in the lambs housed at 37 ^{o}C. No interaction was observed between the use of treated casein and room temperature.

5.2.2.5 Wool growth

Wool growth of the lambs increased ($P<0.001$) in response to supplementation of the basal diet with treated casein. Changing room temperature had no significant affect on wool growth.

Table 5.2 Molar proportions and total concentration of VFA in the rumen fluid of lambs fed a basal diet of cottonseed hulls and supplemented with 0 or 50 g formaldehyde-treated casein (FC). Lambs were housed at either 26 or 37 °C.

Measurements	Supplement							Significance		
	Nil		FC		Means		SEM	Temp	Diet	TxD
	26°C	37°C	26°C	37°C	26°C	37°C				
			Before feeding							
Total VFA (mmol/l)										
VFA Proportions (%)	80.0	69.7	95.0	85.1	88.0	77.0	5.2	*	**	ns
Acetate	81.4	82.2	82.6	83.8	81.6	83.0	1.0	ns	†	ns
Propionate	11.7	12.1	11.4	10.6	11.5	11.4	0.7	ns	†	ns
Bytyrate	6.5	5.0	5.6	4.6	6.0	4.8	0.5	*	†	ns
Iso-butyrate	0.29	0.27	0.32	0.34	0.30	0.31	0.04	†	ns	ns
Valerate	0.27	0.21	0.44	0.36	0.34	0.29	0.04	ns	***	ns
Iso-valerate	0.24	0.21	0.28	0.32	0.26	0.27	0.06	ns	ns	ns
Ammonia (mgN/l)	29	14	64	62	47	38	9	ns	***	ns
			4h after feeding							
Total VFA (mmol/l)	95.6	77.5	101.9	96.8	98.8	87.2	6.5	†	†	ns
VFA Proportions (%)										
Acetate	80.7	82.3	81.5	82.3	81.1	82.3	0.7	ns	ns	ns
Propionate	12.9	12.4	12.3	11.9	12.6	12.2	0.5	ns	ns	ns
Bytyrate	5.9	4.9	5.03	4.9	5.6	4.9	0.5	†	ns	ns
Iso-butyrate	0.07	0.08	0.23	0.23	0.15	0.14	0.04	ns	***	ns
Valerate	0.34	0.26	0.47	0.42	0.41	0.34	0.04	ns	***	ns
Iso-valerate	0.09	0.08	0.17	0.21	0.13	0.15	0.03	ns	**	ns
Ammonia (mgN/l)	232	194	276	215	254	205	21	*	*	ns

5.2.2.6 Rumen fermentation

The results and statistical analysis of the total concentrations of VFA, molar proportions of individual VFA, and ammonia concentration in the rumen fluid of the experimental lambs estimated before and 4 h after feeding during the last week of the experiment are given in Table 5.2. The results of analysis using three temperatures is given in Appendix 5.3 and 5.4.

Addition of treated casein to the basal diet increased the pre-feeding molar proportions of valerate ($P<0.001$) and total VFA concentration ($P<0.01$) in the rumen fluid of the lambs. After feeding, the molar proportions of iso-butyrate ($P<0.001$), valerate ($P<0.001$) and iso-valerate ($P<0.01$) were increased in response to supplementation of the basal diet with treated casein. Increasing room temperature decreased ($P<0.05$) the molar proportions of butyrate in the rumen fluid samples taken before feeding.

Rumen ammonia levels in the rumen fluid of the experimental lambs were increased by addition of treated casein to the basal diet both before ($P<0.001$) and 4 h after ($P<0.05$) feeding. Rumen ammonia levels decreased ($P<0.05$) at the higher room temperature.

5.2.2.7 Protozoa numbers

The results and statistical analysis of pre-trial and end-of-trial rumen protozoa numbers are given in Table 5.3.

Table 5.3 ***Protozoa numbers and percent decrease in protozoa numbers occurring in the rumen fluid of lambs kept at one of three temperatures (25, 27 or 37 ^{o}C) and fed a basal diet of cottonseed hulls supplemented with 0 or 50 g formaldehyde-treated casein.***

Measurement	Supplement						Significance$^{\Phi}$			
	Nil			Treated casein			SEM	T	D	TxD
	25°C	27°C	37°C	25°C	27°C	37°C				
Protozoa Nos. ($x10^{-5}$)										
Pre-trial	7.7	11.7	8.5	8.5	12.1	7.6	1.8	ns	ns	ns
End-trial	1.4	2.9	1.1	1.2	1.0	1.9	0.8	ns	ns	ns
% Decrease	71	70	85	85	93	75	11	ns	ns	ns

$^{\Phi}$ T = temperature, D = diet

No significant differences occurred in protozoa population densities in rumen fluid in response to either increasing temperature or supplementation with treated casein. The decrease in protozoa numbers over time by feeding CSH was highly significant ($P<0.001$) and was similar over diets and temperatures.

5.2.3 Discussion

The decrease in dry matter intake by lambs in response to increased environmental temperature agrees with reports in the literature (Wayman *et al.* 1962, Bianca 1965, Finch 1984). However, dry matter intake (g DM/$W^{0.75}$/d) by unsupplemented lambs at 37 ^{o}C was the same as that of similarly fed lambs kept at a much lower environmental temperature in Experiment 4.6. This indicated that though the THI was high, the lambs may have been able to cope relatively easily with high temperature in the absence of other stresses such as solar radiation (Finch 1984).

One of the difficulties in attempting to simulate heat stress in ruminants is that ruminants grazing pasture are subject to other stresses in addition to temperature. It is probably the combination of stresses such as infrequent watering, disturbance by insect pests, energy expenditure in foraging, air movement, dust, temperature and solar radiation that is critical in determining the stress imposed on ruminants.

In the experiment reported here, the animals were constrained and so their energy expenditure would be far less than in a grazing situation, their food and water were available *ad libitum*. Hence, these factors alone may have influenced their response to increasing environmental temperature. The similarity in feed intake by lambs in this experiment and those used in Experiment 4.6 suggests that temperature stress imposed here was not severe in comparison to what may be observed in a field situation and the lack of interaction found between environmental temperature and supplementation with bypass protein may be due to this factor. The animals were also ensured of a relatively efficient rumen ecosystem through supplementation with minerals and urea which would have assisted in ensuring a relatively high P/E ratio in the nutrients absorbed.

Despite the addition of 1% urea to the basal diet the prefeeding levels of rumen ammonia in lambs in this experiment were low (14 -67 mg N/l). Satter and Slyter (1974) suggested that a ammonia level of 50 mg N/l rumen fluid was needed for efficient microbial growth. In comparison, Australian studies (Krebs and Leng 1984, Boniface *et al.* 1986, Perdok *et al.* 1988) with sheep and cattle fed low quality roughages found that intake and cellulose digestion (in sheep) was optimised at about 200 mg N/l of rumen fluid. The high intake levels achieved in the experiment reported here lend support to the theory of Leng (1990b), that when there is a high P/E ratio in the products absorbed, feed intake will be maximised at a rumen ammonia level lower than that required when the animal relies totally on the products of fermentative digestion for its amino acid sources.

The higher rumen ammonia levels of lambs supplemented with treated casein may indicate that (1) the casein was not completely protected by formaldehyde treatment and that part of the casein was slowly broken down in the rumen; or (2) that considerable deamination of casein amino acids occurred in the animal and the urea produced was recycled.

The hypothesis, that the combination of high environmental temperature and supplementation with bypass protein would result in a large difference in performance of lambs fed a low quality roughage, was not supported by the results of this experiment. One factor that may have influenced these results reported here is the protozoa population in the lambs. Feeding of CSH supported only a low population of protozoa in the rumen. This low population density may have ensured a higher amount of protein from microbial (Veira *et al.* 1983) and feed sources (Ushida *et al.* 1986) digested in the small intestine than would have occurred on an oaten chaff diet. This factor, in combination with the high dry matter intake, may partly account for the high liveweight gains of these lambs on what was considered to be an extremely low quality diet. Research from these laboratories (Soetanto *et al.* 1985, Romulo *et al.* 1988) and that from Demeyer *et al.* (1982) has shown that elimination of protozoa from the rumen of lambs fed low quality roughages produced a liveweight gain response equivalent to adding 10% fish meal to the basal diet.

The small particle size of the CSH may influence feed intake through its effect on rate of passage through the rumen (Balch 1950). This could have resulted in some escape of feed protein due to an increased turnover and outflow of rumen contents.

Thus the animals on the diet of CSH may have had a higher P/E ratio in the nutrients absorbed, possibly 30% higher than would be expected on a low quality forage based diet. This may have removed any nutrient imbalances and therefore any possibility of acetate being in surplus to that which could be utilised by the animal in anabolic processes. It may also account for the relatively high growth rates of the lambs fed this diet.

Although the glucogenic ratios of VFA in the rumen of the lambs in this experiment were low (0.15-0.21) (see Perdok and Leng 1990) the results from Experiment 4.6 showed that lambs fed CSH had high glucose entry rates (85 g glucose/d). In comparison, a diet of wheat straw, 160 g maize, urea and minerals

produced glucose entry rates of only 54 g/d in lambs of a similar age and breed (Habib 1988). The high glucose entry rates in lambs on a diet of CSH indicates a highly efficient rumen system and a high rate of absorption of glucogenic substrates.

The decreased efficiency of feed conversion observed at the higher temperatures was probably a result of the lowered feed intake but have may also indicated that the metabolic rate of these animals was increased (Bianca 1965) or that the efficiency of protein use decreased (McDowell *et al.* 1969). The decrease in wool growth in response to higher environmental temperature of the animals supplemented with treated casein pointed towards a decreased efficiency of protein utilisation.

Follow up studies were undertaken to further elucidate the interaction of temperature and supplementation, using a different basal diet, on the role of environmental temperature in ruminant production.

5.3 Growth of lambs fed a basal diet of ammoniated barley straw with or without bypass protein and sodium propionate and kept at high temperatures

5.3.1 Introduction

In comparison to what would be observed on an apparently higher quality oaten chaff diet, glucose entry rates and feed conversion efficiencies were comparatively high in animals fed CSH, which indicated that this diet was not typical of a low quality forage. It was felt that the high glucose entry rates may have increased the ability of the animals in the experiment to accommodate the high environmental temperature. In the following experiment, ammoniated barley straw was used as the basal diet. Feeding of straw has been shown to support low glucose entry rates (Habib 1988) and low growth rates in lambs and is more typical of a low quality forage available to grazing animals on dry savannahs or pastoral areas in Australia. In the following studies treated casein and/or sodium propionate were included as supplements to the basal diet fed to lambs to

provide additional aminogenic and/or glucogenic substrates and to test their effects in ameliorating the adverse effects of high environmental temperatures on feed intake of poor quality forage.

5.3.2 Experimental

5.3.2.1 Animals and housing

Thirty-two second cross wether lambs with a mean liveweight of 25 kg and approximately 6 months of age were obtained from a flock at pasture. They were placed into metabolism crates in one of 4 rooms. The rooms were ventilated by a forced-draught air fan and continuously illuminated with fluorescent lighting. Before commencement of the study the lambs were drenched with Seponver (Smith Kline Animal Health Products) and Nilvern (ICI Australia) against gastro-intestinal parasites.

5.3.2.2 Experimental design

The experiment involved 32 lambs in a 2 x 4 factorial experiment with two temperatures and four diets (4 lambs/group). The experimental period was 70 days.

5.3.2.3 Diets

The basal diet consisted of *ad libitum* ammoniated barley straw. The barley straw was ammoniated with 4% (w/w) gaseous ammonia at 90 ^{o}C using a commercial oven (An-Str-Verter, FMA, Fuglebjerg, 4250, Denmark) according to the method described by Perdok and Leng (1987). Lucerne chaff (50 g) and a vitamin/mineral premix (17.5 g) were also added to the basal diet. The premix was previously described in Section 4.2.

The four diets used in the experiment were (1) basal, (2) basal plus 50 g formaldehyde-treated casein (treated casein), (3) basal plus 20 g sodium propionate, and

(4) basal plus 50 g treated casein plus 20 g sodium propionate. The casein was treated with formaldehyde to achieve 0.78 g HCHO/16 g N following the procedure detailed in Section 3.3.

Initially, the premix lucerne chaff and supplements were weighed separately and placed on top of the barley straw but were subsequently pelleted together and fed on top of the barley straw. It was observed during the experiment that the lambs would eat the pellets before they ate the barley straw and that there were no instances of animals leaving the pelleted supplements.

5.3.2.4 Temperature and humidity

The average temperature was maintained at either 25±2 ^{o}C or 37±2 ^{o}C. The relative humidity varied between 30 and 60% and between 50 and 60% respectively.

5.3.2.5 Procedures

Before starting the experiment the lambs were adapted for 2 weeks to the metabolism crates during which time they were fed a mixture of lucerne chaff and barley straw and rooms were maintained at ambient temperature.

At the commencement of the experiment lambs were weighed, ranked according to weight and allocated to groups using stratified random sampling. The water supplied for sheep housed at 37 ^{o}C was also maintained at approximately 37 ^{o}C.

Feed intake (g DM) was recorded daily throughout the experimental period. The lambs were weighed at weekly intervals before feeding. During the 10th week of the experiment, a rumen fluid sample was taken from all lambs through a stomach tube, on two consecutive days. The first sample was taken before feeding and the second sample was taken 4 h after feeding. Samples for protozoa, VFA and ammonia analysis were taken following the procedure described in Section 3.4.

Estimation of wool growth: Wool growth was estimated using the dyeband technique described in Section 3.5.

In vivo digestibility. The *in vivo* (whole tract) apparent digestibility of dry matter and nitrogen balance by lambs was measured over a 6 day collection period at the end of the 10 week growth study. Faeces were collected in plastic bags and urine in bottles containing 30 ml glacial acetic acid. The total amounts of faeces and urine were recorded daily. Each sample was mixed and a 10% subsample taken which was bulked over the 6 day period. The total amounts of feed offered and refused were recorded and subsamples bulked for subsequent analyses. All samples of faeces and urine were stored at -20 ^{O}C until analysed. Dry matter and nitrogen content of samples were determined using the procedures given in Section 3.9.

The apparent digestibility of dry matter by the lambs was calculated as the difference between the amount of dry matter consumed and that excreted in the faeces. The nitrogen retention was calculated as the difference between total nitrogen consumed and that excreted in the faeces and the urine. The digestible energy content of the diet was measured using an adiabatic bomb calorimeter and was calculated as the difference of the energy intake and that excreted in the faeces.

Evaporative water loss was calculated during the 6 day digestibility study as the difference between water intake (intake of water from both feed and water) and that excreted in urine and faeces and corrected for evaporation from water troughs.

5.3.2.6 Statistical analysis

The data were initially analysed as a split-plot design, however, for all parameters the main-plot error mean square was homogeneous with the sub-plot error mean square and therefore the data were analysed as a factorial design. The program Neva (Burr 1980) was used to analyse the data (missing observations were estimated by this program) as a 2 x 2 x 2 factorial with 4 replications per treatment combination. The

residuals were examined and in no case was a transformation of data necessary in order to stabilise the variance. For the parameters liveweight gain and dry matter intake the program BMDP 2V for repeated measures was used in order to account for possible correlations between successive observations.

For all parameters measured Dunnett's test (one tailed) was performed to test for significant differences of treatment means from the control lambs kept at 25 ^{o}C and fed the basal diet.

5.3.3 Results

5.3.3.1 Thermal humidity index

The calculated thermal humidity indices (THI) for the rooms at 25 ^{o}C or 37 ^{o}C were between 68 and 72 and 87 and 88 respectively.

5.3.3.2 Intake

The results and statistical analysis of dry matter and digestible energy intake, liveweight change, feed conversion ratio and wool growth are given in Table 5.4 and in Appendix 5.5

The mean weekly feed intakes by the lambs are represented in Figure 5.1.

Straw Intake Intake of straw (g DM/$W^{0.75}$/d) by the lambs was decreased ($P<0.01$) at 37 ^{o}C and in response to supplementation of the basal diet with propionate ($P<0.01$). There was an interaction between environmental temperature and propionate supplementation on straw intake such that the decrease in intake of lambs due to propionate supplementation was only significant at 37 ^{o}C.

Examination of the weekly intakes by the groups held at different temperatures showed a significant linear trend. The difference between the groups was significant

Table 5.4 Dry matter and digestible energy intake, liveweight change, feed conversion ratio and wool growth of lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 °C.

Measurements	Supplement											Significance$^{\Phi}$		
	nil		FC		P20		FC/P20		Means		SEM	T	D	DxT
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C				
Intake														
Straw (g DM/d)	640	577	676	587	633	511	677	425	657	525	32	***	*	*
(g DM/d/$W^{0.75}$)	56	53	51	48	50	44	49	37	50	45	2	**	**	*
Total (g DM/d)	703	640	785	696	711	589	802	550	750	619	32	***	*	*
(g DM/d/$W^{0.75}$)	56	59	60	57	56	51	58	48	57	53	2	*	*	*
Digestible Energy (DEI) (KJ/d)	6447	5567	7497	7147	6401	5529	7771	5907	7029	6037	281	***	***	†
Liveweight change (g/d)	21.7	-20.1	53.5	30.0	26.1	-15.5	56.7	-6.1	39.5	-2.9	8.1	***	***	†
Feed Conversion Ratio# (g/g)	23.7¶	-	16.4	27.0	37.4	-	14.6	-	-	-	7.6	-	-	-
Liveweight Gain/DEI (g/MJ)	4.7¶	-	7.1	4.3	4.2	-	7.3	-	-	-	1.2	-	-	-
Wool Growth (g/d)	3.6	2.7	6.5	5.2	3.7	4.0	6.0	4.6	5.0	4.1	0.3	**	***	*

¶ mean of only 3 lambs as one lamb in this group lost weight
$^{\Phi}$ T= temperature, D= diet
lambs in some groups lost weight and the data from these groups could not be calculated.

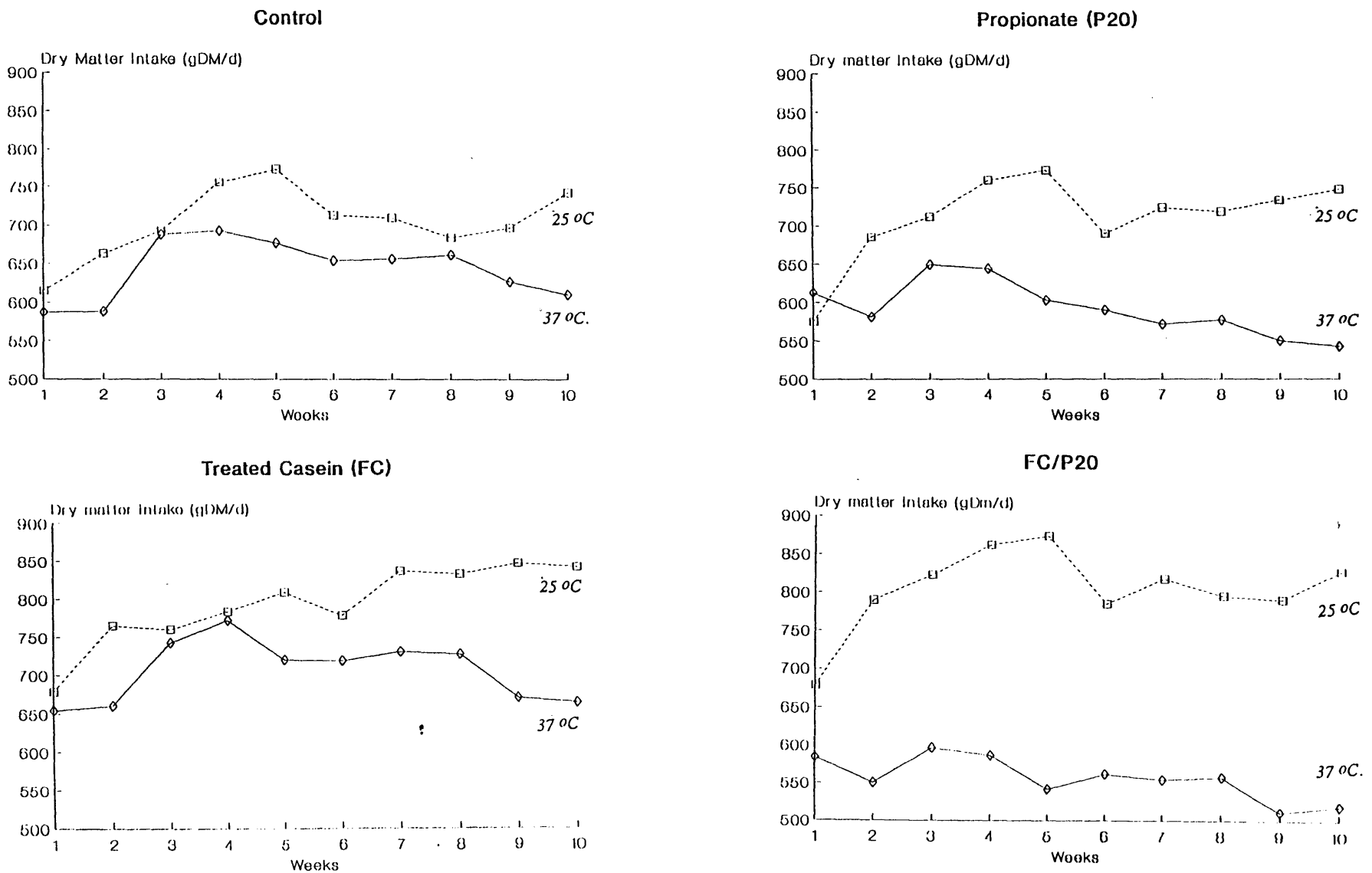

Figure 5.1 *Effects of supplementation with formaldehyde treated casein (FC) and sodium propionate (P20), and of environmental temperature, on the dry matter intake of lambs. Separate plots are shown for lambs housed at 25 °C and 37 °C.*

($P<0.001$) at week 2 and remained significant throughout the trial. The difference in intake between lambs supplemented with or without propionate was significant from week 5 onwards. There was a temperature x propionate interaction which was shown by the Greenhouse-Geisser procedure in the BMDP repeated measures analysis. The difference between intake by the unsupplemented lambs and those supplemented with propionate at 37 ^{o}C was significant ($P<0.05$) at week 4 and remained so until week 9.

Total Dry Matter Intake Intake of total dry matter (g DM/$W^{0.75}$/d) by the lambs decreased ($P<0.05$) at 37 ^{o}C and decreased ($P<0.05$) when lambs were given propionate. There was an interaction between temperature and propionate supplementation with respect to feed intake; the decrease in intake due to propionate supplementation was only significant at 37 ^{o}C.

5.3.3.3 Liveweight

The growth curves of the lambs fed the experimental diets are given in Figure 5.2.

Liveweight gain (g/d) by the lambs was significantly decreased ($P<0.001$) at 37 ^{o}C and increased ($P<0.001$) in response to supplementation of the basal diet with treated casein (Table 5.4, Appendix 5.5).

Examination of the weekly liveweights for the temperature treatments showed a significant ($P<0.001$) linear trend. At week 3 the difference between mean liveweight of lambs kept at the two temperatures was significant ($P<0.05$). This difference was not significant at week 4 but from week 5 onwards the difference was significant ($P<0.01$).

5.3.3.4 Feed conversion ratio

Due to animals on some diets losing weight statistical analysis of feed conversion ratios (FCR) was not performed. The FCR that could be calculated showed

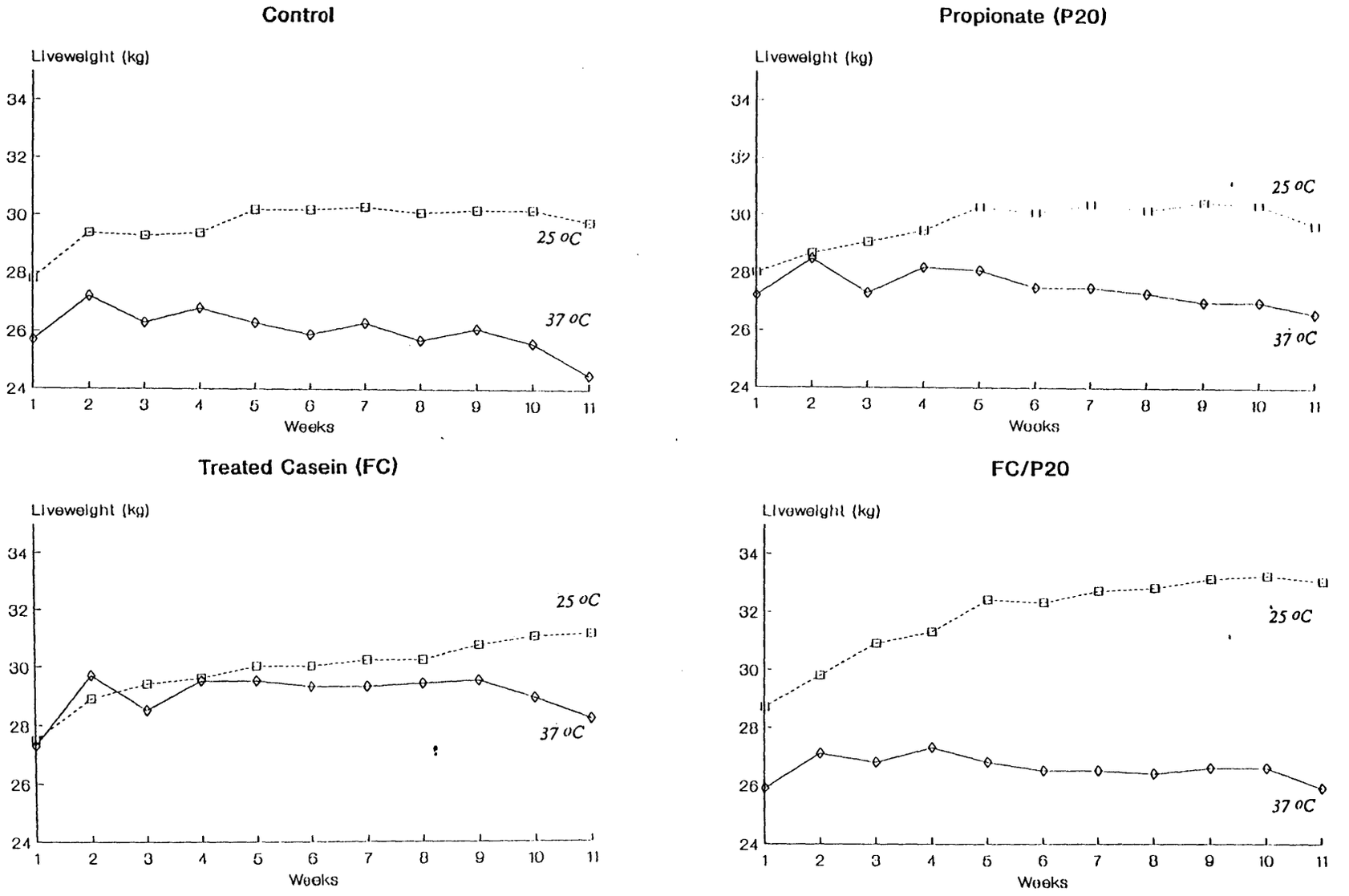

Figure 5.2 *Effects of supplementation with formaldehyde treated casein (FC) and sodium propionate (T20), and of environmental temperature, on the liveweight of lambs. Separate plots are shown for lambs housed at 25 °C and 37 °C.*

that lambs fed the basal diet supplemented with treated casein converted feed into liveweight more efficiently than unsupplemented lambs or lambs supplemented with propionate.

5.3.3.5 Efficiency of gain (liveweight gain/digestible energy intake: g/MJ)

Statistical analysis was not performed on the calculated results as data from several groups were missing due to liveweight losses in those groups. From the results available it appeared that animals receiving treated casein supplements used energy more efficiently than unsupplemented lambs or those receiving propionate. Increasing the temperature to 37 ^{o}C reduced the efficiency of energy use for liveweight gain by the lambs given treated casein to that of the propionate supplemented lambs at 25 ^{o}C.

5.3.3.6 Wool growth

Wool growth of the lambs decreased ($P<0.01$) at 37 ^{o}C and increased ($P<0.001$) in response to supplementation of the basal diet with treated casein (Table 5.4, Appendix 5.5). There was a significant ($P<0.05$) interaction between temperature and treated casein supplementation; the increase in wool growth in response to treated casein supplementation was greater at 25 ^{o}C than at 37 ^{o}C.

5.3.3.7 Digestibility

The results and statistical analysis for *in vivo* digestibility, nitrogen balance and nitrogen loses in faeces and urine are given in Table 5.5.

Digestibility of the total diet increased at 37 ^{o}C ($P<0.05$) and increased in response to supplementation with treated casein ($P<0.001$) and propionate ($P<0.05$).

Table 5.5 *In vivo* digestibility of total dry matter, nitrogen balance and nitrogen losses in faeces and urine in lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde-treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 °C.

Measurements	Supplements											Significance		
	nil		FC		P20		FC/P20		Means		SEM	Temp	Diet	TxD
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C				
In vivo digestibility (%)	57	54	57	60	56	58	59	63	57	59	1.0	*	***	**
Nitrogen balance (g/d)	-0.5	-0.4	2.1	2.0	-0.3	0.1	2.0	1.4	0.8	0.8	0.5	ns	***	ns
N in faeces (g/d)	6.9	5.9	7.9	5.7	6.9	5.0	7.6	4.1	7.3	5.2	0.5	***	*	*
N in urine (g/d)	6.4	5.2	11.0	9.8	6.4	4.5	10.6	9.4	8.6	7.2	0.4	***	***	ns

5.3.3.8 Nitrogen balance

The addition of treated casein to the basal diet significantly ($P<0.001$) increased nitrogen balance. No other treatment differences were significant. Increasing environmental temperature significantly ($P<0.001$) decreased both faecal and urinary nitrogen losses. Supplementation with propionate ($P<0.05$) decreased faecal nitrogen loss whilst addition of treated casein increased urinary nitrogen loss ($P<0.001$). There was an interaction between temperature and treated casein treatments; the nitrogen excretion in the faeces of treated casein supplemented lambs was much greater at 25 ^{o}C than at 37 ^{o}C.

5.3.3.9 Water balance

Water intake (ml/kg liveweight) increased ($P<0.001$) with increasing environmental temperature (Table 5.6).

Water loss via urine in lambs significantly increased ($P<0.01$) from 25 ml/kg to 44 ml/kg with increasing environmental temperature and from 25 to 43 ml/kg with propionate supplementation ($P<0.05$). There was a significant temperature by propionate interaction; the increase in urinary water loss by lambs in response to sodium propionate supplementation was only significant at 37 ^{o}C.

Faecal water loss in lambs decreased ($P<0.01$) with increasing environmental temperature (Table 5.6).

There was a significant ($P<0.001$) increase (from 43 to 56 ml/kg) in the calculated evaporative water loss (ml/kg) with increasing environmental temperature. Lambs supplemented with propionate had significantly ($P<0.05$) lower evaporative water losses than lambs on other diets.

Table 5.6 Water intake and faecal, urinary and calculated evaporative water losses per day in lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde-treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 °C.

Measurements	Supplements								Means		SEM	Significance		
	nil		FC		P20		FC/P20					T	D	TxD
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C				
Water intake (ml/kg)	82	107	87	93	80	118	82	127	83	111	10	***	ns	†
Urine water loss (ml/kg)	27	31	21	23	27	53	24	68	25	44	9	***	*	*
Faecal water loss (ml/kg)	14	15	18	11	14	10	14	10	15	12	2	**	†	†
Evaporative water loss (ml/kg)	41	61	48	59	39	55	44	49	43	56	4	***	*	†

5.3.3.10 Rumen fermentation

The molar proportions of valerate in the rumen fluid of lambs before feeding were increased (P<0.001) when treated casein was added to the basal diet. Four hours after feeding the proportion of acetate in rumen fluid of lambs was lower (P<0.001) in the animals receiving propionate (Table 5.7).

Rumen ammonia levels were significantly (P<0.001) increased by the addition of treated casein to the basal diet (Table 5.7).

There were no significant differences in the ratio of the energy supplied by propionate and total VFA energy (G/E) in the rumen of lambs on the experimental diets before feeding (Table 5.7). Four hours after feeding the G/E ratios of treated casein and propionate supplemented lambs were significantly (P<0.001) higher than unsupplemented lambs. When the means for the groups were examined it was seen that supplementation with treated casein increased the G/E ratio from 0.23 to 0.26 whilst propionate supplementation increased the ratio to 0.40.

5.3.3.11 Protozoa Numbers

Protozoa numbers in the rumen fluid of lambs decreased (P<0.01) with increased environmental temperature (Table 5.7). Supplements to the basal diet had no significant effect on protozoa numbers.

5.3.4 Discussion

The reduction in feed intake by lambs at 37 ^{o}C agrees with reports published in the literature of reduced feed intake by ruminants at high environmental temperatures (Wayman *et al.* 1962, Bianca 1965, Finch 1984). However, the reduction in feed intake by the lambs due to temperature was only 10%. This level of decrease is minor compared with the studies by Lindsay and Loxton (1981) and Hennessy (1984) which

Table 5.7 Total concentrations of VFA, molar proportions of individual VFAs, G/E ratio, ammonia concentration and protozoa numbers in the rumen fluid of lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde-treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 °C.

Measurements	Supplement								Means		SEM	Significance		
	nil		FC		P20		FC/P20					Temp (T)	Diet (D)	DxT
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C				
Before feeding														
Total VFA (mmol/l)	60.8	62.0	73.4	56.1	58.4	57.3	61.4	54.8	63.5	57.5	4.6	†	ns	†
VFA Proportions (%)														
Acetate	72.8	75.4	73.1	72.6	73.7	73.6	73.2	73.4	73.2	73.7	1.0	ns	ns	ns
Propionate	16.9	17.8	18.7	18.5	17.0	18.6	18.0	17.0	17.6	18.0	0.8	ns	ns	ns
Butyrate	8.3	5.2	6.1	5.5	7.4	6.3	5.8	5.9	6.9	5.7	0.8	†	ns	ns
Iso-acids	1.5	1.3	1.6	2.7	1.6	1.2	2.5	2.9	1.8	2.0	0.3	ns	***	*
Valerate	0.4	0.4	0.5	0.7	0.4	0.4	0.6	0.8	0.5	0.5	0.1	ns	***	*
G/E ratio	0.23	0.25	0.26	0.27	0.24	0.26	0.26	0.25	0.25	0.26	0.01	ns	ns	ns
Ammonia (mgN/l)	74	80	102	180	83	66	106	140	91	117	18	†	***	*
Protozoa (x10^5/ml)	4.2	2.4	5.7	2.6	3.5	2.2	4.4	3.1	4.5	2.6	0.8	**	ns	ns
4h after feeding														
Total VFA (mmol/l)	70.9	75.2	81.2	72.0	69.0	73.3	83.9	71.0	76.5	72.9	5.6	ns	ns	†
VFA Proportions (%)														
Acetate	75.8	78.0	75.5	75.8	69.4	64.8	64.9	63.8	71.4	70.6	1.5	ns	***	†
Propionate	16.3	16.4	18.2	17.2	23.5	29.7	28.8	29.2	27.7	23.1	1.2	ns	***	*
Butyrate	6.9	5.0	5.0	5.0	5.7	4.8	4.0	4.4	5.4	4.8	0.5	ns	*	*
Iso-acids	0.6	0.3	0.8	1.4	0.9	0.3	1.7	1.8	1.0	1.0	0.3	ns	***	*
Valerate	0.4	0.3	0.4	0.6	0.4	0.4	0.6	0.7	0.5	0.5	0.1	ns	***	*
G/E ratio	0.23	0.24	0.26	0.25	0.32	0.39	0.39	0.40	0.30	0.32	0.1	†	***	*
Ammonia (mgN/l)	124	129	198	211	101	115	126	236	137	173	28	†	***	ns

found that unsupplemented cattle, fed dry tropical grasses, were able to increase their intake by over 50% when supplements of urea and bypass protein were given.

The intake of the basal diet was not excessively low and was probably due to the ammoniation of the straw which has been shown (Perdok 1987) to increase intake of straw by cattle in comparison with the intake of untreated straw. The level of intake of the basal diet achieved by the animals in this experiment did not allow much scope for large differences in intake when the animals were supplemented.

Propionate, fed at 20 g/d, had no effect on feed intake by lambs at 25 ^{o}C but appeared to create some metabolic disturbance in the animals at 37 ^{o}C and this was not ameliorated by the addition of treated casein to the diet. This suggests that propionate may be increasing metabolic heat production either directly by stimulating propionate oxidation in wasteful cycles or indirectly by stimulating the oxidation of other compounds. However, other causes such as accumulation of propionic acid in blood (which was not measured) or hormonal responses to the extra propionate may also have been involved.

Feed conversion ratio (FCR) of the lambs supplemented with propionate was poor and though intake was depressed at the higher temperature feed intake cannot account for the poor FCR at 25 ^{o}C. This suggests that propionate cannot be readily utilised at high concentrations on these diets. Studies by Elliot *et al.* (1965) showed that, when lambs were fed a basal ration of pelleted alfalfa hay, addition of propionate to the diet gave no response in either rate of liveweight gain or FCR but that the carcasses of the supplemented lambs had a significantly higher fat content. Therefore, on an energetic basis, the propionate supplemented lambs converted digestible energy to liveweight gain with much greater efficiency in that study. These studies were different than the ones reported here in that FCR was not adversely affected by supplementation of the diet with propionate. This may be associated with the difference in the basal diet.

More recent studies with lambs fed a basal diet of ammoniated barley straw (van Houtert 1991) have shown that body fat was higher and water and protein lower when 20 g/d of Na-propionate was added to the basal diet.

Propionate supplemented lambs were able to maintain a higher rate of wool growth at 37 ^{o}C than control lambs even though at 25 ^{o}C wool growth was similar. This indicated that the lambs were able to utilise the propionate and decrease their catabolism of amino acids at 37 ^{o}C, which was reflected in their nitrogen balance and the decrease in urea entry rate (see Section 5.4). These results suggest that the level of propionate may have been too high and resulted in an metabolic disturbance to the animals. It appears that only a small amount of additional propionate can be utilised by the animal. Propionate supplied in high amounts on grain based diets resulted in odd chain fatty acid synthesis with resultant low melting point fat (Garton *et al.* 1972). Again this indicates that propionate, at high concentrations, can result in a metabolic imbalance and the animal responds by depositing it into a fat sink.

The decrease in feed intake by the lambs in response to higher temperatures was not ameliorated by supplementation with protein meals and thus did not support the hypothesis proposed, that protein ameliorates heat stress in ruminants. The possibility that the protein was unprotected was not supported by the wool growth studies which showed a significant increase in wool growth of lambs given treated casein. Wool growth has been shown to be highly correlated with the amount of protein, either from microbial or from dietary bypass protein, reaching the intestine (Reis and Schinckel 1961).

Feed conversion ratio by the lambs given treated casein at 25 ^{o}C was poor in comparison with that of similarly supplemented lambs in Experiment 5.1 (16.4 g/g compared with 7.8 g/g). When the temperature increased to 37 ^{o}C FCR by the lambs increased substantially (to 37.4 g/g) in the experiment reported here as compared with the small increase (to 9.6 g/g) observed in lambs in Experiment 5.1. These results are

surprising when the digestibilities of the two diets are examined. Cottonseed hulls were 45% digestible (see Section 4.6) and contained 17.2 KJ/g, whereas the current diet of ammoniated barley straw was approximately 58% digestible and contained 17.6 KJ/g. Some of the differences in performance of lambs on these diets may then be attributable to (1) feed intake and therefore the palatability of CSH, and (2) the lower protozoa numbers in the CSH fed lambs compared with the lambs on ammoniated barley straw which would lead to a higher P/E ratio in the nutrients absorbed by lambs on the former diet.

The increase in digestibility of the diet by the lambs at the higher temperature has been observed by other workers (Davis and Merilan 1960, Warren *et al.* 1974) and is probably associated with the decreased feed intake and increased rumen retention times occurring in ruminants experiencing heat stress. The increased retention time in the rumen allows the rumen microorganisms greater access to the more resistant fibrous components of the feed. However, a direct effect of heat cannot be ruled out. The increase in digestibility with supplementation suggested that there was an improvement in microbial pool size and therefore growth efficiency of microbes. From this it might be anticipated that the protein to energy ratio was increased.

Contrary to the general conclusions that increased nitrogen excretion is an effect of heat stress (O'Kelly 1973), a significant reduction in both faecal and urinary nitrogen excretion by the lambs in response to the increased temperature was observed in the present experiment suggesting that these lambs were not excessively heat stressed. The work reported by O'Kelly (1973) and Vercoe (1969) was done with cattle fed roughages with moderate to high nitrogen content, and a further study by Vercoe and Frisch (1970) showed no significant effect of heat stress on nitrogen excretion of steers fed a low nitrogen roughage. However, the latter study was conducted for 10 days only and although overall the urinary excretion of nitrogen was not increased, there were significant increases in the urinary nitrogen excretion over the last 5 days of the

experimental period. A study by Bhattacharya and Hussain (1974) on Awasi lambs fed diets containing 16% CP found that nitrogen balance was unaffected by heat stress. In the present study, nitrogen balance was significantly improved in lambs given treated casein, this effect having been found by other workers (Faichney 1971, Kempton and Leng 1979).

Water intake (free water plus feed water) was similar for lambs from all dietary groups at 25 ^{o}C. The amount of water consumed by lambs per kg of liveweight at 25 ^{o}C was similar to that found for heifers kept at 17 ^{o}C by Colditz and Kellaway (1972). At 37 ^{o}C the water intake of lambs was 30, 4, 48 and 55% higher for the control, treated casein (PC), propionate and PC plus propionate groups respectively. The smaller increase in water intake by lambs given treated casein may indicate that these lambs were less heat stressed than the unsupplemented lambs or those supplemented with propionate.

The water intake of lambs given sodium propionate increased markedly at 37 ^{o}C and the amount of urine water loss increased significantly. This could be associated with the intake of sodium (Godwin and Williams 1986, van Houtert 1991) but may also be associated with the metabolic inefficiency caused by propionate supplementation which became more apparent with increasing temperature.

The increase in water intake by the lambs given propionate is similar to that reported for Brahman heifers by Colditz and Kellaway (1972). However, in the studies reported by these authors, the percent increase in water intake by Friesian heifers (a breed less adapted to high temperature than Brahmans) at 38 ^{o}C was 109%. This indicates that in the present experiment the control animals and those given treated casein were not truly stressed by being kept at 37 ^{o}C. This may explain why the response to treated casein in animals at 37 ^{o}C was much less than that reported by Lindsay and Loxton (1981) and Hennessy (1984). In those studies, the cattle were kept under field conditions where they were exposed to solar radiation, which as discussed

previously (Section 2.6.1), increases the heat load on animals to a much greater extent than that of animals held at a similar temperature in a hot room.

Though the total water intake per kg liveweight by the lambs was similar to that of the heifers in the experiment reported by Colditz and Kellaway (1972) the means of water loss was quite different. In the present experiment, the evaporative water loss at 25 ^{o}C by lambs was much higher than the comparable loss by heifers at 17 ^{o}C (mean evaporative water loss by lambs at 25 ^{o}C was 43 ml/kg compared with 11 ml/kg for the heifers). The mean evaporative water loss by the heifers increased to 48 ml/kg at 37 ^{o}C compared with 56 ml/kg by the lambs.

Colditz and Kellaway (1972) observed that the proportion of faecal water loss decreased with increasing temperature (though in absolute terms the amount of faecal water excreted remained the same). In comparison, faecal water loss by the lambs in the present experiment was found to decrease with increasing environmental temperature. These differences in water utilisation may reflect differences between species in their reaction to high temperature.

The trend towards a decrease in the total VFA concentration in the rumen fluid of lambs in response to high temperature agrees with reports found in the literature (Weldy *et al.* 1964, Kelley *et al.* 1967, Olbrich *et al.* 1972) and is almost certainly due to decreased feed intake. Contrary to the findings of Weldy *et al.* (1964) and others, in the present experiment no significant changes in the proportions of individual VFA were found. Addition of propionate to the basal diet markedly increased the proportion of propionate in the rumen fluid of lambs 4 h after feeding, however this increase was not sustained and before feeding no differences in propionate proportions in rumen VFA were found between treatments.

The increase in the glucose energy available from the VFAs was consistent across groups and did not vary with either treated casein or propionate supplementation except in the propionate group shortly after feeding.

Rumen ammonia levels were consistently high due to the treatment of straw (Perdok 1987). Ammonia levels in the rumen were increased by treated casein supplementation and there appeared to be an effect of temperature on the level of rumen ammonia, particularly in casein supplemented lambs. This may indicate that part of the treated casein was degraded in the rumen, or as seen from the data on urea dynamics (Section 5.4), that there was an increased recycling of urea N in the rumen arising from ammonia being produced by deamination of the absorbed casein derived amino acids.

The experiment reported here detailed the response of lambs to high environmental temperatures in terms of feed utilisation and production parameters. To investigate in more depth the effects of high environmental temperature on lamb performance, the following experiment was designed to examine metabolic responses of lambs, and, in particular, metabolic responses that may be associated with high heat production e.g. acetate utilisation and glucose and amino acid (urea) metabolism.

5.4 Measurements of entry rates of glucose and CO_2 in blood, urea kinetics and acetate clearance in lambs at medium and high environmental temperatures

5.4.1 Introduction

In the growth study (Section 5.3) the lambs responded to high environmental temperature by decreasing their feed intake. The responses of the supplemented lambs were different to that initially hypothesised, with the responses by the lambs given propionate being the reverse to what was anticipated. There was no effect of treated protein in reversing the effects of high temperature on feed intake.

Although the animals given treated casein were not able to maintain or increase their feed intake in response to high temperature they became considerably more efficient. At the higher temperature, water intake increased only 5% compared with a 30 % increase of the unsupplemented control sheep.

Whilst the supplements supplied either glucogenic (propionate) or both aminogenic/glucogenic (treated casein) substrates there seemed to be a considerable difference in their utilisation. To further clarify the interactions, isotope studies were undertaken to determine the potential for gluconeogenesis of the two supplements and to assess the extent that casein was degraded (deaminated) and/or contributed to N-balance (protein synthesis) in the animal. Information was obtained on amino acid utilisation by measuring urea synthesis and excretion, whilst carbon dioxide entry rates provided an estimate of heat production by the animals.

5.4.2 Experimental

These studies used the lambs described in Section 5.3 and commenced after completing the growth study whilst the same lambs were still on their original experimental diets and kept under the same conditions.

5.4.2.1 Diets

The basal diet consisted of ammoniated barley straw, 50 g/d lucerne chaff and 17.5 g vitamin/mineral premix (see section 5.3). The amount of barley straw was restricted to 90% of each lambs' previous weeks intake. The four diets used were (1) basal, (2) basal plus 50 g formaldehyde-treated casein, (3) basal plus 20 g sodium propionate, and (4) basal plus 50 g formaldehyde-treated casein plus 20 g sodium propionate.

5.4.2.2 Procedure

At the end of the growth study described above (Section 5.3) the lambs were at hourly intervals by an automatic feeder. They were adapted to this feeding regime for 11 days before the commencement of the isotope study. This feeding procedure has been shown (Kempton 1977, Habib 1988) to give steady state conditions in lambs.

On the afternoon prior to the first isotope injection catheters were inserted into a jugular vein of each lamb. The injection schedule is given in Table 5.7 and the methods are outlined below.

Table 5.7 ***Schedule of events for injection of isotopes and collection of samples.***

Day	Time	Event
1	9 a.m.	Final collection for digestibility study (see Section 5.3), commencement of feeding by automatic feeders.
11	2 p.m.	insertion of catheters into jugular veins
12	8 a.m.	injection of ^{3}H glucose and ^{14}C urea collection of blood and urine samples
14	8 a.m.	injection of ^{14}C bicarbonate collection of blood samples
15	9 a.m.	injection of acetate collection of blood samples

The urea kinetics of lambs were estimated by a single intravenous injection of ^{14}C-urea, followed by collection of urine for 24 h according to the method described by Ford and Milligan (1970) and Nolan and Stachiw (1979). Glucose metabolism was measured following the procedure reported by Judson and Leng (1972) and involved a single tracer injection of ^{3}H glucose. The CO_2 entry rate technique was used for estimating energy expenditure and followed the procedure reported by Young *et al.* (1969).

Injection solutions

(1) ***^{14}C urea and ^{3}H glucose*** : Two mCi of ^{14}C-Urea (Amersham, UK) plus 20 mg urea as a carrier were added with 3.0 mCi of 2-^{3}H-glucose (Amersham, UK) and 20 mg glucose (as carrier) to 250 ml sterile physiological saline (Travenol).

(2) ***^{14}C bicarbonate***: A solution was prepared by adding 1.75 ml of sodium (^{14}C) bicarbonate (54 mCi/mmol) (Amersham, UK) and 20 mg Na_2CO_3 (as carrier). It was made alkaline with NaOH and prepared in 250 ml sterile physiological saline (Travenol).

(3) ***Sodium Acetate***: A solution was prepared by dissolving 8.2 g of sodium acetate in 50 ml of sterile distilled water.

Injection procedure

(1) ***^{14}C urea and ^{3}H glucose:*** approximately 7.5 ml (70 μCi 2-^{3}H-glucose and 60 μCi ^{14}C-urea) was injected into the jugular vein of each lamb. The exact quantity injected was calculated by weighing the syringe before and following the injection. Immediately after injection 2-3 ml of saline was injected into the catheter to wash any residual isotope into the vein.

(2) ***^{14}C bicarbonate***: approximately 7.0 ml of the injection solution was injected into each lamb following the procedure as given above for the ^{14}C-glucose.

(3) ***Sodium acetate***: approximately 35 ml (4 mmol sodium acetate/kg liveweight) of the injection solution, warmed to 37 ^{o}C, was injected into each lamb over a period of 2 to 3 min. It was followed by 3 - 5 ml of a sterile physiological saline solution.

Sampling times

(1) ***Blood sampling for assay of SR of glucose.*** Blood samples (6 ml) were taken from the jugular vein of each lamb by catheter at 30 min intervals for 4 h after the ^{3}H glucose injection and then at 5, 6, 9, 12, 25 and 31 h post injection. The blood was immediately transferred into heparinized tubes and placed on ice until processed following the procedures given in Section 3.10.

*(2) **Collection of urine.*** Urine voided by each lamb over the first 24 h after the ^{14}C urea injection was collected into plastic bottles containing 25 ml glacial acetic acid and 5 ml concentrated HCL. The total volume of urine was measured and a subsample taken and stored at -20 ^{o}C until analysed for urea concentration and specific radioactivity.

*(3) **Blood sampling for assay of SR of CO_2**:* Blood samples (6 ml) were taken from the jugular vein of each lamb at 30 minute intervals for 3 hours and then at hourly intervals until 7 hours after injection of ^{14}C bicarbonate. These samples were processed following the procedure detailed in Section 3.10.

*(4) **Blood sampling for analysis of acetate**:* Blood samples (6 ml) were taken pre-injection and then at 10, 20, 30, 40 and 50 minutes post injection. The blood was immediately transferred into heparinized tubes and placed on ice until processed following the procedures given in Section 3.11.

Processing of samples

The samples of blood and urine were processed following the procedures described in Section 3.10.

Calculations

Tracer terminology and calculations for glucose, urea and carbon dioxide entry rates and acetate clearance are given in Section 3.10 and Section 3.11.

5.4.2.3 Statistical analysis

Statistical analysis procedures were those used in Section 5.3.

5.4.3 Results

5.4.3.1 Glucose metabolism

Plasma glucose No significant differences in plasma glucose concentration were observed in lambs given different supplements (Table 5.8).

Glucose entry rates (GER) Glucose entry rates increased ($P<0.001$) in response to supplementation of the basal diet with treated casein (FC) and decreased ($P<0.001$) in lambs housed at 37 ^{o}C (Table 5.8). There was a significant interaction in the response of lambs to FC supplementation and increasing temperature such that the increase in GER due to the addition of PC was only significant at 25 ^{o}C. No interaction was observed when propionate was given with the treated casein.

Adjustment of GER for the metabolic liveweight ($W^{0.75}$) indicated that the response of GER to increasing temperature was not simply a function of animal size. Adjusting GER for digestible energy intake indicated that the differences in GER in response to temperature was mainly a function of intake.

Table 5.8 Plasma glucose concentration, entry rate of glucose and CO_2, glucose pool size and the $t^{1/2}$ of blood acetate in lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 °C.

Measurements	Supplement											Significance		
	nil		FC		P20		FC/P20		Means		SEM	Temp (T)	Diet (D)	TxD
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C				
Mean Plasma Glucose (mg/100ml)	64.1	62.5	66.3	67.2	65.1	70.1	65.8	69.8	65.3	67.4	3.5	ns	ns	ns
Glucose Entry Rate (GER) (g/d)	57.1	52.7	90.3	65.5	64.2	53.9	87.5	58.2	74.8	57.6	5.0	***	***	*
GER/DEI¢ (g/MJ)	8.8	10.2	11.6	10.7	9.7	10.9	11.3	11.3	10.3	10.8	0.7	ns	*	ns
GER/$W^{0.75}$	4.4	4.8	6.6	5.4	4.9	4.5	6.2	5.0	5.5	4.9	0.4	*	***	*
Glucose Pool (g)	3.9	3.8	5.2	4.3	4.1	4.1	4.5	4.0	4.4	4.1	0.3	ns	*	ns
CO_2 entry rate (g C/d)	169	155	196	180	195	164	201	82	190	145	30	*	ns	ns
(g C/DEI)	26	30	25	29	30	33	26	15	27	27	4	ns	ns	ns
Blood Acetate ($t^{1/2}$ min)	30.4	29.1	25.4	33.8	27.7	28.3	24.6	22.7	27.0	28.5	2.8	ns	ns	†

¢DEI digestible energy intake

There were significant correlations between glucose entry rate and intake of dry matter and digestible energy and liveweight change of lambs and these are given in Table 5.9.

The possibility of different relationships existing between these parameters in lambs housed at the different environmental temperatures were examined but in no case was the difference between the slopes of the regressions significant. The regression equations given represent the combined data from lambs at the different environmental temperatures and the separated data for the different temperature treatments. In all regressions examined there were no significant quadratic components to the slope.

Glucose pool size Glucose pool size increased ($P<0.05$) in response to supplementation of the basal diet with treated casein (Table 5.8). No other treatment responses were significant and no interaction was observed between temperature and supplements.

5.4.3.2 Acetate clearance rates

The addition of propionate to the basal diet decreased the half time for acetate clearance in the blood of lambs with a probability of $P<0.10$ (26 min compared with 30 min). No other treatment responses approached significance and no interaction was observed between temperature and supplements.

5.4.3.3 Urea metabolism

Plasma urea concentration Mean plasma urea concentrations were significantly ($P<0.01$) lower at high environmental temperature (Table 5.10) and increased ($P<0.001$) in response to supplementation of the basal diet with treated casein. No interactions were observed between temperature and supplements.

Table 5.9 *Combined regression equations (of the form Y = a + bx) with the S.E.'s of the regression coefficients between various parameters of glucose metabolism in lambs given ammoniated barley straw and supplemented with either treated casein or sodium propionate and kept at 25 °C or 37 °C.*

	R^2	Significance level
Combined data for 25 and 37 °C		
GER = 0.056(±0.013)DMI + 31.4	37	***
GER = 9.7(±1.6)DEI + 5.0	57	***
MGER = 0.035(±0.014)MDMI + 3.51	19	*
MGER = 8.0(±2.0)MDEI + 1.25	35	***
LW = 1.19(±0.27)GER - 59.7	40	***
LW = 15.2(±5.2)MGER - 60.1	23	**
Data from lambs housed at 25 °C		
GER = 0.048(±0.025)DMI + 39.6	21	ns
GER = 13.7(±4.3)DEI - 23.9	43	**
MGER = 0.035(±0.022)MDMI + 3.61	16	ns
MGER = 9.6(±24.5MDEI + 0.38	24	*
LW = 0.63(±0.28)GER - 7.3	26	*
LW = 10.1(±4.3)MGER - 16.5	28	*
Data from lambs housed at 37 °C		
GER = 0.031(±0.018)DMI + 42.0	17	ns
GER = 7.0(±1.4)DEI + 20.1	64	***
MGER = 0.014(±0.019)MDMI + 4.3	4	ns
MGER = 6.6(±2.0)MDEI + 1.92	46	**
LW = 1.24(±0.7)GER - 73.7	19	ns
LW = 6.2(±11.1)MGER - 32.2	2	ns

GER glucose entry rate (g/d)
DMI dry matter intake (g DM/d)
DEI digestible energy intake (MJ/d)
MGER glucose entry rate ($g/kg^{0.75}/d$)
MDMI dry matter intake ($g DM/^{0.75}/d$)
MDEI digestible energy intake ($MJ/W^{0.75}/d$)
LW liveweight change (g/d)

Table 5.10 Entry and excretion rates of urea in lambs fed a basal diet of ammoniated barley straw and supplemented with formaldehyde treated casein (FC) and sodium propionate (P20). Lambs were housed at 25 or 37 ^{o}C.

Measurements	Supplements											Significance		
	nil		FC		P20		FC/P20		Means		SEM	Temp (T)	Diet (D)	DxT
	25^{o}C	37^{o}C	25^{o}C	37^{o}C	25^{o}C	37^{o}C	25^{o}C	37^{o}C	25^{o}C	37^{o}C				
Mean plasma urea conc (mg/100 ml)	26.9	28.4	40.5	31.9	29.8	18.8	34.9	33.6	33.0	28.2	2.3	**	***	**
Urea entry rate														
(g/d)	17.9	14.9	27.6	20.9	15.6	13.0	24.6	18.8	21.4	16.9	1.8	**	***	†
(g/gN intake)	1.4	1.4	1.3	1.2	1.2	1.3	1.2	1.3	1.3	1.3	0.1	ns	ns	ns
Urea excretion rate														
(g/d)	9.1	8.1	17.1	14.6	7.6	7.5	15.0	14.1	12.2	11.1	1.0	ns	***	ns
(g/gN intake)	0.7	0.8	0.8	0.8	0.6	0.8	0.7	0.9	0.7	0.8	0.06	*	**	ns
Urea degradation rate (g/d)	8.8	6.7	10.5	6.3	8.0	5.5	9.6	4.7	9.2	5.8	1.3	**	ns	ns
% of urea entering body which is degraded	48.8	45.7	38.0	30.3	49.7	41.7	37.9	25.0	43.6	35.7	3.8	**	***	ns

Urea entry rate Urea entry rate significantly decreased ($P<0.01$) in lambs housed at 37 ^{o}C and increased ($P<0.001$) in response to supplementation of the basal diet with treated casein (Table 5.10). No interactions were observed between temperature and supplements.

Urea excretion rate Urea excretion rate increased ($P<0.001$) in response to supplementation with treated casein (Table 5.10). No other treatment differences were significant.

The percentage of urea entering the body pool The percentage of urea entering the body pool which is degraded decreased ($P<0.01$) in response to increasing temperature (Table 5.10). No other treatment differences were significant.

Urea degradation rate Urea degradation rate decreased ($P<0.01$) in response to increasing temperature (Table 5.10). No other treatment differences were significant.

5.4.4 Discussion

The metabolic relationships between nutrients make it difficult to define precisely the role of glucose in the metabolism of sheep fed low quality forages. It has been argued (Leng 1970) that the rate of entry of glucose into the glucose pool in the blood, estimated by isotope dilution of ^{3}H-glucose may give a good indication of the requirement for glucose. The arguments suggest that the synthesis of glucose in excess of an animals need for essential purposes would be energetically wasteful.

In these studies the animals were in a steady state due to hourly feeding so that the measurement of entry rate in the blood represented glucose production/synthesis rate in the body (Leng 1970, Bergman 1983). Use of ^{3}H-glucose has been shown to represent the true flow of glucose in the animal as the amount of recycling of tritium is minimal (Judson and Leng 1972).

The linear relationship found in these studies between glucose entry rate and digestible energy intake is similar to that found by Judson and Leng (1968) in mature sheep and by Bird (1982), Kempton and Leng (1983) and Habib (1988) for growing lambs. Figure 5.3 shows the relationship between glucose entry rate and digestible energy intake obtained in the present experiment and Figure 5.4 show the same result overlaid with results obtained by other workers. From Figures 5.3 and 5.4 it is apparent that the effect of high temperature on glucose entry rate is a result of the effect of high temperature on intake as the data from this experiment and the other studies are similar.

The importance of gluconeogenesis in ruminant nutrition is highlighted by the finding in the present study that 14 to 19 % of the digestible energy, passed through the glucose pool. Habib (1988) reported a value of 14 %, Kempton and Leng (1983) 14.7 % while Elliot (1980) reported values of 17 % for animals on a variety of diets and at different physiological states. A diet of ammoniated barley straw and supplements of either treated casein or propionate would contain negligible amounts of starch to bypass the rumen to be absorbed as glucose by the small intestine. Glucose entry rates of the lambs, therefore, represent glucose generated from *de novo* synthesis, i.e. from gluconeogenesis.

Glucose synthesis or entry rates were stimulated at 25 ^{o}C in lambs given treated casein resulting in an extra 33 g of glucose. According to the calculations of Krebs (1964) this would require the amino acids from the 60 g protein to be deaminated. If this occurred, then an extra 21 g of urea would be excreted by these sheep (Nolan and Leng 1970). The actual value of additional urea excreted by the lambs given treated casein was 8 g urea/d, therefore approximately half of the additional protein given to these animals may have been deaminated. The question arises then whether the function of the extra amino acids given to these sheep via protected protein is for gluconeogenesis, tissue synthesis or both. Certainly there is a strong positive relationship between glucose entry rates and liveweight gain of sheep in this

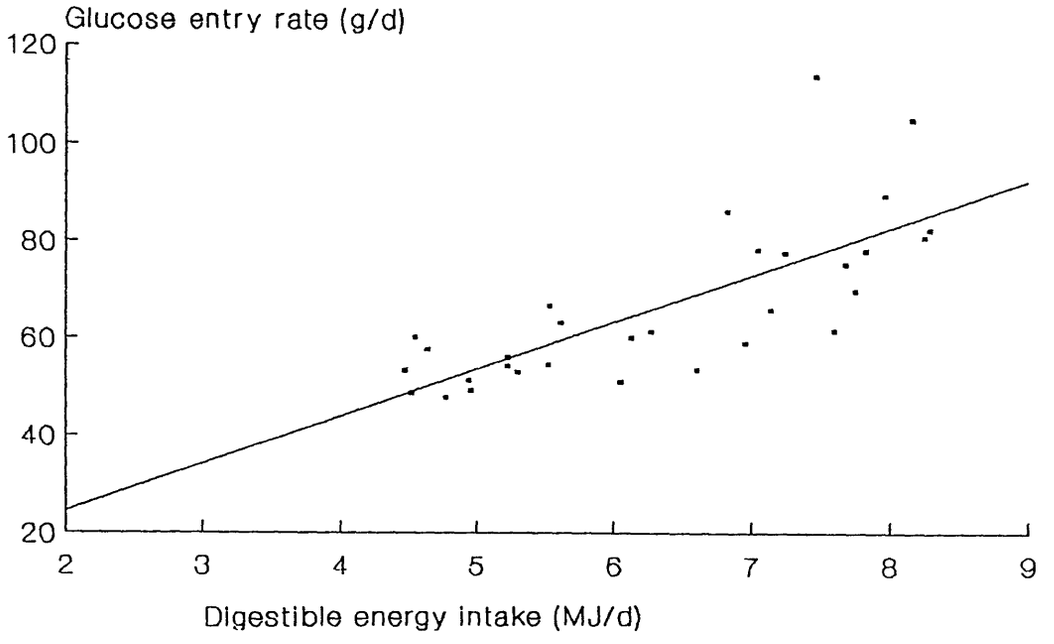

Figure 5.3 *Relationship between digestible energy intake and glucose entry rate in lambs fed ammoniated straw diets with and without formaldehyde treated casein and/or sodium propionate. Lambs were housed at 25 or 37 °C.*

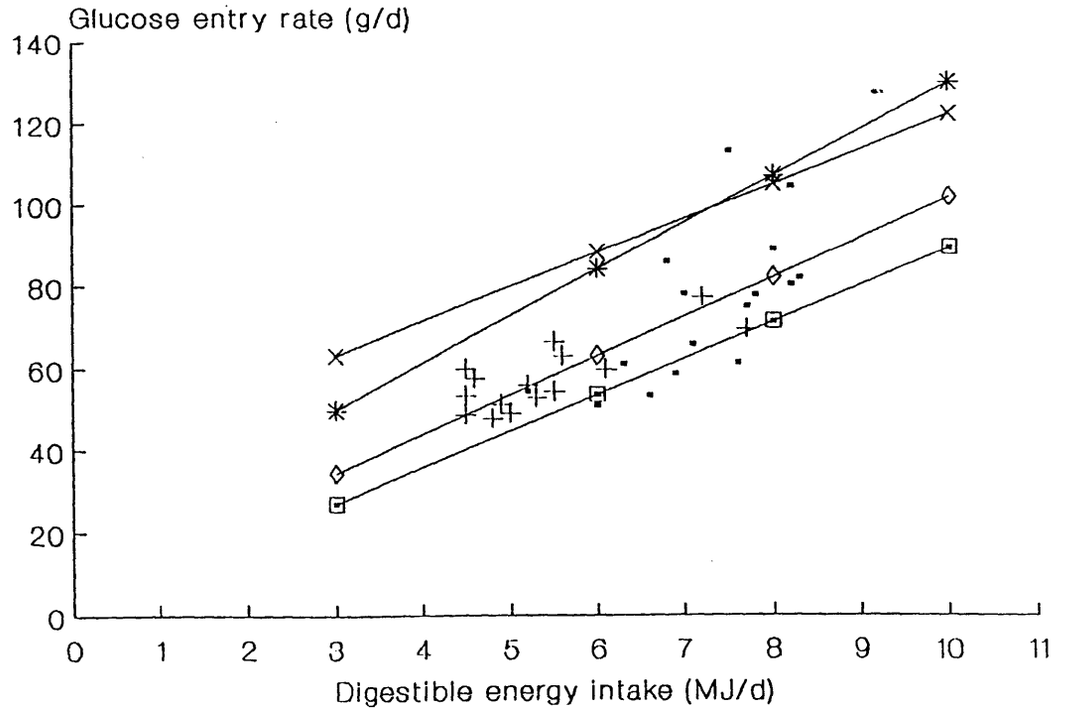

Figure 5.4 *Relationship between digestible energy intake and glucose entry rate in lambs from (• +) Experiment 5.4, (Δ) Judson and Leng (1968), (x) Kempton (1977), (*) Bird (1982), (▣) Habib (1988).*

experiment. This relationship has been reported in the literature (Kempton *et al.* 1983, Habib 1988). The source of the other half of the glucose synthesised by the protein supplemented animals may be from glycerol of fat origin. Fattet *et al.* (1984) reported that lambs fed a basal diet of treated barley straw and given a supplement of fish meal were able to mobilise fat as an energy source whilst using the protein supplement for accretion of body protein. In this instance, the glycerol produced from fat mobilisation may have been used for glucose synthesis.

Addition of propionate to the diet of lambs did not increase glucose entry rate (GER) even though the increase in the molar proportion of propionate in the rumen of lambs was significant when measured 4 h after feeding. The lack of effect of propionate on glucose synthesis is quite unusual as it has been shown to increase glucose synthesis substantially when fed over extended periods of time (Weekes 1991). It appears that the animals may have controlled their glucose synthesis rates to levels commensurate with their requirements.

If propionate is not converted to glucose then its destination in metabolism is either the synthesis of components from TCA cycle intermediates, synthesis of fat or oxidation for energetic purposes. Casein, whilst supplying amino acids for protein synthesis, also supplies some intermediates for the TCA cycle. It can be speculated that propionate utilisation could be altered by the presence of high levels of protein but this was not seen in the glucose entry rates or urea metabolism measured. There appears to have been a block on propionate metabolism to glucose, unrelated to mineral/vitamin deficiency as the animals were supplied with a complete pre-mix.

There was a trend for blood acetate clearance to be faster in lambs given propionate, but in all cases the clearance of acetate was extremely slow in comparison to results reported by Weston (1966). This suggests that there is a low rate of acetate metabolism in these animals for reasons other than glucose or amino acid deficiencies.

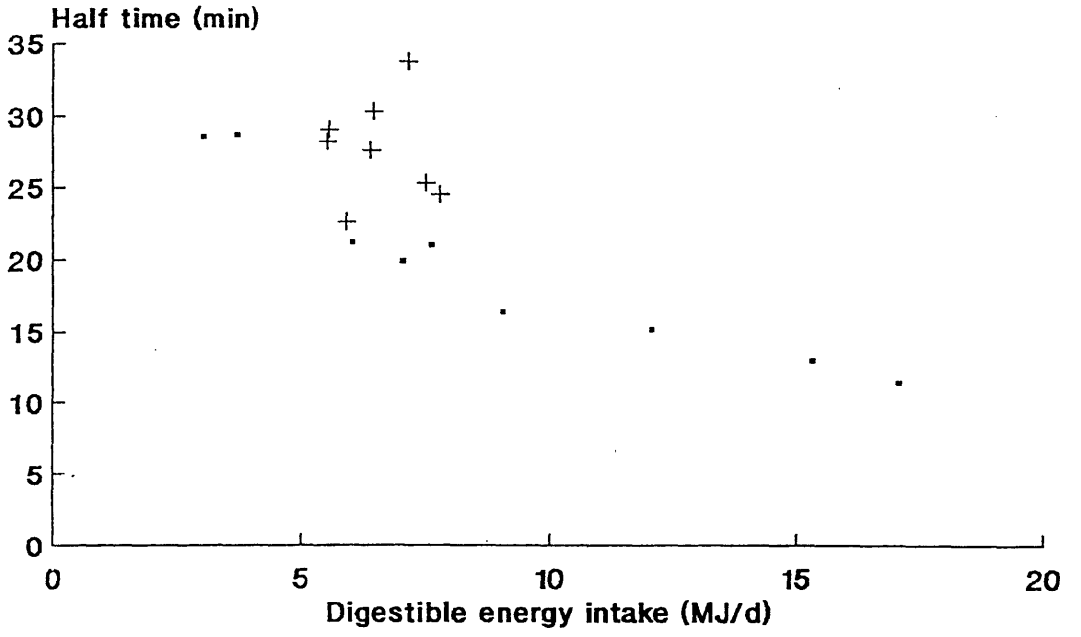

Figure 5.5 *Relationship between the rate of acetate disappearance and the intake of digestible energy from (+) Experiment 5.4 and (•) Weston (1966)*

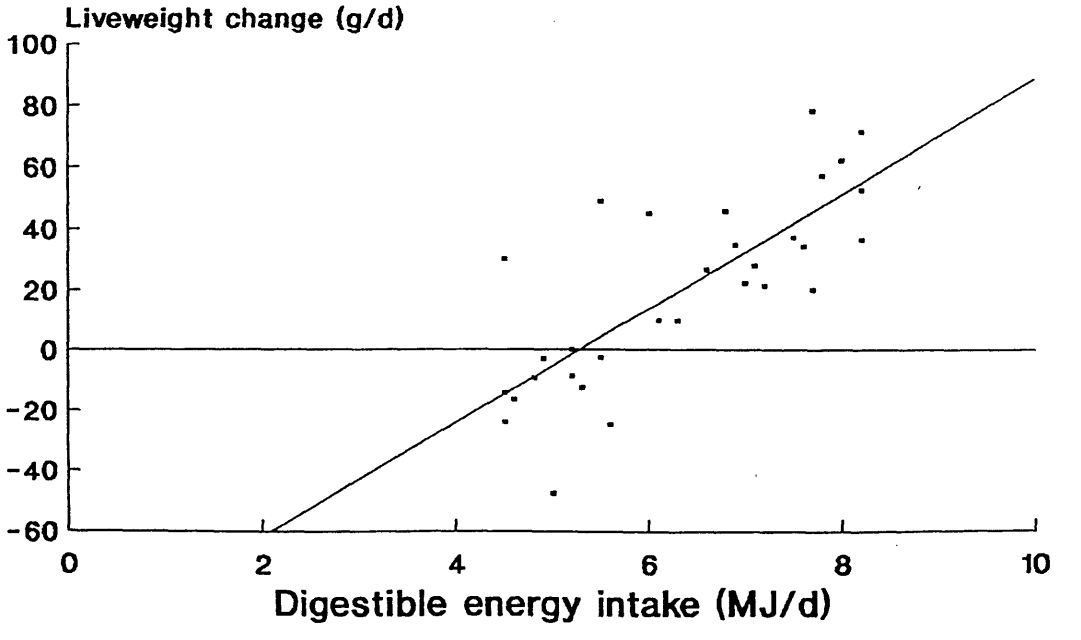

Figure 5.6 *Relationship between digestible energy intake and liveweight gain of lambs fed ammoniated barley straw diets with or without formaldehyde treated casein and/or sodium propionate. Lambs were housed at 25 or 37 °C.*

However, when the data for the $t^{1/2}$ of blood acetate is compared with the data derived by Weston (1966) it appears that acetate clearance is mainly a function of digestible energy intake (Figure 5.5). The effects of supplements were minimal on acetate clearance as supplements provided only small amounts of digestible energy. There appears to be no essential metabolic block on acetate metabolism as hypothesised by Cronjé (1987).

A decrease in carbon dioxide entry rates of lambs was found in response to increasing environmental temperature, however adjustment for the dry matter intake of the animals indicated that the differences in carbon dioxide entry rates were a function of intake.

Urea dynamics followed patterns that could have been anticipated by the effects of supplements on feed intake. In general, animals held at 37 ^{o}C had lower plasma urea concentrations and a urea entry rate than animals held at 25 ^{o}C. Urea excretion rate, when corrected for nitrogen intake, was increased at 37 ^{o}C and may indicate protein breakdown at the higher temperature.

It is extremely difficult to make definite conclusions from the urea entry rates as the proportions of urea entering the body pool from ammonia of dietary origin were unknown. However, the urea excretion rates suggest that considerable amounts of the treated casein were deaminated and lost as urea in the urine.

High temperature apparently reduced urea entering the gut where it would be degraded to ammonia and may be reabsorbed or used by microorganisms. The potential for recycling was thus lowered. This effect of heat stress may be critical when the animals are on very low protein diets and a decrease in the recycling of urea to the rumen may lead to ammonia levels in the rumen below that required for an efficient microbial system. This effect appears to be associated with increased urine volume due to high

temperature. High temperatures decreased both faecal and urinary N output (Table 5.5) and this could be attributable mostly to a deceased feed intake, particularly as the straw contained considerable amounts of ammonia from treatment.

5.4.5 Conclusion

The results of these studies are equivocal. The decrease in feed intake attributable to increasing the temperature from 25 to 37 ^{o}C was relatively small; only 10 % compared with the 50 % difference found by Lindsay and Loxton (1981) and may have been due to one of two factors:

(1) the low feed intake in terms of metabolic liveweight at 25 ^{o}C may indicate that the lambs were already experiencing heat stress; or

(2) that the temperature of 37 ^{o}C was insufficient to stress the Merino-cross animals used, i.e. they would be heat tolerant.

If it is accepted that the animals were stressed at 37 ^{o}C then the results of these studies indicate that the hypothesis is incorrect, i.e. that protein supplementation did not ameliorate a reduction in feed intake in these animals in response to high temperature. This is contrary to the theory published by Leng (1990b).

However, if as the water intake studies strongly suggest, these animals were not excessively heat stressed at the temperatures used, then the conclusion reached by this experimental work become less definite regarding the testing of the hypothesis.

Throughout these studies on lambs at high temperature the decrease in digestible energy intake was highly correlated with production parameters (see Figure 5.6) and to this end the effect of temperature on DEI was the force that drove the other parameters affected by temperature.

Provision of a bypass protein was able to stimulate growth rate and create a more efficient animal over the range of temperatures and basal diets used in these experiments. It appears that the level of DEI by the animal controls the requirements for nutrients, and the balance of nutrients controls the efficiency for which the nutrients available are utilised for various production purposes.

CHAPTER 6

GENERAL DISCUSSION

6.1 Research Findings

Supplementary feeding of ruminants within Australia is normally only carried out in periods of low availability of green pasture, drought or when a target group of animals requires extra feed to meet a physiological or market deadline.

An ideal supplement is one which improves the efficiency of utilisation of the basal diet and either increases intake of the basal diet or at least, does not replace it. Supplements may be considered to act in a catalytic way because they increase the efficiency of use of the basal feed.

As a rule, the use of low protein/high energy grains as supplements to dry standing feed results in the substitution of the basal forage by the grain. For example, Hennessy *et al.* (1983) showed that sorghum grain depressed forage intake in weaner steers fed a basal diet of low-quality grass hay. In contrast, intake of the hay was increased by 44% when a bypass protein supplement was given. The reason for the substitution effect of grain feeding on low quality roughages is believed to be the reduction, due to grain feeding, of rumen pH below a critical level of 5.8. This reduces the cellulolytic activity of rumen microorganisms (Terry *et al.*. 1969).

The control of feed intake in ruminants is complex (see Baile 1975). Intake of forages of low digestibility was believed to be limited by bulk distension of the rumen but other factors such as thermal stress (Weston 1982) and the physiological state of the animal (Weston 1979) may override this. For ruminants fed on low-quality forages it has been suggested that intake is restricted primarily by the protein/energy ratio of the products absorbed from the intestine rather than the distension of the rumen (Egan 1965, Kempton and Leng 1979).

The two main strategies of supplementation are now recognised to be:

(1) to optimise microbial activity in the rumen by ensuring that there are no deficiencies of microbial nutrients. This will maximise the fermentative activity of the microorganisms and extract the maximum possible amounts of carbohydrate from the forage.

(2) to provide critical nutrients that escape rumen fermentation to complement the products of fermentative digestion.

This approach recognises the combined needs of the rumen microorganisms and the host animal for nutrients. For ruminants fed on low quality roughages the critical nutrients required by the animal have been suggested to be the amino acids (for review see Leng 1990b). By meeting the demands of the two systems (microbial and animal) the P/E ratio in the nutrients absorbed by the animal will be increased.

It is important therefore that the source of supplemental protein is relatively resistant to degradation by rumen microorganisms and that it provides amino acids for absorption in the small intestine of the animal.

Provision of a soluble protein source when animals are fed on a low protein, low N diet, could result in a lowering of the P/E ratio of the nutrients absorbed.

Fermentative degradation of protein results in relatively inefficient formation of ATP and subsequently low microbial growth efficiency (Russell *et al.* 1991).

There is still considerable controversy in the literature as to whether the effect of proteins of low solubility on animal production is due to: (1) the slow release of amino acids and ammonia in the rumen thus leading to a more efficient microbial growth and subsequently more microbial protein reaching the intestine to be absorbed, or (2) increased dietary amino acids absorbed from the intestines, or (3) a combination of both factors. Recent work by Maeng *et al.* (1989), has shown that rumen microorganisms growing on starch or sugar based substrates will respond to the substitution of 25% urea with protein by increasing their growth rate by 35 to 46%. However, when cellulose was used as the energy source, the partial substitution of urea with protein only increased microbial cell yield by 7%.

Evidence is accumulating that the response of ruminants fed on low quality diets to bypass protein i.e. protein that escapes degradation by rumen microorganisms (Ørskov *et al.* 1971, Kempton and Leng 1979), is primarily due to the increase in dietary amino acids reaching the intestine. Fraser *et al.* (1991) showed that in lambs grazing on high quality temperate grasses or legumes, the production of microbial protein is not sufficient to support maximum growth, and that these animals responded to extra protein reaching the small intestine by increasing their liveweight gain. It has been demonstrated that intake by sheep can be stimulated by infusing protein and amino acids directly into the duodenum (Egan 1965, Egan and Moir 1965, Egan 1970). Feed intake of sheep has also been reported to increase in response to supplements of bypass proteins. However, lack of an intake response to supplemental proteins that are absorbed in the intestines has also been reported (Redman *et al.* 1980, Perdok and Leng 1990).

The lack of uniformity of feed intake responses to bypass protein by ruminants fed low quality roughages makes it necessary to develop a thesis that will explain these differences.

Recently it has been proposed that voluntary intake of feed by ruminants is determined by the rate at which the efficiency of ME utilisation declines with increasing consumption level (Ketelaars and Tolkamp 1991). Ketelaars and Tolkamp (1991) proposed that the metabolic acid load was the main cause of such differences. Central to this proposal is that ruminants, via the effect of acid load on intercellular pH, strive to maximise the efficiency of use of O_2 and will do this even if it means reducing intake to submaintenance levels.

Leng (1990b) noted that it was only when animals had depressed appetites on roughage based diets that a major response in feed intake occurred to a supplement that increased P/E ratios. This led Leng (1990b) to hypothesise that, at times, a metabolic heat increment induced by the need to oxidise acetate could precipitate heat stress in animals in tropical areas. The work in this thesis has attempted to test this hypothesis.

The research work in the thesis presented here followed a pattern which examined the effects of bypass protein on feed intake. Firstly a basic trial was performed to determine that level of responses to a bypass protein that could be obtained using lambs. Secondly the potential for developing bypass protein from a highly fermentable source such as lupins was examined. Thirdly, the research attempted to define the mode of action of the supplement and whether the supplement provided aminogenic or glucogenic substrates. Lastly, the effects of supplementation of lambs held at high temperatures and their response to a increase in the P/E ratios of nutrients absorbed in the small intestine was examined.

Redman *et al.* (1980) found that Hereford steers, fed on a basal diet of oaten chaff, did not respond to increases in amino acid availability in the intestine. Lambs fed

a basal diet of oaten chaff were shown to increase feed intake, liveweight gain, wool growth and feed conversion efficiency when supplemented with formaldehyde treated casein. Provision of a soluble nitrogen source (untreated casein) did not affect liveweight gain, feed intake or feed conversion efficiency. It was therefore shown that supplementation with a bypass protein was beneficial to growing lambs, it was then of interest to determine if treated lupins could be used as a source of bypass protein.

Within Australia there is a lack of suitable sources of protein that can be used directly as supplements to increase dietary amino acid availability for grazing animals. Lupins have recently become popular as a supplementary feed because of their high protein and energy content, digestibility, palatability and ease of feeding. The protein in lupins is relatively soluble (Hume 1975) but at high levels of intake some lupin protein may escape rumen degradation and increase the P/E ratio of the products absorbed in the intestine (Smith and Kenney 1987).

As discussed above, a supplement for grazing ruminants ideally needs only be used in small amounts to increase the P/E ratio of the products absorbed by the animal. Formaldehyde was used in an attempt to protect lupins from rumen degradation. The results showed that formaldehyde treatment had no significant effect on the solubility of lupin meal in the rumen and thus may have had no effect on the amount of lupin protein reaching the small intestine. A subsequent growth study showed that lambs, fed a basal diet of oaten chaff and supplemented with 100 g/d treated or untreated lupin meal, had liveweight gains and feed conversion efficiencies similar to that of lambs supplemented with 10 g/d urea. It was concluded that lupins, when fed at these levels do not lead to an increase in dietary amino acids absorbed from the intestine. To increase the P/E ratios absorbed from the intestine it would be necessary to include lupins as a larger proportion of the diet.

There are critical levels of rumen ammonia below which intake and digestibility of the diet is will be lowered (see Section 2.4.1). Provision of a nitrogen source that is effective and safe to feed may be of considerable importance for the pastoral industry in certain periods of the year. Traditionally, urea has been used for this purpose but it is difficult to feed and may result in livestock losses due to toxicity if animals over-consume. Lupins offer a safe alternative being easy to feed, palatable and with a high protein and energy content. However, unless fed in large amounts, the value of lupins is in the provision of a soluble nitrogen source for situations when rumen ammonia levels are too low to support efficient microbial growth, and not as a bypass protein.

In situations where rumen ammonia levels are below the critical level for efficient microbial growth, provision of urea generally increases feed intake and reduces liveweight losses (see for instance, Lindsay and Loxton 1981). However, feeding of urea to animals on poor quality feeds is only a survival or maintenance strategy as the amount of microbial protein is still insufficient to meet the animals requirements for growth and lactation (Ørskov 1970, Fraser *et al.* 1991). The further provision of a source of amino acids that bypasses ruminal degradation is therefore essential (Lindsay and Loxton 1981, Preston and Leng 1987).

The response in feed intake by lambs to treated casein, though significant, was not in the range expected. If the work reported by Maeng *et al.* (1989), holds true in an *in vivo* situation, the liveweight gain responses produced in these lambs by supplementation with treated casein may be due, at least partly, to the provision of a slowly degradable source of amino acids in the rumen thereby increasing the efficiency of microbial growth, rather than as a direct effect of intestinally digested protein. Alternatively, both factors may be involved.

In an attempt to differentiate in-rumen from in-animal responses, a different fibrous basal diet which was devoid of starch was used in a further growth study with

lambs. Cottonseed hulls (CSH) are of low digestibility (45%) and low nitrogen content and considered to be a very low-quality roughage.

In these studies, the effects of bypass protein supplementation was compared with the effects of feeding supplements of sodium propionate and sodium acetate, to clarify responses to both aminogenic or glucogenic substrates.

Glucose has been recognised as a potentially limiting nutrient for ruminants, particularly those on low quality forages that promote low propionate to acetate ratios in the rumen (Lindsay 1959, Annison and White 1961, Leng *et al.* 1977, Nolan *et al.* 1986). Central to the importance of glucose to ruminants fed low-quality diets is the hypothesis that glucose is needed to supply glycerol and NADPH to enable fat synthesis from acetate. If amino acids are diverted to gluconeogenesis this will reduce the potential for protein deposition and will decrease N retention. Without a sink for acetate in excess of the animals requirement the acetate load would be a metabolic embarrassment to the animal as acetate cannot accumulate in blood without creating acidotic conditions. This in itself may lead to a decrease in feed intake.

In an imbalanced feeding situation, where there is insufficient glucose available to utilise the acetate produced, the animal has a number of metabolic strategies that it may draw on. It can initiate acetate oxidising cycles which are not coupled with ATP formation, i.e. futile cycles (see MacRae and Lobley 1982), it may excrete some of the acetate in the urine (Ørskov and Macleod 1990) which is unlikely to be significant under normal feeding conditions, or if the previous strategies are not sufficient to reduce acetate accumulation, the animal may reduce its feed intake.

Liveweight gain, feed intake, wool growth, glucose synthesis (Judson and Leng 1968) and acetate clearance (Weston 1966) were measured in lambs fed CSH and supplemented with aminogenic and/or glucogenic substrates. An additional

supplement, sodium acetate, was included in an attempt to assess the response of lambs to very high acetate levels.

The results of the growth study showed that CSH were capable of supporting relatively high liveweight gains in lambs when provided with urea, lucerne and minerals. Productivity and efficiency of feed conversion of lambs was increased by supplementation with protected casein. This supports the concept that the lambs were responding to a post-ruminal source of amino acids which provided them with a better balance of nutrients absorbed.

The CSH were consumed at a high level by lambs and the protozoa population densities in the rumen were either absent or extremely low. This could have been a contributing factor in the relatively high liveweight gain and high feed conversion efficiencies of lambs fed CSH diets. Studies from these laboratories (Bird and Leng 1984, Habib 1988, Romulo *et al.* 1988, Bird *et al.* 1990) have shown that unsupplemented animals without protozoa in their rumen have similar liveweight gains to animals with protozoa and supplemented with a bypass protein. The small particle size of the CSH, because it led to rapid communition, may have contributed to the high feed intake.

The relatively high productivity obtained by feeding a basal diet of CSH was considered an important finding of this research. In a grazing situation when the amount of feed on offer is scarce CSH could be used to augment the dry standing feed.

The feeding of a bypass protein supplement led to an increase in the efficiency of feed utilisation but its mode of action was not fully understood. The lambs responded to the addition of the bypass protein by increasing their liveweight gain, wool growth and feed conversion efficiency. However, there were no interactions between propionate and treated casein on their effects on production parameters. Lambs given propionate increased their intake of dry matter but did not have higher liveweight gains

than unsupplemented control sheep. The additional acetate in this diet which already produced high levels of acetate in the VFAs in the rumen did not appear to have any adverse affects on the parameters measured.

The metabolic study showed that glucose entry rates in lambs fed CSH were high and were not altered significantly by addition of bypass protein, propionate or acetate. Clearance of an acetate load injected into the jugular vein of the lambs was measured and also showed no significant differences between the lambs given either the basal diet or the diet supplemented with bypass protein, propionate or acetate.

The conclusions drawn from this experiment were that because of the high intake, absence of protozoa and the high glucose entry rates in these lambs the nutrients absorbed were relatively well balanced to meet the needs of the lambs. The animals therefore appeared to be in receipt of the nutrients needed for their physiological state and level of production. It was not possible from these results to precisely define the roles of the different substrates. It was shown, however, that only when the P/E ratio of the products absorbed from the intestine was increased by feeding a bypass protein was there a significant increase in liveweight gain, wool growth and feed conversion efficiency.

In the two studies in which a known bypass protein source was fed, intake of dry matter of lambs was only stimulated in one experiment. These studies were examined in the light of the hypothesis proposed by Leng (1990b), i.e. that environmental temperature will affect an animals response to supplementary feeding. It was noted, that when intake of the basal diet by lambs increased in response to supplementation with bypass protein, the experiment was being carried out in summer. Conversely, when feed intake of lambs was not increased by supplementation, the experiment was done during the winter. Although other factors may have been involved in these

differences in intake response it was decided that there was enough evidence to suggest that environmental temperature may have been an interacting factor.

When *ad libitum* CSH were fed to lambs housed at 26 ^{o}C or 37 ^{o}C liveweight gain, wool growth and feed conversion efficiency of the lambs were increased by feeding treated casein. However, the hypothesised interaction between supplementation with bypass protein and environmental temperature was not significant, though feed intake of lambs given treated casein was maintained at 37 ^{o}C to the same level as that of unsupplemented lambs at 26 ^{o}C and their liveweight gain was 10% higher. The small numbers of animals used may have contributed to the lack of significance in these results.

Although feeding of CSH produced extremely high proportions of acetate and low proportions of propionate in the rumen it was also shown, in the previous experiment, to support high glucose entry rates in the lambs. The high glucose entry rates may have been related to microbial protein supply which would be relatively high due to the low protozoa population densities in the rumen. One theory of inefficient use of acetate in ruminants on roughage diets is based on the observation that these diets only provide very small amounts of glucose precursors (both propionate and amino acids). Therefore, the high glucose entry rates produced in lambs by CSH feeding may have been providing the necessary metabolic substrates for efficient use of any surplus acetate. The low protozoa population densities and high feed intakes of lambs on this diet would allow considerably more microbial and dietary protein to flow into the intestines than on a similar diet with high protozoa densities (Ushida *et al.* 1986). The high feed intake, high glucose entry rates, and potentially high P/E ratio in the products absorbed, make this diet atypical of the low quality straws available to many of the ruminants in tropical areas. For this reason straw was used for the next experiment.

When straw was fed to lambs housed at 25 or 37 ^{o}C the lambs at 37 ^{o}C had significantly lower intakes than those housed at 25 ^{o}C. Provision of a bypass protein did not prevent this decline in dry matter intake. Nevertheless, the lambs given treated casein were able to increase their liveweight gain at the higher temperature compared with unsupplemented lambs or those given propionate, all of which lost weight.

The feed intake of the lambs at 37 ^{o}C was only 10% less than that of the lambs at 25 ^{o}C. The work reported by Kempton and Leng (1979) and Lindsay and Loxton (1981) showed increases in feed intake due to supplementation with urea and a source of bypass protein in the order of 60 to 96%.

It is apparent when viewing the data presented here that the lambs used as controls in all these experiments were already supplemented to a far greater degree than animals in the field *viz* their mineral requirements were met and any deficiency of rumen ammonia was allayed by supplementation with either ammonia-treated straw or urea. Therefore, the starting point in terms of efficiency of the animal system was already appreciably higher than that found in animals fed low quality roughages. The magnitude of the increase/decrease in intake due to supplementation and to high environmental temperature may be much lower than would be found when the basal diet does not supply critical nutrients. The responses obtained to bypass protein in these experiments with high temperature could possibly be much greater under a field situation where the starting point in terms of the animal system is much lower.

The metabolic studies showed that high temperature through its effect on feed intake, effected glucose entry rates and nitrogen metabolism of the lambs. High temperature reduced the amount of urea apparently entering the gut (i.e. recycling of urea back to the rumen). This effect may be important for ruminants on low-quality roughages that are already critically affected by low rumen ammonia levels. The

consequence of a reduced recycling of urea to the rumen may be a reduction of dry matter intake of animals already experiencing a protein and energy deficit.

Propionate supplementation of these lambs did not increase glucose entry rates though propionate is known to be a major glucose precursor (Bergman 1983). The decrease in feed intake, especially at 37 ^{o}C, of the lambs given propionate demonstrated that some metabolic disturbance, perhaps an increase in the animal's heat production, was caused by the additional propionate.

The breed of the lambs (Merino cross) used in these experiments would have contributed to their ability to withstand thermal stress. Work with cattle has shown that *Bos indicus* cattle have greater heat tolerance than *Bos taurus* breeds, and that a significantly higher temperature was required for an increase in rectal temperature to occur in the *B. indicus* cattle compared with *B. taurus*. Genetic differences, plus the absence of other stresses such as solar radiation, humidity and infrequent watering may partly explain why differences in the magnitude of response to supplementation with bypass protein are seen between laboratory versus field studies.

From the studies on effects of high temperature on lambs it was shown that at the higher temperature bypass protein supplements were able to increase productivity and maintain feed intake of lambs fed straw. A further indication of the benefits attained by bypass protein supplementation was that supplemented animals increased their water intake in response to high temperature to a much smaller degree than animals given nil or propionate supplementation. It would appear that in a grazing situation where the animal is not supplemented with essential minerals or bypass protein that the impact of a supplement that provides these nutrients would be much greater than that observed in these experiments.

6.2 Future Research

There is a major need to test the hypothesis that imbalanced diets lead to additional heat production which, at times, may cause ruminants to reduce feed intake. Continuing studies in this area should use geographical distribution of experimental sites to test this hypothesis rather than attempting to do it in controlled environmental temperature rooms. Animals in the experiments should be kept outdoors with access to some shade.

A suitable experiment would involve selection of animals from one source and provision of feed from one source and then a replicated experimental design in, for example, subtropical Australia and temperate Australia.

Future studies should use control animals that are fed the basal diet with no supplements. Treatment groups would include basal diet plus minerals/urea (to ensure an adequate rumen), basal diet plus minerals/urea and bypass protein. Results from experiments of this nature would be directly applicable to the field.