Chapter 7. Effect of increasing levels of bypass protein on the performance of Merino lambs at high ambient temperature

7.1. Introduction

There was a trend in the results of Experiment 5 for sheep experiencing a moderate level of heat stress and fed oaten chaff plus 1% urea and 100g/d of CSM (CSM+ diet) to eat more than those on oaten chaff plus 2% urea (control diet) alone. However, the differences in DMI observed in Experiment 5 were small and non significant. The relatively high DMI (2.5 - 3% of liveweight) observed in Experiment 5 probably allowed a very small margin for bypass protein supplementation to result in a significant additional increase. The presence of urea in the basal diet, which would be expected to have maintained a sufficient level of ammonia in the rumen to support microbial fermentation and growth, and to thus enable these animals to maintain relatively high feed intakes. The rumen ammonia concentrations recorded in Experiment 5 were well above the level of 50 mg NH_3-N/l suggested by Satter and Slyter (1974) and Russell and Strobel (1987) as an optimal level for microbial growth.

When urea was withheld from the basal diet but ambient temperature was maintained (during the second stage of Experiment 5), the total DMI of sheep on the CSM+ diet was significantly higher than that of those on oaten chaff. However, the actual difference was only about 100 - 130 g/d, meaning that intake of the basal diet did not differ significantly between treatments. Despite the absence of urea in the basal diet, the DMI was even slightly higher than when urea was provided. At a higher level of heat stress (Experiment 3), on the other hand, DMI was very low and bypass protein supplementation again did not significantly alleviate the depression in intake. The level of heat load imposed in Experiment 3 was apparently too high for DMI to be stimulated by 100 g CSM/d.

The evidence from all current experiments, however, is thus still insufficient to either accept or reject the hypothesis that bypass protein supplementation can minimise the total heat load of ruminants fed low quality basal diets at high ambient temperatures and thus enable the animals to maintain or even increase their feed

intakes. The 'quality' of the basal diet used in Experiment 5 was high enough to allow these sheep to maintain high feed intakes. In the current experiment, a lower quality roughage (barley straw) and varying levels of a preformed bypass protein supplement (Norpro; 40% N disappearance after 24h nylon bag incubation; Resksupaphon, 1996) were used to overcome these possible deficiencies. A mineral supplement was also used to ensure that rumen microbial requirement are met.

7.2. Materials and methods

7.2.1. Experiment 6

Sixteen Merino wethers aged 5-6 months and weighing 19.6±0.36 kg were housed individually in metabolism crates within 2 climate chambers at an ambient temperature of 33-39°C and 40-50% relative humidity. Lighting was provided for 12 h from 06.00 h to 18.00 h daily

The animals were randomly divided into 4 groups which were randomly allocated to 4 different dietary treatments as described in Table 7.1.

Table 7.1. Levels of urea and "Norpro" used to supplement the basal diet of chaffed barley straw + minerals in each treatment.

Treatment	Urea (g/kg Chaff)	Norpro (g/d)
1	20	0
2	18	50
3	16	100
4	14	150

All diets were designed to be isonitrogeneous in terms of having similar rumen degraded protein (RDP) concentrations, but differed in the amount of bypass protein they supplied to the small intestines. Because barley straw is known to be deficient in essential minerals, especially sulphur (Hills, 1996; unpublished), Na_2SO_4 and micromineral mix (Pfizer 422) were supplemented to all diets at the levels of 2 g Sulphur/kg (SCA, 1990) and 1 g/kg chaff respectively.

Ad libitum feed and water intakes were measured daily, while respiration rate (RR) and rectal temperature (RT) were recorded four times during the experiment (at 14.00

- 15.00 h). Rumen fluid samples to be analysed for NH_3-N and VFA were collected before feeding on day 21, and 2 h after feeding on day 22. Total collections of faeces and urine were performed for 7 consecutive days during week 4 to estimate dry matter digestibility, N balance and microbial yield from the rumen. Ten percent of daily faecal excretion was sampled and stored at -20°C. Urine was collected in a bucket containing 1000 ml of tap water and 15 ml of 50% H_2SO_4 (to maintain a pH of 2-4), and 5% of daily urinary excretion was sampled and stored at -20°C for determinations of urinary N and allantoin.

Thawed faecal samples were dried at 70°C for 48 h and N content was determined by Organic Nitrogen Determinator (LECO FP-228). The concentrations of ammonia N and VFAs in the rumen fluid were determined using Technicon Auto - Analyser and gas chromatography (PACKARD MODEL 427), respectively. The urinary allantoin concentration was determined by a colorimetric method as described by Chen and Gomez (1992) Estimations of total urinary excretion of purine derivatives, purine absorption and microbial N supply were described in section 1.3. Metabolisable energy intake (MEI) was calculated by multiplying DMI and estimated energy density (M/D, MJ/kgDM) which was estimated as 0.17 %DMD - 2 (SCA, 1990).

Data were analysed by one-way analysis of variance (ANOVA) on the Minitab 10.1 Statistical Package (Ryan *et al*, 1985).

7.3. Results

Respiration rate and rectal temperature

Under the current experimental conditions, increasing the level of Norpro (a bypass protein-rich supplement) in the diet did not affect the RR and RT of the sheep, which averaged 160 resp/min and 40.4°C respectively (Table 7.2).

Table 7.2. Respiration rate (RR, respirations/minute), rectal temperature (RT, °C), water intake (WI, n l/d), DMI (DMI, g/d), water to feed intake ratio (W/F, ml/gDM), dry matter digestibility (DMD, %), estimated* MEI (MEI, MJ/d) and liveweight gain (LWG, g/d) of sheep fed varying levels of Norpro supplement (mean±sem) at 33-39°C.

Parameter	Norpro supplementation (g/d)				P
	0	50	100	150	Value
RR	151±14	163±10	153±13	174±11	0.54
RT	40.5±0.0[illegible]	40.5±0.26	40.5±0.15	40.2±0.13	0.38
WI	3266±44[illegible]	2873±47	3294±209	2854±58	0.50
DMI:					
- straw	531±34	535±27	591±29	572±23	0.40
- total	531±34	580±27	683±29	709±23	0.00
W/F	6.1±0.65	5.0±0.16	4.8±0.34	4.0±0.08	0.01
DMD	48±2.6	48±1.1	46±1.3	47±1.1	0.79
MEI	3.22±0.0[illegible]	3.56±0.22	3.97±0.29	4.21±0.21	0.03
LWG	-32±7	-8±8	24±11	33±6	0.00

* see materials and methods

Water and feed intake

As shown in Table 7.2, mean WI ranged from 2.9 l/d in sheep receiving 50 and 150 g/d of Norpro to 3.3 l/d in sheep on the control diet and in those fed 100 g/d Norpro. These differences in WI, however, were non-significant between treatments.

Supplementation with Norpro significantly increased total DMI of Merino sheep fed a barley straw basal diet. Animals supplemented with 100 g/d of Norpro had a significantly higher DMI than those given the control diet or those supplemented with 50 g/d of Norpro, but increasing the supplement to 150 g/d did not lead to any further significant increase in DMI. Supplementation with Norpro did not significantly change the intake of the barley straw basal diet (Figure 7.1).

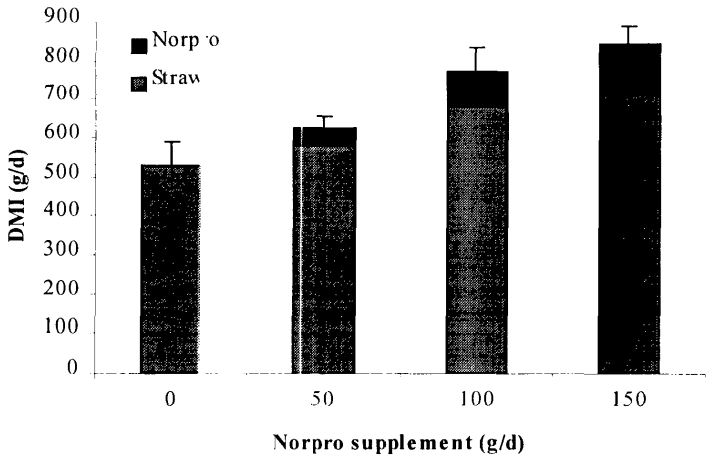

Figure 7.1. Effect of increasing level of Norpro supplement on the intake of chaffed barley straw by Merino lambs at 33-39°C (vertical bars = SEM of total DMI).

Increasing levels of Norpro significantly and progressively reduced the water to feed ratio (Table 7.2) from 6.1 ml/gDM on the control (basal) diet to 4.0 ml/gDM in the group supplemented with 150 g Norpro/d.

Dry matter digestibility

DMD in all treatments, which ranged from 46 to 48% (Table 7.3), was not significantly affected by Norpro supplementation.

Liveweight gain

Norpro supplementation significantly ($P<0.01$) improved LWG of Merino lambs. Lambs on the control diet and on the 50 g/d Norpro supplement lost weight throughout the experiment, while those on the 100 and 150 g/d of Norpro supplement gained weight at the rates of 24 and 33 g/d respectively (Table 7.2 and Figure 7.2).

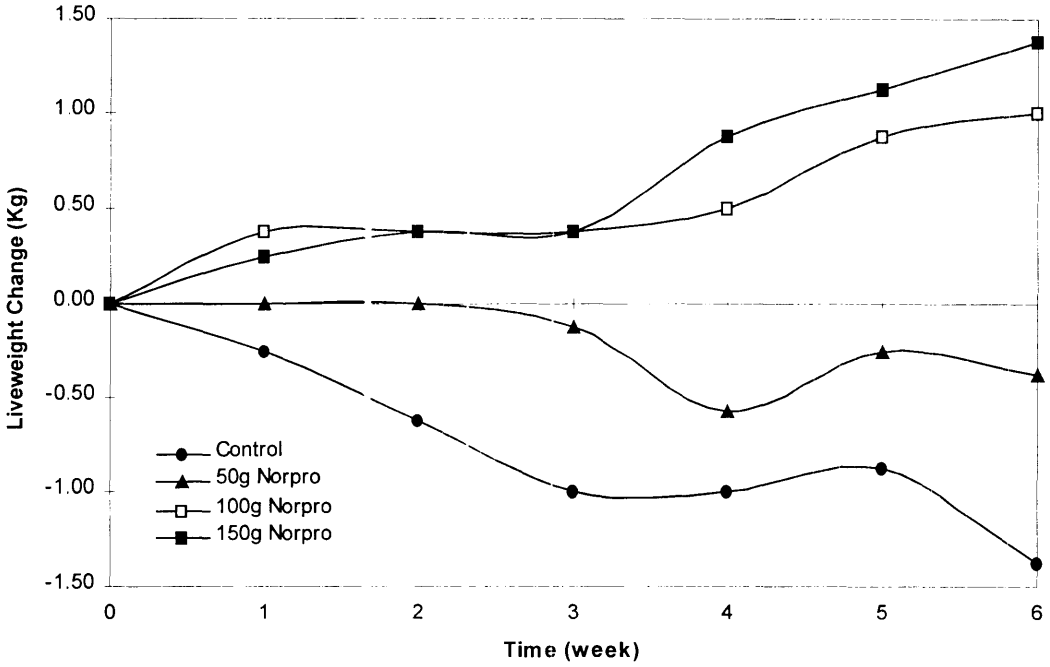

Figure 7.2. Liveweight change of Merino lambs given basal diet of barley straw plus minerals and increasing levels of Norpro supplement (g/d) over a six-week period

When the mean liveweight change of sheep in each treatmetn was plotted against its corresponding digestible DMI (Figure 7.3), the increase in liveweight gain was significantly correlated the mean DDMI.

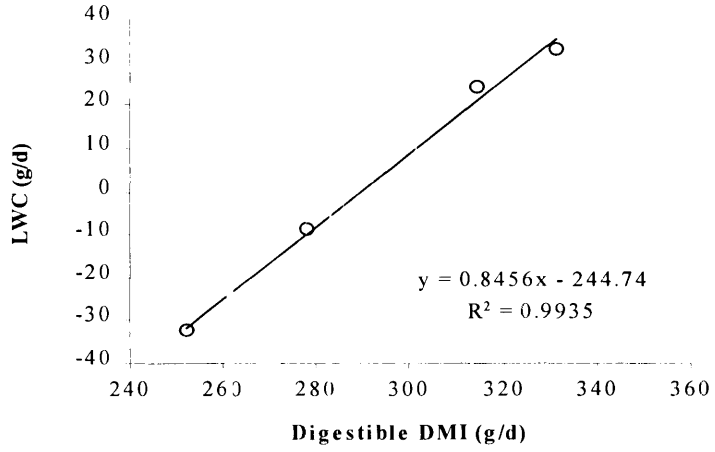

Figure 7.3. The relationship between digestible DMI and liveweight change (LWC) in Merino lambs during Experiment 6.

Rumen ammonia concentration

The concentration of ammonia in the rumen fluid before feeding was significantly higher (P<0.01) in sheep supplemented with Norpro than in sheep on the control diet, but there was no significant difference in the concentration between the three levels of Norpro supplement provided. At 2 h after feeding, on the other hand, rumen ammonia concentrations were relatively similar in all treatments (Table 7.3).

Table 7.3. Rumen ammonia concentration (NH_3-N, mg N/L), nitrogen balance (NB, g/d), urinary excretion of allantoin (mmol/d), purine derivatives (PD, mmol/d) and microbial N supply (MNS, gN/d) of sheep consuming increasing levels of Norpro supplement (mean±sem).

Parameter	Norpro supplementation (g/d)				P value
	0	50	100	150	
NH_3-N:					
- before feeding	75±14	115±7	110±11	151±15	0.01
- 2 h after feeding	241±35	265±16	274±29	257±14	0.81
NB:					
- N intake	9.8±.44	11.4±.34	13.8±.38	15.2±.30	0.00
- N in faeces	3.4±.23	3.9±.21	5.0±.20	5.2±.13	0.00
- N in urine	4.9±.93	5.9±.43	6.5±.44	7.0.34±	0.12
- N retention	1.5±.70	1.5±.39	2.3±.37	3.0±.06	0.10
Allantoin excretion	2.6±.63	3.6±.26	4.6±.40	4.8±.21	0.01
PD	3.3±.73	4.6.±.33	5.8±.54	6.1±.26	0.01
MNS*	2.3±.82	3.6±.32	4.8±.47	5.1±.24	0.01

*calculated from urinary allalntoin excretion (see materials and methods).

Nitrogen balance

N intake and N excreted in faeces were significantly increased (P<0.001) by the increasing levels of Norpro supplementation. Differences in urinary N excretion and N retention, on the other hand, only approached significance (P= 0.12 and P= 0.10 respectively). N retention was twice as high in sheep supplemented with 150 g/d of Norpro as in sheep on the control diet.

Microbial outflow rate

The rate of purine derivative excretion (calculated from the daily excretion of allantoin), and thus the calculated microbial protein outflow rate, were significantly (P=0.01) increased by the increasing levels of Norpro supplement (Table 7.3). The microbial N supply was significantly higher in supplemented sheep and reached a plateau at a level of supplementation of 100 g/d. Further increase in supplement level did not significantly increase microbial N supply.

7.4. Discussion

The means of RR and RT recorded for the lambs in the current experiment were relatively high (160 respirations/minute and 40.4°C respectively), indicating that the lambs were under a moderate level of heat stress. Thus while RT was constantly higher than the suggested normal value of 39.1°C for adult sheep under thermoneutral conditions (Bianca, 1968), it was also considerably lower than the suggested upper limit for safety for ruminants of 41.5°C (Yeates *et al.*, 1975). The lambs also consumed considerably more water (2.9-3.3 l/d) than did similar animals at 17°C in Experiment 3.

Supplementation with a bypass protein-rich supplement (Norpro) did not significantly reduce either RR, RT or WI. The ratio of water to feed intake, however, was significantly lower in sheep supplemented with Norpro than those in the control diets. The reduced water/feed ratios in sheep on the Norpro supplemented diet does not appeared to be related to the reduced requirement for cooling purposes because RR and RT were not significantly reduced. It is possible that the amount of water required for digestion and metabolism is reduced when Norpro was supplemented.

DMI and LWG, on the other hand, were significantly improved by the addition of Norpro to the barley straw basal diet. Supplementation with Norpro (a formaldehyde-treated soybean meal) would be expected to have improved the level of dietary amino acids absorbed in the small intestines, and to have thus increased the ratio of protein to energy in the absorbed nutrients and consequently to have minimised the animals' total heat load, which in turn would be expected to have increased DMI and LWG (Lerg, 1990). The protein : energy ratio would have been

further improved by the addition of microbial amino acid supply to the small intestine.

The optimum level of Norpro supplementation in terms of DMI, microbial N supply and LWG appeared to be 100 g/d. However, at this level of supplementation, the lambs gained only at a rate of 24 g/d. Factors other than bypass protein, such as MEI or the balance between protein and energy, should also be considered as having potential to increase the rate of liveweight gain of sheep under the current experimental conditions. Thus Bolam *et al.* (1996) reported that increasing the daily MEI by 4, 8 and 12%, by continuous infusions of acetate or glucose, tended (the slope was 0.24 for both acetate, r^2 = 0.5, and glucose, r^2 = 0.4) to proportionally improve the nitrogen balance in sheep supplemented with 5 g/Kg$W^{0.75}$.d of casein. When the MEI of the lambs in the current experiment was estimated (Table 7.2) and compared with the corresponding estimate of their requirement (of 6 MJ/d to grow at 100 g/d; SCA, 1990), the ME intake of the current lambs was concluded to be insufficient.

Rumen ammonia N concentrations before feeding in all treatments were well above the level of 50 mg NH_3N/L suggested to be adequate for rumen function by Satter and Slyter (1974). In contrast, the concentration exceeded 200 mg/l at 2 h after feeding, a level which has more recently been suggested to be appropriate for a straw-based diet (Preston and Leng, 1987; Kanjanapruthipong, 1995). While the ammonia concentrations at 2 h after feeding were very similar between treatments, the concentrations before feeding were significantly higher ($P<0.01$) in sheep given the Norpro supplement (at all levels) than in those given the control diet. Even though the intake of soluble nitrogen was maintained as close as possible to a similar level in all treatments, the total nitrogen intake (Table 7.3) necessarily increased with increasing levels of Norpro supplement. Urea recycling to the rumen could have contributed to the significantly higher rumen ammonia N in all supplemented sheep. The amount of urea recycled into the rumen is positively related to the rate of digestion of organic matter in the rumen and urea concentration in plasma (Kennedy and Milligan, 1980; Nolan, 1993). Unfortunately, plasma urea N was not measured in the current experiment.

The increasing N intake following supplementation with increasing levels of Norpro, resulted in more N excreted in faeces and also tended to increase N excreted in the urine. Consequently, N retention only tended ($P = 0.10$) to increase with

Norpro supplementation. The utilisation of the additional nitrogen was probably limited by energy availability.

The microbial N supply, estimated from the daily excretion of urinary allantoin, was significantly increased by the increasing levels of Norpro supplementation; from 2.3 gN/d in sheep fed the control diet to 3.6, 4.8 and 5.1 gN/d in those fed the control diet plus 50, 100 and 150 g/d of Norpro respectively. As Norpro supplementation significantly increased total DMI (Table 7.2), the increases in allantoin excretion in the present study can be attributed to the increased organic matter intake (Vercoe, 1976; Chen *et al.*, 1992; Dewhurst and Webster, 1992; Balcells *et al.*, 1993). Susmel *et al.* (1994), also found that allantoin excretion in Simmental cows fed fescue hay increased from 26 mmol/d in cows supplemented with a low N concentrate (no urea supplement; CP content = 92 g/kgDM) to 32 mmol/d in those supplemented with a high N concentrate (20 g/kg; CP content = 161 g/kg). In the experiment of Susmel *et al.* (1994), the digestibility increased with urea supplementation and thus the higher excretion of allantoin was again related to intake of digestible organic matter.

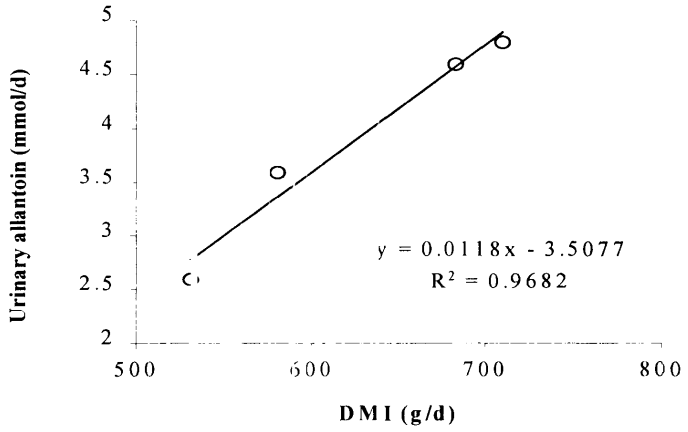

Figure 7.4. Relationship between dry matter intake (DMI) and urinary excretion of allantoin in Merino lambs in Experiment 6 (data were plotted in the order of dietary treatments).

The calculated microbial N supply recorded in the present experiment was comparable to the value of 5.2-7.7 gN/d reported by Kanjanapruthipong (1995) for sheep fed the oaten chaff supplemented with increasing levels of urea. Kanjanapruthipong (1995) suggested that the higher rumen ammonia due to urea supplementation reduced protozoal and fungal growth. As a consequence, the ingestion of bacteria by protozoa and bacterial turnover within the rumen would have been reduced, and the rate of microbial growth increased, resulting in a marked

increase in purine derivatives excreted in the urine. Even though the rumen ammonia concentrations in the present study were successfully adjusted to a similar level in all treatments at 2 h after feeding, the concentrations were significantly elevated by Norpro supplementation before feeding (Table 7.3). These prolonged increases in ammonia levels and the fact that the Norpro supplement contained some soluble carbohydrate, would be expected to have increased the rate of microbial growth and thus the allantoin excretion rate.

Increasing levels of Norpro supplementation appeared to have significantly increased the DMI, microbial N supply and LWG in the present study. However, the actual increase in LWG was only 24-33 g/d at a relatively high level (about 25-40 g/d) of protein meal (Norpro) supplementation. It is apparent that the primary limitation to production is inadequate feed intake. The low degistibility of the basal diet was probably the most important limitation to higher DMI. Even though the DMI was relatively high (2.5 - 3.5% of liveweight), low digestibility (mean DMD = 47%) resulted in low digestible DMI. As a result, estimated ME intakes (Table 7.2), were close to the estimated MEm of 3.5 MJ ME/d (SCA, 1990). In the following experiment (Chapter 8), additional energy (in the form of the soluble carbohydrate in barley grain) was supplemented in addition to Norpro bypass protein.

Chapter 8. Effects of soluble carbohydrate on the responses of Merino lambs to bypass protein supplementation at high ambient temperature

8.1. Introduction

The feed intake and liveweight gain of Merino lambs fed a barley straw basal diet supplemented with "Norpro" bypass protein (Experiment 6) reached a peak at a level of supplementation of 100 g/d. The primary limitation to increase feed intake beyond this point was probably the gut fill. Despite the intakes of 100 - 150 g/d of Norpro protein supplement (5.2% N, 40% degradable), the actual liveweight gain was less than 50 g/d.

Orskov and Grubb (1978) suggested that if the potential digestibility of a basal diet is low, the available nitrogen from a protein or NPN supplement cannot be utilised efficiently by the rumen microbes, presumably due to limited availability of fermentable carbohydrate. On the other hand, on a basal diet with higher potential digestibility and therefore an adequate supply of fermentable carbohydrate, the available nitrogen can be efficiently utilised and as a result, feed intake can be increased. The DMD of the basal diet used in Experiment 6 was only 46-48%, and the intakes of fermentable organic matter was inadequate to enable optimum utilisation of available N. It was thus concluded that at such a high intake of protein supplement in Experiment 6, the ME intake was not sufficient to support high growth rate. Any increase in maintenance energy requirement at high ambient temperature (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brinks and Ames, 1975; Ames and Brinks, 1977) may have exacerbated the problem.

In the present experiment, increasing levels of barley grain (a source of soluble carbohydrate), in addition to 100 g/d of "Norpro", were supplemented to the basal diet of barley straw + 2% urea, to increase the overall degradability of the diet. It was hypothesised that the additional soluble carbohydrate from barley grain would provide energy substrate for the rumen microbes, and that as a result the DMD, and the rates of microbial protein synthesis and of liveweight gain would be improved.

8.2. Materials and methods

8.2.1. Experiment 7

Sixteen Merino wethers aged 10 months and weighing 19.5±0.35 kg were housed individually in metabolism crates within 2 climate chambers at an ambient temperature of 33-39°C and 40-50% relative humidity. Lighting was provided for 12 h daily from 06.00 h to 18.00 h

The animals were randomly divided into 4 groups, each of which was fed for 6 weeks *ad libitum* on a basal diet of barley chaff + 2% urea and one of the 4 protein (Norpro) and/or energy (barley grain) supplements described in Table 8.1.

Table 8.1. The levels of "Norpro" and/or barley grain supplemented to a basal diet of barley straw treated with 2% urea and minerals in Experiment 7.

Treatment	"Norpro" (g/d)	Grain + 1% urea (g/d)
1	0	100
2	100	0
3	100	100
4	100	200

All diets were supplemented with Na_2SO_4 and Pfizer's 422 micro-mineral mix at the levels of 2 g S/kg and 1 g per kg chaff respectively.

Feed and water intakes were measured daily. The basal diet was offered twice daily (morning and afternoon) at a level at least 20% in excess of the previous day's intake, while the supplements were offered once daily (with the morning feed). Drinking water was freely available at all times.

Respiration rate (RR) and rectal temperature (RT) were measured 3 times during the experiment to monitor the level of heat stress. Total collections of faeces and urine to measure digestibility, N balance and microbial protein production were performed for 7 consecutive days during week 5. Rumen samples for ammonia N and VFA were taken before feeding, and at 2-3 h after feeding on day 42. The methods for urine collection and for determinations of rumen ammonia and VFA, faecal N, urinary N and allantoin were described in Chapter 7 (section 7.2). Calculations of microbial N supply and MEI were also described in section 7.2. All animals were weighed weekly before feeding to allow liveweight gain to be estimated.

Data were analysed by oneway ANOVA for a completely randomised design on the Minitab 10.1 statistical package (Ryan *et al.*, 1985).

8.3. Results

Respiration rate and rectal ten perature

RR and RT ranged f:om 143-175 respirations/minute and 40.2-40.4°C respectively. No significant eff:cts we:e observed either on respiration rate or rectal temperature (Table 8.2).

Table 8.2. Respiration rate (RR, respirations/minute), rectal temperature (RT, °C), water intake (WI, ml/d), water to feed intake ratio (W/F, ml/gDM), dry matter intake (DMI, g/d), estimated MEI (MEI, MJ/d) and liveweight gain (LWG, g/d) of sheep fed varying levels of Norpro and energy supplements (mean±sem).

Parameter	Norpro (N) and grain (G) in the diet (g/d)				P
	100N+0G	0N+100G	100N+100G	100N+200G	Value
RR	143±13	170±16	165±8	175±13	0.44
RT	40.4±0.18	40.2±0.17	40.3±0.19	40.2±0.13	0.90
WI	3683±309	2902±81	3126±155	3868±385	0.08
W/F	5.9±0.85	4.7±0.17	4.6±0.29	5.3±0.78	0.46
DMI:					
- staw	553±65	533±31	509±29	476±36	0.58
- total	645±65	621±31	688±29	744±36	0.18
MEI	3.2±0.6	3.9±0.1	4.1±0.2	4.1±0.6	0.50
LWG	2±15	9±3	32±4	48±7	0.01

Water intake and feed intake

Water intake tended to be lower (P=0.08) in lambs fed 100 g of barley grain compared with those that received the other supplements. The ratio of water to feed intake, however, was not significantly different between treatments.

DMI (Table 8.2) of lambs fed 100 g/d of "Norpro" did not differ significantly from that of lambs supplemented with 100 g/d of barley grain. The DMI of lambs offered 100 or 200 g/d of barley grain in addition to 100 g/d of "Norpro" were also

not significantly higher than that of lambs supplemented with 100 g/d of "Norpro" alone. Similarly, estimated ME] was not significantly increased by dietary treatments.

Even though not significantly so, grain supplementation tended to depress the intake of the basal diet (Table 8.2 and Figure 8.1).

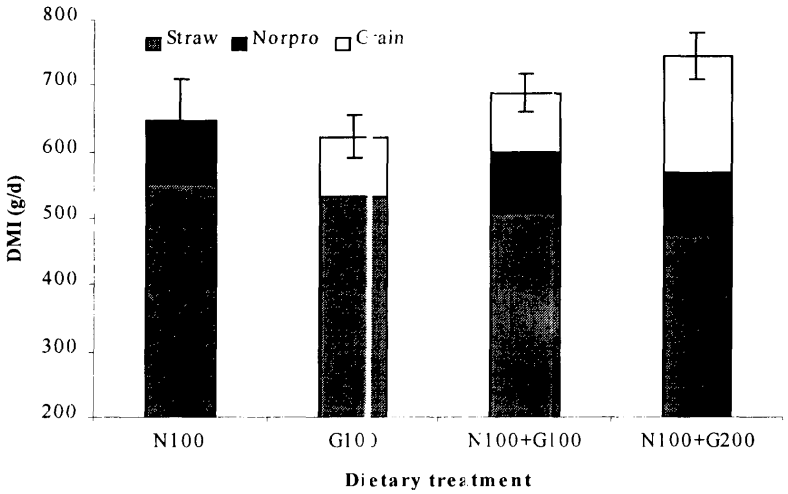

Figure 8.1. Effect of increasing level of Norpro supplement on the intake of chaffed barley straw by Merino lambs at a high ambient temperature (vertical bars = SEM of total DMI).

Liveweight gain

Lambs given 100 g of "Norpro" or 100 g grain barely maintained liveweight during the experimental period. Supplementation with 100g or 200 g of grain in addition to 100 g "Norpro" per day significantly (P=0.01) improved liveweight gain to 32 and 48 g/d respectively (Table 8.2 and Figure 8.2).

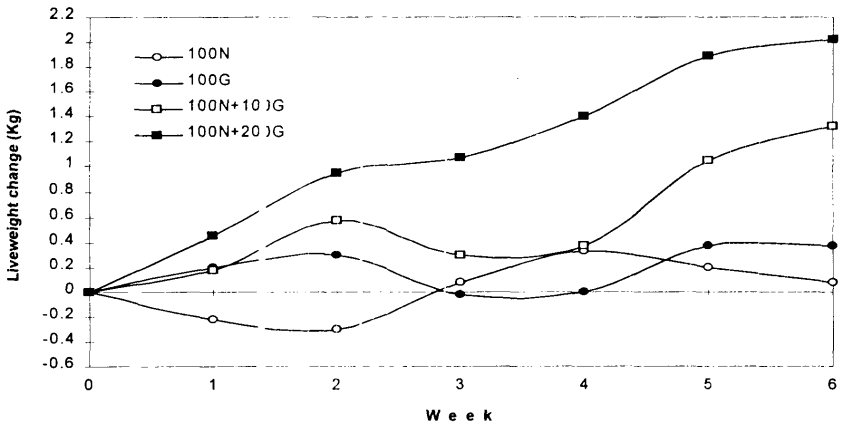

Figure 8.2. Liveweight change of Merino lambs fed barley straw supplemented with different levels of "Norpro" (N) and barley grain (G) over a six-week period

As shown in Figure 8.3, the liveweight change of sheep in Experiment 7 was significantly correlated (R^2 0.79) with digestible organic matter intake.

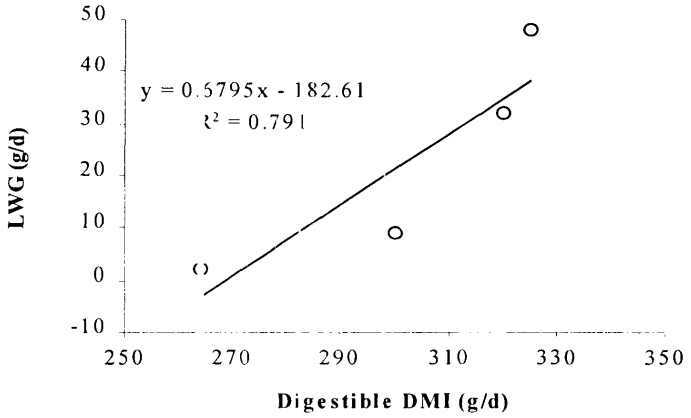

Figure 8.3. The relationship between digestible DMI and LWG of Merino lambs in Experiment 7 (mean DDMI in each treatment was plotted against its corresponding LWG).

Rumen ammonia concentration

The concentrations of ammonia in rumen fluid before feeding differed significantly between treatments. The concentration in sheep given 100 g "Norpro" was significantly higher than in those given 100 g grain or 100g "Norpro" + 200 g grain, but non significantly different from that of sheep fed 100 g "Norpro" + 100 g grain. The differences in rumen ammonia concentrations between sheep fed 100 g grain, 100 g "Norpro" + 100 g grain and 100 g "Norpro" + 200 g grain were not significant. Similarly, the values at 2 h after feeding did not differ significantly between treatments.

Table 8.3. Rumen fluid ammonia concentration (NH_3-N, mg N/L) and pH, dry matter digestibility (DMD, %), nitrogen balance (NB, g/d), urinary excretion of allantoin (mmol/d), and purine derivatives (PD, mmol/d) and microbial N supply (MNS, gN/d) of sheep fed varying levels of Norpro and grain supplements (mean±sem).

Parameter	Norpro (N) and grain (G) in the diet (g/d)				P
	100N+0G	0N+100G	100N+100 G	100N+200G	Value
NH_3-N:					
before feeding	135±31	58±3	105±5	79±11	0.02
2 h after feeding	337±20	334±11	418±29	365±48	0.32
pH	6.71±0.11	6.78±0.03	6.62±0.02	6.56±0.14	0.39
DMD	41±2.8	49±1.6	47±0.4	43±3.7	0.18
NB:					
- N intake	14.2±1.02	10.7±.63	15.4±.50	16.6±.50	0.00
- N in faeces*	5.3±.32	4.4±.24	5.5±.27	6.6±.21	0.00
- N in urine	8.6±.68	4.4±.40	6.2±.75	6.2±.75	0.01
- N retention	0.3±1.38	1.9±.23	3.6±.64	3.9±1.22	0.10
Allantoin	5.2±.19	3.9±.49	5.1±.73	7.3±.48	0.01
PD	6.5±.24	4.9±.61	6.4±.91	9.1±.59	0.01
MNS	5.4±.21	3.9±.62	5.3±.85	7.8±.53	0.01

*** means for faecal N allow for the one-day between when feed was put out and faeces were collected**

Dry matter digestibility

Even though the DMD values ranged from 41% in the sheep fed Norpro alone to 49% in those supplemented with grain alone, there were no significant (P>0.05) differences between treatments

Urinary output

Urine output varied between days CV 7.25%). The mean CVs for each treatment were 11% or less (i.e. 8.23 for Treatment 1, 4.78 for Treatment 2, 5.41 for Treatment 3 and 10.8% for Treatment 4, respectively). However, one lamb in treatment 4 had urine outputs ranging from 2.1 to 3.87 L/d (mean = 2.78, sd 0.78 L/d; CV = 28.2 %).

Nitrogen balance

N intake was significantly lower in sheep supplemented with grain only than in those on the other treatments. On the other hand, the N intakes of sheep fed Norpro only, Norpro + 100 g grain or Norpro + 200 g grain/d did not differ significantly. Increasing N intake significantly increased ($P<0.01$) N excreted in the urine and faeces, and tended ($P=0.10$) to increase N retention.

Microbial N supply:

The rate of urinary excretion of purine derivatives and estimated microbial N supply in the small intestines were significantly higher in sheep given 100 g/d of Norpro plus 200 g/d grain (9.. mmol/d and 7.8 gN/d) than in those supplemented with 100 g/d grain alone (4.9 mmol/d and 3.9 gN/d, respectively). The means for other treatments , however, did not differ significantly (Table 8.3).

Rumen fluid pH.

The pH of the rumen fluid in sheep given 100 g "Norpro"/d and those fed 100 grain/d did not differ significantly. The pH tended to decline ($P > 0.05$) when 100 g/d or 200g grain/d was supplemented on top of 100g "Norpro"/d.

Rumen fluid VFA.

The total VFA, the ratio of acetogenic to glucogenic (C2+C4/C3) acids and the molar proportions of VFA (Table 8.4) did not differ significantly between treatments. The total values ranged between 58 to 69 mmol/litre and the molar proportions of acetic, propionic and butyric acids were respectively 74.5-77.6.%, 16.4 - 17.5% and 6 - 7%.

Table 8.4. Rumen fluid VFA profiles (means of samples taken before feeding and 2 h after feeding) of Merino sheep in Experiment 7.

Parameter	Norpro (N) and grain (G) supplemented (g/d)				P
	100N+0G	0N+100G	100N+100G	100N+200G	Value
Total (mmol/L)	58.4±10.(	67.5±2.7	69.0±4.3	69.0±6.4	0.61
(C2+C4)/C3	5.0±0.3	4.7±0.2	4.6±0.2	4.8±0.3	0.81
Molar percentage					
Acetic (%)	76.2±0.9	74.5±10.0	74.6±0.7	74.7±1.1	0.61
Propionic (%)	16.4±0.7	17.5±0.8	17.4±0.5	17±1.0	0.80
Isobutyric (%)	1.2±0.3	0.7±0.2	0.9±0.1	1.0±0.2	0.46
Butyric (%)	5.0±0.4	6.3±0.3	6.0±0.4	6.0±0.1	0.08
Isovaleric (%)	0.8±0.2	0.6±0.1	0.7±0.1	0.8±0.1	0.69
Valeric (%)	0.3±0.0	0.4±0.0	0.4±0.0	0.5±0.0	0.12

8.4. Discussion

As environmental conditions in Experiments 6 and 7 were similar, it is not surprising that the levels of heat stress of the corresponding groups of lambs, as indicated by respiration rate and rectal temperature, were also generally similar. The respiration rates and rectal temperatures observed in the current experiment (143-175 resp./min and 40.2-40.4°C) were about the same as those reported in mature Suffolk ewes at 40°C (151 resp./min and 40.2 °C; Ames *et al.*, 1971), but were lower than those observed in Experiment 3 (180-202 resp./min and 40.8-40.9°C). These lower values were probably related to the fact that a lower relative humidity was applied in the current experiment (40-50%) than in Experiment 3 (60-70%).

Water intakes, on the other hand, were generally higher than in Experiment 6. The intake of water tended to be lower ($P = 0.08$) in lambs supplemented with 100 g/d of "Norpro" than in the other treatment groups. Similarity between treatment groups in the ratio of water to feed intake indicates that water intake reflected the need for digestion as influenced by DMI. However, as the water to feed ratio in the present study was about 2 times higher than that observed in crossbred lambs at 17°C (see Experiment 3) or in feral goats housed at 25°C and fed a pelleted diet containing 80% roughage and 19.5% grain (Dahlanuddin and Thwaites, 1993), it is concluded that a significant part of the water ingested was required for thermoregulation when ambient temperature is high. Dahlanuddin *et al.* (1996) showed that the water:feed ratio was significantly lower in goats on a concentrate diet that on a roughage diet at high ambient temperatures, but increasing levels of Norpro and grain supplements in the current experiment did not reduce the water:feed ratio.

Dry matter intake did not differ significantly between treatments. Grain supplementation up to 200 g/d did not significantly reduce the intake of the basal diet of barley chaff, though there was a progressive downward trend (Figure 8.1). As the grain supplement was always mixed with 1% urea and minerals before feeding, soluble nitrogen concentration in the rumen (indicated by the non-significantly different NH_3-N concentration at 2 h after feeding, Table 8.2) was not reduced by grain supplementation and thus the digestibility and the intake of the basal diet were not depressed significantly.

However, rumen ammon a N concentrations before feeding were significantly lower in lambs offered 100 g grain (58 mg N/L) than in those supplemented with 100 g Norpro (135 mgN/L). The concentrations in other treatments, even though the differences were non-significant, tended to be lower as the amount of grain was increased. These values suggest that the availability of starch (from the grain) might have increased the ruminally digestible energy the total diet, and thus increased the requirement for soluble N to support microbial fermentation (Orskov and Grubb, 1978).

Lambs fed 100g/d "Norpro" or 100 g/d grain could only barely maintain liveweight, while those supplemented with 100g "Norpro"+100 g grain and 100g "Norpro"+200 g grain per day grew significantly faster, at rates of 32 and 48 g/d respectively. Compared with the results of Experiment 6, where the plateau of liveweight gain of lambs (24 g/d) was reached when 100 g/d of "Norpro" was supplemented, provision of soluble energy (in the form of grain) in addition to 100 g/d of "Norpro" in the current experiment further improved the rate of liveweight gain. The balance of protein to energy in the absorbed nutrients should also have been improved in Experiment 7. The non-significant difference in liveweight gain between lambs supplemented with 100 g "Norpro" and those supplemented with 100 g grain suggests that the response to "Norpro" supplementation observed in Experiment 6 was partly due to improvement in total organic matter intake as suggested by the relationship between digestible DMI and liveweight change (Figures 7.3 and 8.3). Thus, to achieve a maximum response to bypass protein supplementation under these conditions, sufficient organic matter intake to provide adequate metabolisable energy intake must firstly be achieved, e.g. by inclusion of energy concentrates or prior treatments of the basal diet to increase its potential digestibility. In the current experiment, treatment of the basal diet to improve its potential digestibility was not possible, due to time and facility limitations. As an alternative, barley grain was supplemented to improve the overall digestibility of the diet.

The intake of ME (Table 8.2) tended to increase with grain supplementation. However, their ME intakes were only sufficient to meet their estimated ME requirements of 3.9-4.0 MJ ME/d, and thus their respective live-weight changes are predictable.

The pH of the rumen fluid (which ranged from 6.6 to 6.8) did not differ significantly between treatments and appeared to be close to the upper limit of the

normal range (5.5 to 7.0; Church, 1970; Theodorou and France, 1993). It is often reported that the pH of rumen fluid is reduced by grain supplementation due to rapid fermentation of starch, which results in an elevated concentration of lactic acid. Supplementation of barley grain up to 200 g/d in Experiment 7 was apparently within the 'safe' level and the microbes thus appeared to have obtained adequate concentrations of substrates necessary for maintaining a normal rumen environment and therefore a normal pH of rumen fluid.

Nitrogen intake was significantly lower in sheep supplemented with grain only than in those supplemented with Norpro only, Norpro + 100 g grain or Norpro + 200 g grain. The high N intakes increased the faecal and urinary N excretions; faecal N loss was proportional to N intake, but urinary N loss tended to be reduced by grain supplementation. As expected, N retention tended (P = 0.10) to increase with grain supplementation in addition to 100 g Norpro. In fact, sheep fed Norpro only, retained only 0.31 g N/d, while those fed grain only retained almost 2 gN/d, and N retention increased to 3.6 and 3.9 gN/d when 100 and 200 g/d of grain was supplemented in addition of 100 g/d of Norpro (Table 8.3). This indicated that the soluble carbohydrate component of the grain increased the supply of energy which is expected to have increased the utilisation of available nitrogen by microorganisms in the rumen (McLennan *et al.*, 1995).

Total VFAs were not significantly affected either by Norpro or grain supplementation, even though the values tended to be lower by 10 mmol/l in sheep offered 100 g Norpro than in the other treatments. The total production of VFAs observed in the present experiment (58 - 69 mmol/L) is lower than the range (70-130 mmol/L) considered to normal by France and Siddons (1993). The concentration of of VFA in the rumen fluid is related to microbial yield (see section 1.2, Figure 1. 5); when the efficiency of microbial cell synthesis increases, the amount of fermentable OM used for cell synthesis is increased while the amount available for VFA production is reduced (Leng and Nolan, 1984). In the present study, this relationship cannot be established because rumen volume and thus the efficiency of microbial cell synthesis was not measured.

The molar proportions of VFA in the current experiment were high in acetate (76%) but lower in propionate (16%) and butyrate (6%) than generally observed in ruminants fed high fibre diets (70%:20%:10%; France and Siddons, 1993). These low values were probably a result of the relatively low digestibilities of the current diets

and also due to a relatively low ME intake. Even though nutrients essential for microbial fermentation, especially N and S, were included in the basal diet the digestibilities recorded were very low (41-49% DMD; Table 8.3).

The low potential digestibility of the basal diet used can be expected to have limited the supply of digestible organic matter for microbial growth. The availability of soluble carbohydrate from the grain supplement in Experiment 7, in addition to 100 g/d of Norpro, appeared to have improved the rate of microbial growth. The microbial N supply was twice as high in sheep fed 100 Norpro plus 200 g grain than in those fed 100 g grain only. The higher microbial N supply again appeared to be due to increased intake of organic matter. The increases in intakes of digestible DM due to supplementations of 100 or 200 g/d of barley grain in addition to 100 g/d of Norpro in the present experiment were very small (56 and 61 gDDM/d repectively). Thus, it is not surprising that improvement in the rates of liveweight gain were only small (even though statistically significant) in Experiment 7. The major limitation to growth was again appeared to be the low DMI, which was constrained primarily by the limited physical capacity of the gut to accomodate the bulky, low digestibility basal diet.

As low DOMI and thus low MEI appeared to be the primary explanation for low growth rate observed in Experiments 6 and 7, it is necessary to find strategies to improve total DOMI, so that by improving the balance of nutrients absorbed through supplementation, the growth rate can be increased. In the following chapter (Experiment 8) the effects of increasing the potential intake of a similar basal diet (by grinding and pelleting) on digestion and metabolism will be examined and discussed.

Chapter 9. Effects of grinding and CSM supplementation on feed utilisation by Merino sheep at high temperature

9.1. Introduction

It was observed (Experiment 7) that wethers fed barley straw *ad libitum* and supplemented with either 100 g/d of "Norpro" or 100 g/d of barley grain barely maintained liveweight during a 6-week feeding trial. Supplementation with 100g or 200 g of barley grain in addition to 100 g "Norpro" per day significantly (P=0.01) improved liveweight gain to 32 and 48 g/d, respectively. It appeared most likely that these responses were due to an increased supply of digestible energy in the form of carbohydrate from the grain.

One of the factors affecting the response of ruminants to bypass protein supplementation is the nature of the basal diet (Leng *et al.*, 1977). In the proposed experiment, a similar basal diet to that used in Experiment 7 was ground (hammer-milled) to reduce its particle size and, hopefully, to increase the amount of metabolisable energy available for productive purposes. From their extensive review, Berger *et al.* (1994) concluded that grinding and pelleting consistently increased feed intake, liveweight gain, and feed efficiencey. Feed digestibility on the other hand, is generally depressed as particle size decreases due to a reduced mean retention time in the gut, but the efficiency of microbial protein synthesis may be increased due to an increased particulate dilution rate and increased feed intake, provided that rumen ammonia N is not limiting (Berger *et al.*, 1994).

The net energy content of forages is increased by grinding, largely as a result of a reduced heat increment and the fact that less energy is required for eating and ruminating (Berger *et al.*, 1994). Orskov and MacLeod (1990) also suggested that a high 'energetic cost of digestion' is responsible for the lower efficiency of ME utilisation in roughage based diets compared with concentrate diets. Therefore, reducing the work involved in digestion, for example by grinding or pelleting long roughages, could be expected to reduce the time spent eating and ruminating and to thus increase the efficiency of utilisation of absorbed energy for tissue growth.

In the proposed experiment, the following hypotheses were investigated:

1. That feed intake, microbial protein production and liveweight gain of sheep fed ground basal diet will be higher than in those given the same basal diet in chaffed form (both with equal contents of soluble nitrogen).
2. That bypass protein will further increase feed intake of sheep fed the pelleted basal diet due to an improved P/E ratio of absorbed nutrients, and as a result, the overall performance of these sheep will be better than that of sheep not supplemented with bypass protein.

9.2. Materials and methods

Experiment 8

Sixteen mature Merino wethers previously used in Experiment 7 were housed individually in temperature and light controlled rooms after a rest period of 4 months at pasture. Owing to limits placed by the Animal Care and Ethics Committee on the time for which sheep may be housed in metabolism crates, all animals were housed in floor pens for the first 4 weeks and then moved to metabolism crates for the last 2 weeks of data collection. Ambient temperature in the climate rooms was set at 19-21°C during a 2-week preliminary period, and was then gradually increased to 33-39°C during a 1-week temperature adaptation period. The temperature of 33-39°C was maintained for the rest of the 8-week experimental period. The relative humidity in the chambers was maintained at 40-50% during the rest of this period and lighting was provided from 06.00 to 18 00 h daily.

The experimental sheep were randomly allocated to 2 groups of 8, each was fed *ad libitum* a basal diet of either chaffed or ground-pelleted barley straw treated with 2% urea. The barley straw used in the present Experiment was similar to that used nin Experiments 6 and 7. Four randomly selected animals from each basal diet were supplemented with 100 g/d cottonseed meal (a source of bypass protein containing 7 % N and 58% N degradability), and the other 4 were left unsupplemented. All diets were supplemented with sulfur (2 g S/kg basal diet) and Pfizer 422mineral and vitamin supplement (1g/kg basal diet). Feeds were offered twice daily (at 09.00 and 17.30 h respectively) at a level of at least 15% in excess of previous day's intake.

CSM supplement (100 g/d) was offered once daily in a separate container (placed on the corner of the basal diet's container) at the same time with the morning basal diet. Drinking water was freely available at all times.

Feed and water intake were measured daily, while respiration rate (RR) and rectal temperature (RT) were recorded four times during the experimental period. Rumen fluid was collected before feeding and 2 h after feeding on day 30 to measure the concentrations of rumen ammonia nitrogen and volatile fatty acids. Rumen fluid samples for measurement of pH and protozoal numbers were taken 2 h after feeding on the last day of the experiment. A total collection of faeces and urine to measure dry matter digestibility, nitrogen balance and urinary excretion of allantoin was conducted for 7 consecutive days during week 6. All sheep were weighed weekly before feeding to estimate liveweight gain.

The methods for urine collection and for estimating concentrations of rumen ammonia and VFA, faecal N, urinary N and allantoin were as described in Chapter 7 (section 7.2). Estimations of total urinary excretion of purine derivatives, purine absorption and microbial N supply were made as described in section 1.3. Metabolisable energy intake (MEI) was calculated by multiplying DMI and estimated energy density (M/D, MJ/kgDM) which was estimated as 0.17 %DMD - 2 (SCA, 1990).

The degradability of CSM used in the present study was determined by the nylon bag method of Perdok (1987). Twenty nylon bags containing approximately 3 g of CSM dry matter were incubated in the rumen of 4 sheep (5 bags per sheep), and one bag was taken from each sheep at 2, 4, 8, 16 and 24 h after incubation. All bags were washed immediately under running tap water for approximately 10 minutes and dried at 70°C for 24 h for determination of DM and N contents.

All data were analysed by a two-way ANOVA using the Minitab 10.1 program (Ryan et al., 1985).

9.3. Results

Protein degradability of CSM

The protein degradability of the cottonseed meal used in the present study (Table 9.1) was estimated based on the model of Orskov and McDonald (1979):

$$P = a + (bc/(c+k))(1 - e^{-(c+k)/t})$$

where P = the protein degradability (%), a = the protein disappearance at zero time, b = the difference between a and the asymptote, c = the rate of disappearance of the protein per hour, k = the rate of passage, and t = the time of incubation (hours).

Table 9.1. Protein degradability * of the CSM at different rates of passage, calculated from the N disappearance at different incubation times in the rumen of sheep fed oaten chaff *ad libitum*.

Time of incubation (hours)	N disappearance (%)	
	measured (p)	fitted ($p^\wedge$)
2	37.8	37.3
4	42.9	43.7
8	54.7	54.5
16	70.1	69.9
24	79.5	79.6

Rate of passage (k)	0.04	0.05	0.06	0.07	0.08
Degradability (P)	69.1	65.5	62.5	59.9	57.8

*calculated using a curve fitting software (Neway Program, Rowett Research Institute, 1992).

Respiration rate and rectal temperature

Neither RR nor RT differed significantly between treatments, the respective values ranged from 150-171 respirations/minute and 40.1-40.3°C (Table 9.2).

Feed and water intakes

Grinding / pelleting and CSM supplementation significantly increased the DMI of both the basal and the total diet (Table 9.2 and Figure 9.1).

Table 9.2. RR (resp./min.), RT (°C), DMI (g/d), estimated ME intake (ME, MJ/d), WI (L/d), water/feed ratio (W/F, ml/gDM), DMD (%) and LWG (g/d) of Merino wethers fed chaffed or ground-pelleted barley straw with or without CSM supplement at high ambient temperature (mean±SE, N=4).

	Chaff		Pellet		P value		
	Control	CSM-	Control	CSM+	P*	CSM	Int.
RR	154±14	155±17	150±6	171±6	.61	.37	.40
RT	40.2±.08	40.1±.5	40.2±.19	40.3±.08	.51	.63	.47
DMI:							
- basal diet	705±34	721±59	1015±76	1286±64	0.00	0.03	0.06
- total	705±34	814±59	1015±76	1378±64	0.00	0.00	0.06
MEI	3.9±0.38	5.3±0.74	5.5±0.26	6.7±0.94	0.06	0.11	0.91
WI	3.44±.44	3.87±.23	3.99±.16	5.11±.34	0.01	0.03	0.28
W/F	4.9±0.75	4.8±0.32	4.0±0.18	3.7±0.37	0.05	0.72	0.93
DMD	44±2.2	50±4.1	43±2.9	40±3.4	0.12	0.75	0.19
LWG	-45±11	-28±13	16±20	68±13	0.00	0.04	0.26

* P = pellet effect, CSM = CSM supplementation effect, Int. = interaction

The intake of basal diet was 44% higher in sheep fed pellets than in those offered chaff as the basal diet (1150 vs 713 g/d). The interaction between basal diet and supplementation tended to be significant (P=0.06); CSM supplementation improved total DMI by only 110 g/d when chaff was the basal diet, but it increased the total total DMI by 364 g/d when pellet was fed as the basal diet. The total DMI of sheep fed pellet + CSM was 82% higher than the DMI of sheep fed chaff only. The estimated ME intakes tended to be higher on pellets than on chaff (P = 0.06), and on the CSM supplemented than on the control diets (P = 0.11), but the interactions between basal diet and CSM supplementation was non-significant.

Water intake (Table 9.2) was also significantly higher in sheep fed the basal diet of pellets (P=0.01) and in those supplemented with CSM (P=0.03). The interaction between basal diet and CSM was not significant. The ratio of water to feed intake, on the other hand, was significantly reduced (P=0.05) by grinding the basal diet, but not by CSM supplementation. Sheep fed the pellet basal diet consumed 1 ml less water per gram DMI than those fed chaff basal diet. The interaction between basal diet and CSM was also non-significant.

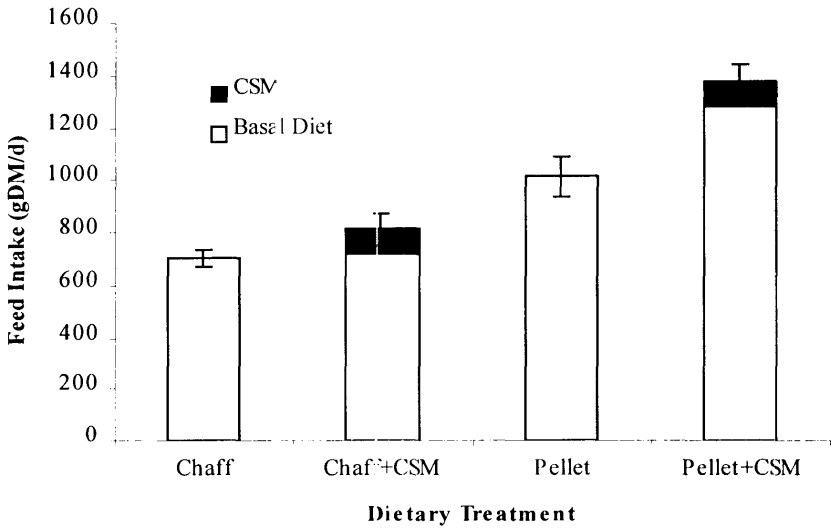

Figure 9.1. Effect of grinding/pelleting and CSM supplementation on DMI of Merino wethers at a high ambient temperature (vertical bars = SEM of total DMI).

Digestibility

DMD was not significantly affected by either grinding (P=0.12) or CSM supplementation (P=0.75), although the mean DMD values were 6% lower in sheep fed the pelleted basal diet than in those fed chaff (Table 9.2).

Rumen ammonia, VFA and pH

Rumen ammonia concentration before feeding, did not differ significantly between treatments, but at 2 h after feeding the concentrations were significantly higher (P<0.05) in sheep given chaff than in those given pellets. The concentrations were also higher (P<0.05) in the groups supplemented with CSM than in those fed chaff or pellets alone.

Table 9.3. Rumen ammonia, VFA and pH of Merino wethers fed chaffed or pelleted barley straw with or without CSM supplement at high ambient temperature (mean± SE, N=4).

	Chaff		Pellet		P value		
	Control	CSM+	Control	CSM+	P*	CSM	Int.
NH_3-N (mg/L):							
before feeding	129±14	92±7	110±14	120±23	0.75	0.38	0.15
2 h after	254±23	317±10	183±17	257±40	0.02	0.02	0.84
pH	6.84±.14	6.81±.04	6.73±.06	6.71±.06	0.24	0.78	0.99
VFA:							
Total (mmol/L)	80±6.6	6[illegible]±5.0	77±3.0	78±2.7	0.31	0.21	0.11
(C2+C4)/C3	4.8±0.4	4.[illegible]±0.1	4.2±0.2	4.4±0.3	0.10	0.70	0.72
Proportions:							
Acetic (%)	76±1.2	7[illegible]±0.3	71±2.1	73±0.8	0.02	0.75	0.39
Propionic (%)	17±1.2	1[illegible]±0.4	19±0.6	18±1.1	0.14	0.73	0.94
Isobutyric (%)	0.1±0.0	0.[illegible]±0.1	0.1±0.0	0.2±0.0	0.00	0.00	0.00
Butyric (%)	6±0.3	6±0.2	7±0.6	8±0.4	0.01	0.37	0.66
Isovaleric (%)	0.3±0.1	0.[illegible]±0.1	2±2	0.3±0.1	0.47	0.42	0.30
Valeric (%)	0.2±0.0	0.[illegible]±0.0	0.2±0.1	0.3±0.1	0.66	0.13	0.87

* P = pellet effect, CSM = CSM supplementation effect, Int. = interaction

The pH of rumen fluid, taken at 2 h after feeding, did not differ significantly between treatments: the values ranged from 6.71 to 6.84. Likewise, the total VFA concentration, the ratio of glucogenic to acetogenic and the molar proportions of VFAs (except isobutyric) did not differ significantly between treatments. There was a significant interaction between CSM and basal diet whereby the concentration of isobutyric acid was increased significantly by CSM on both diets but the effect was much greater when the basal diet was chaffed barley straw (Table 9.3).

Microbial N supply

The daily excretion of urinary allantoin was significantly ($P<0.05$) higher in sheep fed the pelleted basal diet than in sheep fed chaff. The effects of CSM supplementation and of the interaction between basal diet and CSM supplementation were non-significant. Based on the daily excretion of allantoin, total purine

derivatives excretion and microbial outflow rate were calculated and the results are presented in Table 9.4.

Table 9.4. Urinary excretion of allantoin (mmol/d) and estimates of the excretion of total purine derivatives (PD, mmol/d) and microbial N supply (MNS, gN/d) of Merino wethers fed chaffed or pelleted barley straw with or without CSM supplement at high ambient temperature (mean±SE, N=4).

	Chaff		Pellet		P value		
	Control	CSM+	Control	CSM+	P*	CSM	Int.
Allantoin	3.8±.47	5.8±.79	6.6±1.19	7.7±1.26	0.04	0.15	0.63
PD	4.8±.58	7.3±.99	8.3±1.48	9.6±1.57	0.04	0.15	0.63
MNS	3.6±.61	6.1±.92	7.0±1.39	8.2±1.46	0.04	0.15	0.60

* P = pellet effect, CSM = CSM supplementation effect, Int. = interaction

Liveweight gain

Liveweight gain was affected both by grinding/pelleting ($P<0.01$) and CSM supplementation ($P<0.05$), but the interaction between these two factors was non-significant. The wethers fed the basal diet of chaff all lost weight, but those fed pellets gained weight. Again, the effect of CSM supplementation was non significant when chaff was the basal diet, but when the ground-pelleted diet was offered, the liveweight gain was 52 g/d higher in wethers supplemented with CSM than in those given the basal diets. The liveweight change of sheep in the present experiment significantly correlated with digestible DMI (Figure 9.2).

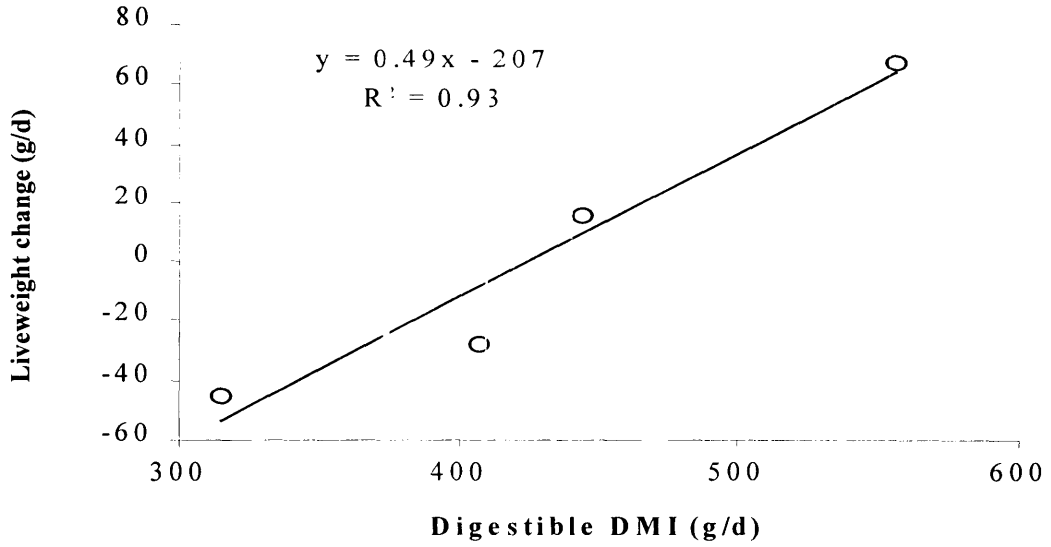

Figure 9.2. The relationship between digestible DMI and liveweight change of Merino wethers in Experiment 8.

9.4. Discussion

The ranges of RR and RT of sheep during Experiment 8 (Table 9.2) were similar to those exhibited by the sheep during Experiments 6 and 7. This is as expected, because the same temperature and humidity regimen as used in Experiments 6 and 7 was applied in Experiment 8. It is therefore assumed that the levels of heat stress experienced by the sheep during these 3 experiments were also very similar.

The estimated values of protein degradability of the cottonseed meal used (7.3% N), were 57.8, 59.9, 62.5, 65.5 and 69.1% at *k* values of 0.08, 0.07, 0.06, 0.05 and 0.04 per hour, respectively. The rumen digesta retention time in sheep was not measured in the present experiment (fed oaten chaff *ad libitum*). However, *k* value of 0.06 (measured for sheep fed dried grass *ad libitum;* Orskov and McDonald, 1979) may be used which corresponds to the degradability value of 62.5%.

DMI was significantly higher in the sheep fed the ground diet (pellet) than in the sheep fed chaff. This is consistent with general findings (see Berger *et al.*, 1994) that a reduction in particle size of the basal diet increases the rate of passage (reduced mean retention time) of digesta which enabled more feed to be ingested.

The interaction between basal diet and CSM supplementation was significant on DMI; the effect of CSM supplementation did not improve DMI of sheep given chaff, but significantly increased DMI of sheep given the pelleted diet. It has been reported that one of the factors affecting the response to bypass protein supplementation is the protein content of the basal diets used, i.e. if the basal diet already contains an adequate amount of protein, additional bypass protein cannot be expected to result in a significant response (Leng *et al.*, 1977). The basal diets used in the current experiment were similar in protein content (4% CP), thus the difference in responses to bypass protein between sheep fed chaff and those fed pellet cannot be explained by differences in the nutrient compositions of the basal diets. Rather, it was more likely to be related to the physical form (particle size) of the basal diet (chaff vs ground/pellet). However, grinding can be expected to have reduced particle residence time and hence increased the proportion of rapidly fermented substrates, which in turn improved the balance of nutrients.

The theory that bypass protein can improve the utilisation of any excess acetate produced from the fermentation of a low quality diet and thus improve DMI of ruminants under tropical conditions (Leng, 1990) does not seem to apply to the barley straw basal diet used in Experiment 8 unless the straw was ground. This indicates that it is not only chemical composition that limits the efficiency of utilisation of so called "low quality" roughages, but the physical form is also a significant constraint. It is apparent that the lower DMI of sheep on chaff in the present sudy was not due to a higher fibre content or to the nutrient balance of the diet, but it was most likely to be due to a limited capacity of the rumen and a longer retention time of feed materials in the rumen. Thus grinding, which reduced the particle size of the diet, increased DMI to a level at which nutrient imbalance was the primimary limitation to feed intake. When supplemented with CSM, sheep on the pellet further increased ($P<0.01$) their intake of the basal diet to a rate of almost twice that of sheep fed chaff only. Additional amino acids from CSM would have improved the P/E ratio of the diet and, and these results support the view of Leng (1990) who suggested this as a factor which may stimulate DMI. The P/E ratio in the sheep fed pellet+CSM could have also been improved by a higher rate of microbial outflow resulting from grinding and pel eting (see Table 9.3).

WI was significantly higher in sheep fed the pelletted basal diet, most likely due to increased DMI. However, the water to feed ratio was significantly reduced by pelleting, suggesting that reducing the particle size of the basal diet reduced the need for water for digestion. CSM supplementation, on the other hand, further increased WI in sheep fed the pellet basal diet. The increase in protein and/or mineral content of the diet due to CSM supplementation may also have increased the need for water to dispose of excess nitrogen and minerals.

It is generally reported (reviewed by Berger *et al.*, 1994) that grinding reduces digestibility due to increased feed intake and rate of passage. Goetsh *et al.* (1997) reported that grinding and pelleting tended ($P=0.11$) to reduce OMD of Bermuda grass and ryegrass hays by sheep. In the present experiment, DMD did not differ significantly between diets. Owing to the low potential digestibility of the barley straw used (DMD = 46-48% in Experiment 6, 41-49% in Experiment 7 and 40-50% in the current experiment), the longer retention time of chaff cannot be expected to increase digestibility in this case. On the other hand, the rate of fermentation is generally increased by grinding (Moore, 1964; Berger *et al.*, 1994), probably as a

result of increased exposure of feed surface to microbial attack. Grinding of the barley straw basal diet actually increased the rate of wash-out of the slowly degraded material.

As expected, rumen ammonia concentrations in all treatments were significantly higher at 2 h after feeding than before feeding. The concentrations at 2 h after feeding were significantly higher on CSM supplemented diets than on the control, and on chaff than on pellets. The higher concentrations on the CSM+ were probably due to a higher soluble nitrogen intake, as CSM also contain a considerable amount of rumen degradable N (more than 50% of total N, see Table 9.1). The lower ammonia concentration in sheep fed the pellet is interpreted as indicating that a higher requirement for ammonia N (to support higher microbial growth) existed on the ground/pelleted diet.

The pH of rumen fluid at 2 h post feeding did not differ significantly between treatments. The pH range (6.71-6.84) recorded in the present experiment was well within the suggested normal range (Theodorou and France, 1993; Dijkstra, 1994; Orskov, 1994) of 6 - 7, even though Moore (1964), Berger *et al.* (1994) and Orskov (1994) suggested that grinding reduced the rate of secretion of saliva and thus rumen pH was likely to be less well buffered.

The concentrations and molar proportions of VFAs in rumen fluid did not differ significantly between treatments. The total VFA concentrations, which ranged from 67-80 mmol/L, appeared to be at the lower end of the normal range of 70 to 130 mM/L suggested by France and Siddons (1993), but higher than the values recorded in Experiment 7. The concentrations of VFAs in Experiment 8 were also relatively high in acetate (71-76%), but low in propionate (17-19%) and butyrate (6-8%), compared to the corresponding ratio of 70: 20: 10 quoted by France and Siddons (1993). The results did not support the suggestion of Males (1964) that grinding may reduce the ratio of acetate to propionate as a result of increased rate of fermentation. It is possible that the rate of fermentation of barley straw in the present study was not significantly increased by grinding.

The increased DOMI due to pelleting in the present study would have increased the availability of substrates required for microbial growth. Microbial populations in the rumen are associated with the liquid-small particle pool, feed particles and the surface of the rumen wall (Owens and Goetsch, 1986). Any increase in the rate of

passage due to grinding in the present experiment would be expected to have resulted in:

1. An increase in the outflow rate of microbial cells both in the liquid phase and attached to particulate matter, but not those attached to the rumen wall.
2. A reduction in the energy used for microbial maintenance in the rumen. It is known that a considerable amount of ruminally digestible energy (15-55%) is used for microbial maintenance (Owens and Isaacson, 1977; Hespel and Bryant, 1979; Owens and Goetsch, 1986). The amount of digestible energy available for microbial growth is thus increased by reducing the microbial residence time in the rumen (Owens and Goetsch, 1986).

Thus the significant increases in liveweight gain which resulted from grinding and CSM supplementation in the current experiment may have been associated with:

1. Increased intake of digestible organic matter
2. Increased microbial outflow rate (Table 9.4), which is expected to have altered the protein to energy ratio of absorbed nutrients (Preston and Leng, 1987; Leng, 1990)
3. Increased efficiency of ME utilisation due to a reduced maintenance requirement of microbes in the rumen (Owens and Isaacson, 1977; Hespel and Bryant, 1979; Owens and Goetsch, 1986 Orskov, 1994), and less energy being wasted on activities associated with feeding, digestion and metabolism (Orskov and McLeod, 1990).

In conclusion, it is apparent that reducing the particle size of the basal diet improved DMI to a greater degree than that achieved by CSM supplementation in the absence of grinding or pelleting. The DMI increased further in sheep fed ground and pelleted barley straw when CSM was supplemented. These results support the suggestion of Leng (1990) that an improved P/E ratio of the absorbed nutrients (as might be due to CSM supplementation) would increase DMI on a diet of low quality roughage. This theory appeared, however, not to apply on a basal diet that had large-sized particles (i.e. chaff), because possible increases in DMI were restricted by a slower degradation rate.

Chapter 10. Effect of grinding and CSM supplementation on acetate metabolism in Merino sheep housed at high temperature

10.1. Introduction

It was apparent that increases in DMI and LWG due to CSM supplementation were greater in sheep fed a basal diet of ground, pelleted barley straw than in those fed the same material as chaff (Chapter 9). It was hypothesised that when chaff was offered, the increase in feed intake due to CSM supplementation was limited by rumen capacity. On the other hand, the smaller particle size of the ground basal diet enabled the animal to obtain digestible energy at a greater rate, and when the excess of acetogenic substrates on the basal diet alone was balanced by additional amino acids from the CSM, feed intake could be further increased because extra rumen capacity was available.

However, the necessary data were not collected during Experiment 9 to allow a conclusion to be made as to whether the better DMI and LWG of sheep fed the ground/pelleted basal diet were due to an increased efficiency of utilisation of acetate towards synthetic purposes (i.e. fatty acid synthesis) instead of being wastefully oxidised. An experiment using the isotope dilution technique was thus carried out to test whether the improvements in DMI and LWG observed during Experiment 8 were due to an increased efficiency of utilisation of acetate.

The hypothesis tested in this experiment was that CSM supplementation will reduce the rate of acetate oxidation (i.e. improve the use of acetate for productive purposes), by supplying more amino acids and increasing the availability of potential glucose precursors.

10.2. Material and methods

Experiment 9

The experiment was carried out to estimate the rate of acetate oxidation, and the contribution of acetate to total heat production, in Merino wethers maintained at high ambient temperature.

All sheep from Experiment 8 were maintained for another 10 days, at similar environmental conditions. The sheep were fed a basal diet of either chaffed or ground-pelleted barley straw with or without supplementation of 100 g/d of CSM. Feeding management during isotope infusion period was similar to that described in Experiment 8 (section 9.2). Because only 8 sheep could be infused at one time, the sheep were randomly divided into 2 groups of 8. On day 3, jugular catheters were inserted into both jugular veins in the first 8 sheep (sheep 1-8), and on day 4, a mixture of two tracers, ^{14}C-sodium bicarbonate (1mCi) and D-(2-^{3}H)-glucose (2mCi), was infused for 10 h at a rate of 0.22 ml/min and blood samples to be used for determination of blood bicarbonate (and the estimation of the rates of acetate oxidation) and glucose production were collected at 3, 4, 5, 6, 7, 8, 9 and 10 h after the beginning of the infusion. On day 5, 1-^{14}C-sodium acetate (1mCi) was infused into the same 8 sheep for another 10 h at a similar rate as that of the first 2 tracers. Blood samples were again taken at 0, 3, 4, 5, 6, 7, 8, 9 and 10 h after the beginning of the infusion to measure the plateau specific radioactivity (S. R.) of bicarbonate carbon. On the same day (day 5) jugular catheters were inserted into the other 8 sheep (sheep 9-16). On day 6, a mixture of two tracers, ^{14}C-sodium bicarbonate (1mCi) and D-(2-^{3}H)-glucose (2mCi), was infused into these new 8 sheep for 10 h at a rate of 0.22 ml/min. On day 7, 1-^{14}C-sodium acetate (1mCi) was infused into the new sheep (sheep 9-16) for another 10 h at a rate similar to that of the first 2 tracers. The procedures for tracer infusions and blood sample collections were similar to those described for sheep 1-8.

The collection and preparation of blood samples to be used for determination of blood bicarbonate ($H^{14}CO_3^-$) production were carried out according to the procedures described by Leng and Leonard (1965). About 6 ml of blood from each sheep was collected into a wide-necked McCartney bottle containing a small tube, into which 1

ml of 1.0 M NaOH was placed immediately after the blood samples were collected. After the bottle was sealed tightly, 1 ml of 1 M H_2SO_4 was injected into the blood through the lid. The sample was stored at room temperature for approximately 16 h to enable CO_2 released from the blood to be absorbed into the NaOH solution. Blood bicarbonate was isolated by precipitation as $BaCO_3$, and beta emission from ^{14}C in the $BaCO_3$ was determined by counting a dried, weighed amount of $BaCO_3$ in 10 ml of a scintillation cocktail by means of a Liquid Scintillation Analyzer (PACKARD 1900CA TRI-CAB). The scintillation cocktail contained 40 g of Cab-o-sil (thixotropic gel powder), 4g PPO (2,5 diphenyloxazole) and 0.2 g of POPOP [1,4 bis-2(-5-phenyloxazol)-benzene] per litre of toluene .

The glucose from plasma was isolated as the penta-acetate derivative and specific radioactivity was determined by the method of Jones (1965) as follows:

1. Centrifuged blood plasma samples in labelled McCartney bottles (1 ml each) were deproteinised by mixing with 100 mg glucose carrier, 8 ml distilled water, 5 ml 0.3M $Ba(OH)_2.8H_2O$ and then 5 ml of 0.3M $ZnSO_4.7H_2O$.
2. After standing for 30 min, the bottles were centrifuged at 3000 rpm for 10 min; supernatant was collected into labelled 50 ml beakers through Whatman No. 41 filter paper.
3. Three drops of glacial acetic acid were added into each beaker, samples were then cooked in a boiling water bath until all water had evaporated.
4. 60-70 mg of anhydrous sodium acetate and 1 - 1.5 ml acetic anhydride was added to each beaker.
5. All beakers were sealed with aluminium foil and placed in an oven at 90-95°C for approximately 30 min and then cooled.
6. The seals were removed and distilled water was added to make the volume in each beaker up to 18 ml.
7. Samples were boiled on a hot plate (beaker was held with tongs and moved in a circular motion to avoid bumping) until oily globules disappeared.
8. All beakers were cooled at room temperature until crystals of glucose penta-acetate appeared and distilled water was then added to the 20 ml mark and left overnight in a cool room.
9. On the following day, crystals were washed with cold water and collected on No. 1 filter paper placed on a $BaCO_3$ planchette-making device.

10. Crystals were put back into the same beakers and after adding 15-18 ml distilled water, all samples were again dissolved on a hot plate as described in steps 7-9.
11. Using a spatula, crystals were dropped into a tared and labelled scintillation vial, dried at 100°C for at least 2 h, cooled in a desiccator and weighed.
12. Five ml of liquid scintillation cocktail [4 g PPO (2.5-diphenyl oxazole) + 0.2 g POPOP (1,4-bis-2-(5-phenyloxazolyl) benzene) in 1 l toluene] was added into each vial, dissolved and counted for ^{3}H using Liquid Scintillation Analyzer (PACKARD 1900CA TRI-CAB).

10.3. Calculations:

To enable the tracer results to be analysed, the following assumptions were made: 1). the chemical and physiological behaviour of tracers is exactly the same as that of the tracees, and 2). the compartment size of a tracee remains constant, i.e. the rate of input is the same as the rate of output (Shipley and Clark, 1972). These assumptions enabled the a calculation to be made of the net flux of tracee into the compartment into which tracer is infused.

During a continuous infusion of tracer, it is further assumed that the tracer will mix instantaneously with the tracee in the primary compartment, and the ratio of tracer : tracee (the S.R) will reach a plateau some time after infusion (Figure 10.1). Thus, net flux of blood bicarbonate and glucose in the present experiment were calculated according to the procedure described by Shipley and Clark (1972) as illustrated below.

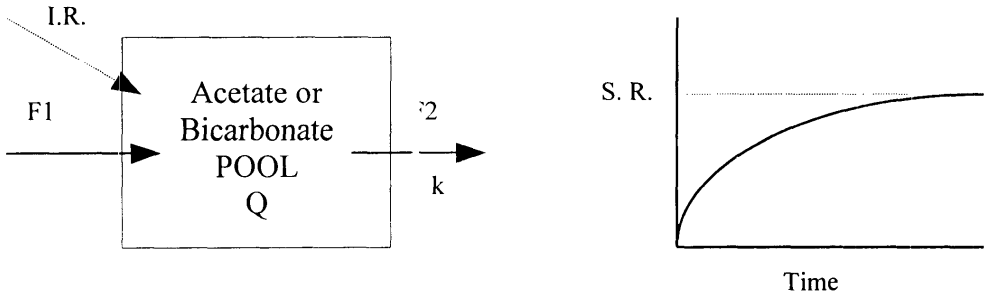

Figure 10. 1. A single compartment model showing the relationships between infusion rate (IR), specific radioactivity (S. R.) and the net flux (F1=F2) of blood bicarbonate and glucose during a continuous infusion (based on Shipley and Clark, 1972).

As illustrated in Figure 10. 1, the net flux of glucose or bicarbonate can be calculated as :

$$\textbf{E. R.} = \textbf{I. R. / S. R.}$$

where E. R. = net flux (g/min); I. R. = infusion rate (nCi/min) and S.R. = mean specific radioactivity at plateau (nCi/g).

As the content of the compartment is being replaced at the rate of k per unit time, the compartment size of glucose was calculated using the following formula:

$$\textbf{Q} = \textbf{E. R. / } k$$

where Q = compartment size (g); E. R. = net flux (g/min) and k = output rate constant.

Because the S. R. of blood acetate was not measured in this experiment, the net flux of acetate could not be calculated. However, the proportion of acetate oxidised could be calculated from the amount of acetate ^{14}C flowing through the bicarbonate pool during the labelled acetate infusion as follows:

Flow of acetate ^{14}C through HCO_3 pool = E. R. of HCO_3-C (gC/d) x S. R. of HCO_3 (nCi/gC)

The fraction of acetate oxidised was calculated from the following formula:

$$\textbf{Fraction Oxidised} = \frac{\textbf{Rate of } ^{14}\textbf{C flow through bicarbonate (nCi/d)}}{\textbf{Rate of infusion of } ^{14}\textbf{C-acetate (nCi/d)}}$$

Energy expenditure (**Y**, kJ/h) was estimated from the net flux of HCO_3 (**X**, l/h) using the equation given by Corbett *et al.*, (1971):

$$\textbf{Y} = 0.55\,\textbf{X} + 5.74$$

For the purpose of this calculation, the unit of HCO_3 'entry rate' was converted from mgC/min to L/h using ideal gas laws which assume that the volume of 1 mol (12 g) of CO_2 gas at standard temperature and pressure (STP: 273°K, 1 Atm) occupies 22.4 L. The volume of CO_2 at the ambient temperature of the current experiment (36°C) was calculated using Charles' Law, viz.

$$\textbf{V1 x T1} = \textbf{V2 x T2}$$

where V1= volume of gas at STP, T1= standard temperature (273°K),

V2 = volume of gas at 36°C, and T2 = the current temperature in °K (273+36).

10.4. Statistical Analysis

Data were analysed by analysis of variance for a 2 x 2 factorial design (2 basal diets and 2 levels of bypass protein supplementation) on the Minitab 10.1 statistical program (Ryan *et al.*, 1985).

10.5. Results

10.5.1. Feed intake

DMI during Experiment 1 was significantly affected by both grinding/pelleting and CSM supplementation (Table 10.1). The calculated digestible DMI, on the other hand, was significantly higher in sheep supplemented with CSM but the effect of grinding/pelleting was non-significant.

Table 10.1. DMI (g/d) and DDMI (g/d) of Merino sheep fed chaffed or ground barley straw with or without CSM supplement.

	Chaff		Pellet		P value		
	Control	CSM+	Control	CSM+	P*	CSM	Int.
DMI	668±19	747±51	751±154	1184±82	0.01	0.02	0.08
DDMI	298±19	368±16	332±79	474±52	0.18	0.05	0.47

* P = pellet effect, CSM = CSM supplementation effect, Int. = interaction

10.5.2. Specific radioactivity

The specific radioactivity of blood bicarbonate approached a plateau at 3 - 4 h after the infusion of ^{14}C-sodium bicarbonate and 1-^{14}C-sodium acetate began. During both infusions, the plateau remained relatively steady until infusion was terminated (Figures 10.2).

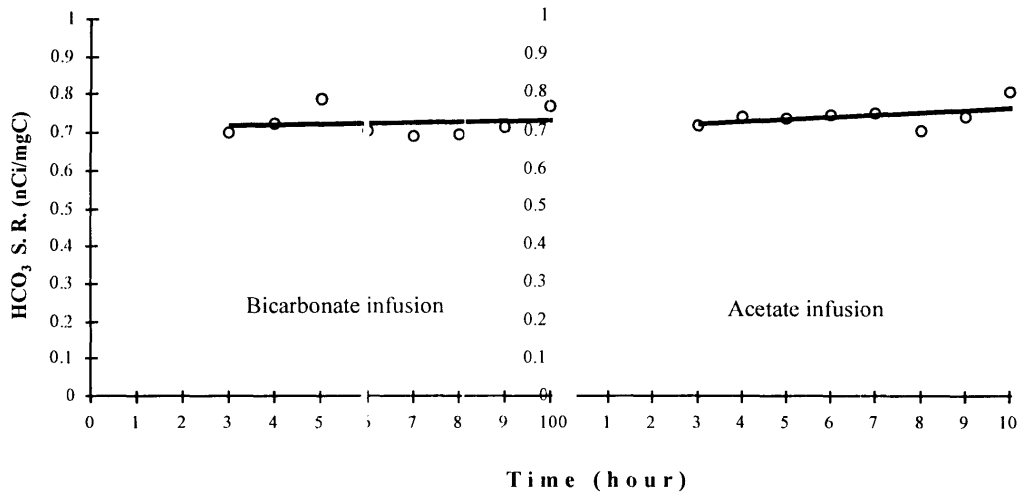

Figure 10.2. Specific radioactivity of blood bicarbonate (HCO_3 S.R.) during continuous infusions of ^{14}C-sodium bicarbonate and 1-^{14}C-sodium acetate.

10.5.3. Net flux / entry rate

The means of S.R. at plateau were used to calculate the net flux of blood bicarbonate and glucose, and the results are presented in Table 10.2.

Net flux of bicarbonate:

The net flux of ^{14}C-bicarbonate was significantly higher in sheep fed pellet + CSM than in those fed other diets. Even though the effects of pelleting and supplementation on bicarbonate net flux were significant, the net flux in sheep fed pellets only did not differ significantly from those of both groups of sheep fed chaff. Thus a significant effect of CSM on bicarbonate net flux occurred only in sheep on the pelleted diet. However, when the net flux was expressed as 'gC/kg DDMI', the values did not differ significantly between treatments. Similarly, the estimated energy expenditure per ME intake did not differ significantly between treatments.

Table 10.2. The net flux of bicarbonate (HCO_3) and glucose (Glucose), the proportion of acetate oxidised and estimated energy expenditure (E) in Merino sheep fed chaffed or ground barley straw with or without CSM supplement.

	Chaff		Pellet		P value		
	Control	CSM+	Control	CSM+	Pellet*	CSM	Int.
HCO_3 :							
- gC/d	150±6.5	157±10.4	156±25.3	223±8.3	0.05	0.04	0.08
- gC/kg DDMI	458±86	407±53	475±168	778±181	0.17	0.36	0.21
E (KJ/h)	213±5.6	219± 7.3	218±19.3	275±7.0	0.05	0.04	0.08
E/ ME intake	1.4±0.1	1.2±0.2	1.5±0.3	1.2±0.2	0.78	0.29	0.97
Glucose :							
- g/d	54±5.1	62±7.2	76±3.9	101±7.4	0.00	0.01	0.14
- g/kg DDMI	161±31	163±14	220±47	343±65	0.02	0.18	0.18
Acetate oxidised (%)	78±3.5	67±3.5	73±6.0	73±5.5	0.91	0.37	0.35

* Pellet = pellet effect, CSM = CSM supplementation effect, Int. = interaction

***Net flux of glucose*:**

Glucose net flux was significantly higher in sheep fed pellets (mean = 88.4 g/d) than in sheep fed chaff (mean = 58.5 g/d). The effect of CSM was again non-significant when chaff was the basal diet. The net flux of glucose in sheep fed pellet + CSM was 33% higher than in those fed pellets only, or almost twice the net flux in sheep fed chaff only. On the other hand, the net flux of glucose per kg of DDMI was only affected ($P<0.05$) by grinding/pelleting; the net flux was 73% higher in sheep on pellets than in those on chaff (281 vs 162 g/kg DDMI respectively).

Acetate oxidation:

The proportion of acetate oxidised (estimated from the net flux of bicarbonate and the S. R. of bicarbonate during acetate infusion), on the other hand, was not affected either by grinding/pelleting or by CSM supplementation. As shown in Table 10.2, most of the acetate was oxidised and, at a maximum, only about 22-33% was deposited in tissues.

10.6. Discussion

The net flux (irreversible loss) of bicarbonate-C in sheep on the chaff only, chaff plus CSM and pellets only did not differ significantly. The mean values of the net flux of bicarbonate in the mature Merino sheep used in these 3 treatments were lower than those found for 35-40 kg Border Leicester x Merino sheep on molasses-based diet (178 gC/d) in the study of Rowe (1978). The net flux in sheep fed pellets plus CSM, on the other hand, was significantly higher (223 gC/d) than those of sheep in the first 3 treatments, and was similar to that recorded by Rowe (1978) for Border Leicester x Merino sheep fed lucerne chaff.

The net flux was probably an underestimate of the total rates of entry of materials due to recycling of labelled C via CO_2-fixation reactions (Figure 10. 3). The similar net flux of bicarbonate carbon per kg DDMI (Table 10.2) indicates that this net flux was a function of feed intake.

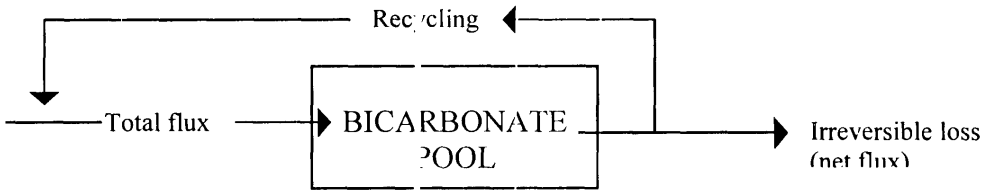

Figure 10. 3. A single compartment model of the blood bicarbonate pool (based on Nolan and Leng, 1974)

Knowledge of the proportion of acetate C flowing through the bicarbonate pool that is oxidised enabled the proportion of acetate C being used for fatty acid and other syntheses to be calculated (Figure 10.4). The S. R. of blood acetate was not determined and so the net flux and total rate of acetate carbon (g/d) flowing to the bicarbonate pool and to fatty acid synthesis could not be calculated directly.

The proportions of acetate oxidised recorded in the current study (67-78%) were comparable to the values of 78% reported by Pethick *et al.* (1981) for 54 kg Clun Forest ewes fed 1 kg of a 50:50 lucerne and cocksfoot hays plus 100 g rolled oats daily. If, as suggested by Pethick *et al.*, (1981), acetate oxidation accounted for 40% of respiratory CO_2, the contribution of acetate to CO_2 in the present study would be 27-31%, This percentage is higher than the values reported by Cronje (1987) who

found that 20 - 25% of blood bicarbonate carbon was derived from acetate in 28 kg crossbred Merinos, fed basal diets of chaffed wheat straw plus urea (B) or B plus 0.62 mol propionate/d and supplemented with 40, 120 or 200 g/d of protein meal,.

In the study of Cronjé (1987) the net flux of acetate in sheep supplemented with 120 g/d protein meal (without propionate) was 109.5 mmol/h or 63 g acetate C/d. Using this value as a guide, the flows of acetate carbon through blood bicarbonate and into tissues in sheep fed pellet plus 100 g/d CSM in the present experiment would be as illustrated in Figure 10.4.

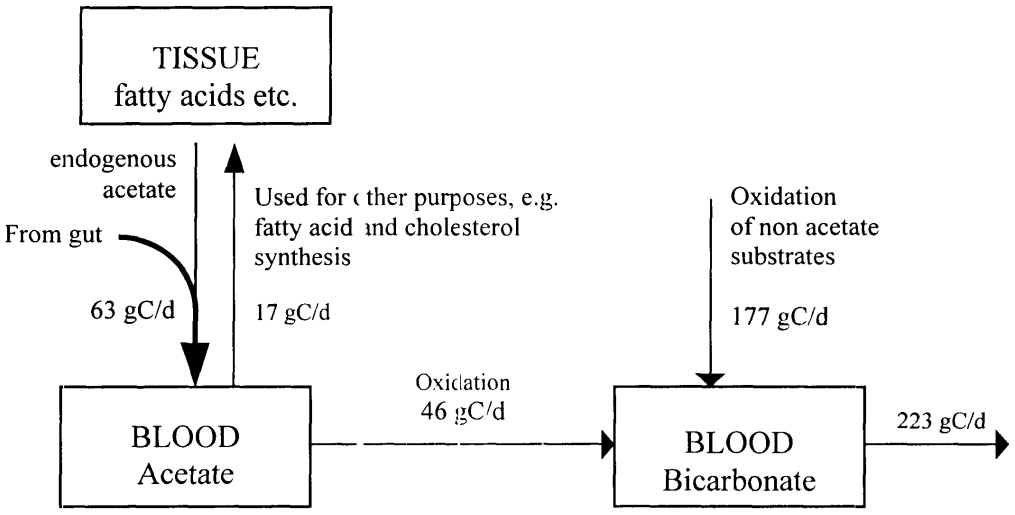

Figure 10.4. A 3-compartment model describing the acetate metabolism in sheep fed pellets plus 100 g/d of CSM during Experiment 8.

Figure 10.4 suggests that the proportion of CO_2 derived from acetate recorded in the present experiment were comparable to those reported by Cronje (1987).

The net flux of glucose, on the other hand, was significantly increased by both grinding and CSM supplementation. The mean glucose net fluxes recorded in the present experiment (Table 10.2) were similar to those reported by Cronjé (1987), i.e. for sheep fed oaten chaff supplemented with 40, 120 and 200 g protein meal without propionate (54.6, 76.1 and 93.7 g/d respectively) or with 0.62 mol propionate/d (75.1, 103.7 and 120.8 g/d respectively).

The rate of glucose production is a function of digestible energy intake (Leng, 1970; Steel and Leng, 1973a,b; Brockman, 1993) and thus of the availability of its precursors in plasma (Lindsay, 1978; Brockman, 1993). In the present experiment,

the increase in glucose production rate due to pelleting may be related to the increased supply of absorbed propionate and microbial amino acids for gluconeogenesis. Additional amino acids from the UDP of CSM supplement could have further increased the rate of gluconeogenesis.

The glucose production observed in the present study, however, is contradictory to that reported by Mahyuddin and Teleni (1994) who found that glucose net fluxes in Merino wether lambs fed lucerne pellets at maintenance energy level (M) and in those fed M + 60 g formaldehyde-treated casein did not differ significantly (26.0 vs 30.2 mmol/h). Because the glucose uptake by the hind-limb muscle was twice as high in the latter group, they suggested that additional amino acids from casein were probably used for protein synthesis, rather than as sources of energy. Thus the discrepancy in glucose production between the two studies may be explained by one of two reasons. First, the protein content in the basal diet used by Mahyuddin and Teleni (1994) was much higher than that in the present study. Second, there was a difference in temperature imposed that can affect the nutrient requirements of animals, especially energy (NRC, 1981b).

In diets where glucose or NADPH is limiting, increased production of glucose resulting from protein supplementation, for example, might increase the proportion of acetate being converted to fatty acids and thus to reduce the rate of acetate oxidation (MacRae and Lobley, 1982; Preston and Leng, 1987; Leng, 1990). Thus Walsh *et al.* (1990) reported that fish meal supplementation of young cattle on silage diets increased the rate of NADPH production from glucose and of fat synthesis, probably due to increased availability of gluconeogenic precursors. van Houtert and Leng (1993) also reported that the rate of fat deposition in Dorset Horn x (Border Leicester x Merino) lambs fed a basal diet of ammoniated barley straw was increased by supplementation of either 50g/d of casein or 20g/d of propionate. Scollan and Jessop (1991) found that the rate of acetate oxidation was lower in sheep fed barley than in sheep fed sugarbeet pulp, and the rate of acetate conversion to fatty acid was increased by increasing the level of protein supplementation. In addition, Scollan and Jessop (1995) reported that acetate incorporation into fatty acid in sheep fed sugarbeet pulp was increased by 43% by protein supplementation.

However, the significantly higher glucose production in sheep fed the pelleted diet supplemented with 100 g/d CSM in the present study did not increase the proportion of acetate being deposited in tissues. In the study of Cronjé (1987), on the

contrary, the fraction of acetate oxidised was decreased during supplementation of protein and propionate and thus a higher fraction of acetate was deposited in tissues. The difference may be due to the higher ambient temperature set in this experiment, which may have increased the requirement for energy for maintenance (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brink and Ames, 1975; Ames and Brink, 1977). Most available acetate was thus oxidised to meet the energy requirement. Also, the growth rate of Merino sheep in the current experiment was lower than that of the sheep in the experiment of Cronje (1987) and less acetate would be expected to have been deposited as body fat.

Cronjé (1987) showed that the flux of acetate was increased by protein supplementation. If the net flux of acetate in the present experiment was increased by CSM supplementation, the proportion of acetate which was converted to fatty acids might have also been increased. However, because the total production of acetate was not measured in the current experiment, this possibility remains speculative.

Estimated energy expenditure (Table 10.2), when calculated as kJ/h, was higher (P=0.05) on pellet than on chaff, and also higher (P=0.04) on the CSM supplemented diets than on the control. However, the ratios of estimated energy expenditures : ME intake did not differ significantly between treatments, and thus there was no suggestion that the efficiency of ME utilisation was altered by diet. All sheep appeared to have experienced a negative energy balance (energy expenditures were higher than ME intakes). However, because the ME intake was estimated using DMD values of similar treatments in Experiment 8, ME intakes in sheep on pellets only and in those on pellets + CSM were likely to be underestimated because DMI was lower (by 364 and 102 g/d, respectively) during the infusion period (Table 10.1) than in Experiment 8 (Table 9.1). Relatively low DMI in Experiment 9 may have increased the DMD and thus to have under-estimated the ME intake.

Despite difficulties of interpretation brought about by lack of acetate production or plasma acetate flux measurements, glucose precursors (e.g. amino acids) appeared to be sufficiently available in the present study for fatty acid synthesis from acetate to have been been limited by availability of NADPH. The high rate (67 - 78%) of acetate oxidation recorded in these sheep appears most likely to have been a consequence of either a low energy intake or a high energy requirement for maintenance and thermoregulation under heat stress. In the light of this suggestion, it is concluded that the increases in the rate of fatty acid synthesis and/or reductions in

the rate of acetate oxidation reported by other workers (e.g. Cronje, 1987; Walsh *et al.*, 1990; van Houtert and Leng, 1993; Scollan and Jessop, 1995) when additional amino acids were supplied were probably related to the higher growth rates of the animals they used. These growth rates would be expected to have resulted in more acetate having been used for fat deposition in these experiments compared to that of animals in the present study.

IV. General discussion

It is apparent that low productivity of ruminants in the tropics is due to a combined effect of high ambient temperature and humidity, and the low quality of available feedstuffs. Even though genetic improvement has been suggested to be able to improve productivity, this long-term benefit appears to be less attractive and not feasible for small-scale farmers in tropical developing countries.

Thus, several nutritional strategies have been put forward to improve production of tropical ruminants. Leng (1990), for example, theorised that supplementation with bypass protein would correct a marginal amino acid deficiency in ruminants fed low quality roughage, and as a result, feed intake and the overall productivity may be increased. However, this theory appeared to have been derived from results of studies in which environmental conditions and quality of basal diets used may have varied considerably, and as a consequence, it seemed important to examine it under more controlled conditions. Studies were therefore carried out to establish the effects of supplementation with bypass protein on the performance of ruminants fed low quality roughage.

11.1 The preliminary study

The preliminary study (Experiments 1 and 2) was initiated with a hypothesis that addition of locally available protein-rich supplements (e.g. tofu by-product, whole kapok seed and copra meal) to tropical grasses or agricultural byproducts would correct dietary nitrogen deficiency, and improve the digestibility and thus the productivity of local ruminants (Preston and Leng, 1987). Thus, Bamualim (1991) showed that the liveweight gain of goats can be increased from 20 - 60 g/d (a commonly observed rate under traditional management systems) to 100 - 150 g/d if feeding management is improved. However, when these supplements were given to Kacang goats, a common ruminant species in the Indonesian small holder farming system, on top of a native grass or rice straw basal diet, liveweight was barely maintained.

One of the factors that may be related to the poor response of those goats to protein-rich supplements, especially when rice straw was the basal diet, is that the digestibility of the basal diet was very low, resulting in low DOMI. The digestibilities of neither native grass nor rice straw were significantly improved by addition of any of the supplements. Moreover, rumen ammonia, the principal source of nitrogen for most species of rumen bacteria (Hungate, 1966), was consistently below the suggested adequate level of 50 mg NH_3-N/L rumen fluid (Satter and Slyter, 1974). This, together with possible deficiencies in essential minerals (e.g. sulfur; because no mineral supplement was provided), could have impaired rumen function (Johnson and Djajanegara, 1989; Leng, 1990).

Another factor possibly involved is the feeding habit of the goat, which might have confounded the dietary treatments imposed. Goats usually browse on trees and shrubs (Devendra and Burns, 1983), and in the present study they were very slow to adapt to pen feeding and to the newly introduced supplements. The consequences were a low DMI and weight loss. Depletion of body tissue reserves during weight loss could have upset the physiological mechanisms regulating metabolism in these goats, which in turn may have limited the utilisation of dietary nutrients. In the succeeding experiments, these aspects were taken into account and corrected, and animals of homogeneous genotype and which had high growth potential were selected. Essential substrates for the microbes were also supplemented in the basal diet, so that confounding between effects on rumen metabolism with those related to tissue metabolism could be eliminated.

11.2. Improving the intake and utilisation of feed at high ambient temperature

During the preliminary study with Kacang goats, it appeared that low DMI was the major factor leading to low productivity. The study which followed was thus directed toward strategies to improve the intake and utilisation of nutrients in ruminants consuming low quality roughages. Considering the importance of bypass protein for the growing ruminants (Preston and Leng, 1987), supplements rich in bypass protein, i.e. CSM and "Norpro" protein-rich supplement, were used throughout. Sheep of homogeneous genotype (Border Leicester x Merino crossbred or pure Merino), age and growth potential were used, and all experiments were conducted at high ambient temperatures to simulate a range of tropical environments.

All experimental diets used in Experiments 3-8 were designed to provide sufficient RDP as reflected by comparable rumen ammonia nitrogen concentrations in each experiment. This should have eliminated, or at least minimised, any confounding of treatment effects by differences in rumen ammonia concentration. For the same reason, essential minerals, especially S were also provided in the diets to optimise rumen function.

11.2.1. High temperature and bypass protein interaction

The overall theory examined throughout this study was that amino acids from undegraded dietary protein supplements would promote efficient use of acetate for fatty acid synthesis during growth. The expected result was that heat generated from inefficient use of acetate due to 'futile cycling', tissue turnover and other non-productive reactions within the body in animals consuming high fibre, low protein diets would be minimised, and so the total heat load on the experimental animals would be reduced. As a result, feed intake and LWG would be improved (Leng, 1990).

In Experiment 3, at normal ambient temperature, supplementation with 100 g/d of CSM significantly improved liveweight gain. At high ambient temperature, on the other hand, the sheep receiving CSM did not perform any better than those fed the control diet, even though Leng (1989) suggested that the response to bypass protein supplementation would be greater at higher ambient temperature. Leng (1990) theorised that provision of bypass protein might minimise metabolic heat production at high ambient temperature as a result of a better balance of energy:protein in the absorbed nutrients, and that, as a consequence, feed intake and growth rate might not be depressed and might even be improved. The animals in Experiment 3 were heat-stressed to a considerable degree (as indicated by relatively high respiration rates and rectal temperatures) and yet the effects of heat load (imposed by high ambient temperature) on feed intake still apparently outweighed any beneficial effect of CSM supplementation in reducing endogenous heat load.

In order to establish an ambient temperature regime which was less stressful than that used in Experiment 3, and more representative of field conditions in the tropics, ambient temperature in Experiment 4 was set at 35°C by day, but was

reduced at night. To test a range of night temperatures, the night temperature was progressively reduced at night by 2°C every 3 days for a total of 30 days. Feed intake increased as night temperature declined, and animals on the CSM+ diet gained more weight than those on the control diet. Response to CSM in terms of feed intake started to occur when night temperature reached 23°C on the current regime.

Reduced night temperature may have reduced the time spent panting (for evaporative cooling) and therefore increased the time available for eating. In addition, the cooler nights would be expected to have reduced the overall heat stress and body temperature and thus to have had less of a depressing effect on feed intake. Liveweight gains during Experiment 4 were significantly higher in sheep supplemented with CSM than in those fed the control diet.

The results of the first stage of Experiment 5 demonstrated that the intake and digestibility of urea-supplemented oaten chaff by sheep housed under high day but lower night temperature did not differ significantly from those of sheep housed in a constant high ambient temperature. Apparently, these sheep were able to maintain relatively high feed intake without urea supplementation, i.e. intake was maintained at similar level in the second stage as that in the first stage of Experiment 5. Respiration rates (which were 62-186/min in stage 1 of Experiment 5, and 154-181 in stage 2) and the corresponding rectal temperatures (39.6-40.1 and 39.4-39.8) indicated that the animals were under a moderate degree of heat stress.

Quality of basal diet

When urea was removed from the basal diet in the second stage of Experiment 5, CSM supplementation (100 g/d) significantly improved total DMI. The absolute increase, however, was very similar to the amount of CSM supplemented (109 g/d at 35/35°C temperature and 132 g/d at 35/20°C temperature), meaning that there was only a very small increase in intake of the basal diet. This indicates that the balance of nutrients (especially proteir and energy) provided by oaten chaff (9%CP) might have enabled these sheep to maintain a relatively high level of feed intake. The high intake of the basal diet provided only a small margin for the CSM supplement to further improve the basal diet intake. This confirms a suggestion that the response to bypass protein depends on the 'quality' of the basal diet (Kempton *et al.*, 1976; Ortigues *et al.*, 1990; McLennan *et al.*, 1995). Liveweight gain, measured from the

beginning of stage 1 to the end of stage 2 of Experiment 5, on the other hand, was significantly higher in sheep supplemented with CSM. Because the high DMI and the difference in DMI between treatment was small, improvement in liveweight does not appear to be well correlated with digestible DMI (R^2 = 0.35; see Figure 6.2). CSM supplementation may have improved the availability of dietary protein being available for growth, and ratio of protein to energy in the absorbed nutrients, as suggested by Leng (1990).

11.2. 2. Effects of increasing levels of bypass protein

In Experiment 6, when a low quality basal diet (barley straw chaff; 40-50% DMD) was used, feed intake and liveweight gain of Merino wethers responded progressively to the level of bypass protein supplementation. The optimum level of bypass protein in this experiment appeared to be 100 g/d of "Norpro" (formaldehyde protected soybean meal; expected UDP content = 60%). Bypass protein in the "Norpro" supplement would be expected to have improved the P:E ratio in the absorbed nutrients, resulting in significant increases in DMI and LWG. However, as the "Norpro" supplement also contained a considerable amount of energy, the response could have been due both to additional protein and energy (McLennan *et al.*, 1995), as indicated by a significant correlation between MEI and liveweight change (Figure IV. 1).

The absolute response to the "Norpro" supplement in terms of liveweight gain was less than 50 g/d, even at the highest level (150 g/d) of inclusion. Practically, this would not be economical in the tropics, because protein-rich supplements are generally more expensive than energy-rich supplements, and other strategies thus need to be explored to further improve the liveweight gain at an optimum level of bypass protein supplementation.

11.2. 3. Effects of additional soluble carbohydrate

When an energy-rich but lower protein supplement was provided in the form of barley grain were provided at different levels in conjunction with 100 g/d of "Norpro" in Experiment 7, total DMI did not differ significantly between treatments. Liveweight gain, on the other hand, was significantly improved by energy

supplementation. While lambs supplemented with "Norpro" or barley grain only barely maintained liveweight, supplementation with 100g "Norpro"+100 g grain and 100g "Norpro"+200 g grain per day significantly improved the rate of liveweight gain to 32 and 48 g/d respectively. This suggests that digestible energy was a primary limitation in Experiment 6, due to either low DOMI or increased maintenance energy requirements under heat stress due to the additional needs for thermoregulation (Graham *et al.*, 1959; Blaxter and Wainman, 196; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brink and Ames, 1975; Ames and Brink, 1977; Ames *et al.*, 1980; Beede and Collier, 1986). The fact that the liveweight gains of lambs supplemented with 100 g "Norpro"/d, and those supplemented with 100 g grain/d were similar, suggests that the response to "Norpro" supplementation observed in Experiment 6 was partly due to improvement in total DOMI as indicated by the relationship between digestible DMI and liveweight gain (Figures 7.3 and 8.3). Under the conditions of Experiment 6, extra bypass protein was not required.

Black and Griffiths (1975) demonstrated a linear relationship between nitrogen balance and ME intake, indicating that when N absorption is high, the rate of protein synthesis can be increased by providing sufficient energy. Thus, McLennan *et al.* (1995) suggested that grain supplementation would be beneficial to correct protein : energy ratio when intake of protein meal is high. The reductions in urinary and faecal N losses when barley grain was supplemented in addition to Norpro protein meal (Chapter 8) are consistent with such effects.

11.2.4 Effects of particle size of the basal diet

Grinding and pelleting improved the intake of the basal diet to a greater degree than that did CSM supplementation (Experiment 8). Because the potential degradability of the basal diet (barley straw; 44% DMD) was so low, the limitation imposed by low rate of rumen emptying was probably greater than any possible constraint imposed by an imbalance in the nutrient supply.

Digestibility of ground feed is generally lower than that of long feed, due to increased DMI and reduced mean retention time (Berger *et al.*, 1994). However, the DMD recorded in Experiment 8 did not differ significantly, suggesting that the potential degradability of barley straw was so low that any possible difference in the

rate of passage between the chaff and ground basal diets would not significantly change digestibility.

It seems likely that the increase in total DMI in the present study was sufficiently large as to outweigh the reduction in digestibility that resulted from grinding and pelleting. CSM supplementation to the pellet basal diet appeared to have corrected any marginal deficiencies, resulting in a further increase in DMI. An increased DMI is expected to have improved the availability of digestible energy, which is required for deposition of additional amino acid from CSM, and thus liveweight gain (Blaxter, 1962 Orskov, 1982; Black *et al.*, 1987b). An improved protein to energy ratio of absorbed nutrients may improve the utilisation of acetogenic substrates for synthetic purposes, minimise heat production and improve DMI and LWG (Leng, 1990).

However, when acetate metabolism was studied (Experiment 9), the results revealed that the better performance of sheep supplemented with CSM was not due to more efficient use of acetate for fat synthesis. In fact, a significantly higher rate of glucose production (which is expected to provide sufficient NADPH for fat synthesis) in sheep fed the pellet + CSM diet did not reduce the proportion of acetate oxidised. Based on a computer simulation, Black *et al.* (1987a) suggested that improved acetate utilisation due to protein supplementation was not directly related to NADPH production. Black *et al* (1987b) further concluded that additional amino acids may divert NADPH utilisation from fatty acid synthesis to protein synthesis.

It is possible that bypass protein may have increased the flux rate of acetate (due to increased feed intake) and thus, at similar rates of oxidation, the rate of acetate conversion to fatty acids may have been actually increased by CSM supplementation (Cronje, 1987). Because acetate production was not measured in the present study, this possibility must remain speculative. Also, van Houtert *et al.* (1993) found that the net flux of acetate in plasma of sheep fed a basal diet of oaten hay alone or supplemented with pellets containing, fish meal (75 g/d), VFA (acetate or propionate each equivalent to 1.5 MJ gross energy/d) or combination of fish meal and VFA did not differ significantly.

The better performance of sheep fed pellet + CSM may also have been a result of the combined effects of increased DMI and an improved balance of nutrients available for microbial growth. The soluble part of the supplemented CSM would have provided additional amino acids, peptides and branched-chain fatty acids, each

of which is essential for maximum growth of some rumen microbes (Hume, 1970a; Hume, 1970b; Leng and Nolan, 1984; Oosting, *et al.*, 1995). The effects of high microbial growth and of a possible increase in dilution rate resulted in a significant increase in microbial N flowing to the small intestines (Table 9.3) and thus improved the amount of amino acids available for absorption. If the absorbed amino acids were not utilised as a source of NADPH for fat synthesis or oxidised for energy through the TCA cycle (Fig. 2.4), then they would be expected to have been deposited as tissue protein (Black *et al.*, 1987b).

The overall effect of bypass protein observed in the present study appeared to be an improvement in digestible DMI, resulting in increased availability of nutrients and thus rate of liveweight gain (see Figures 7.3, 8.3 and 9.3). When the ME intakes during Experiments 3, 5, 6, 7 and 8 were plotted against their corresponding liveweight changes (Figure IV.1), the results suggest that low rate of liveweight gain observed was most likely to be primarily due to inadequate MEI.

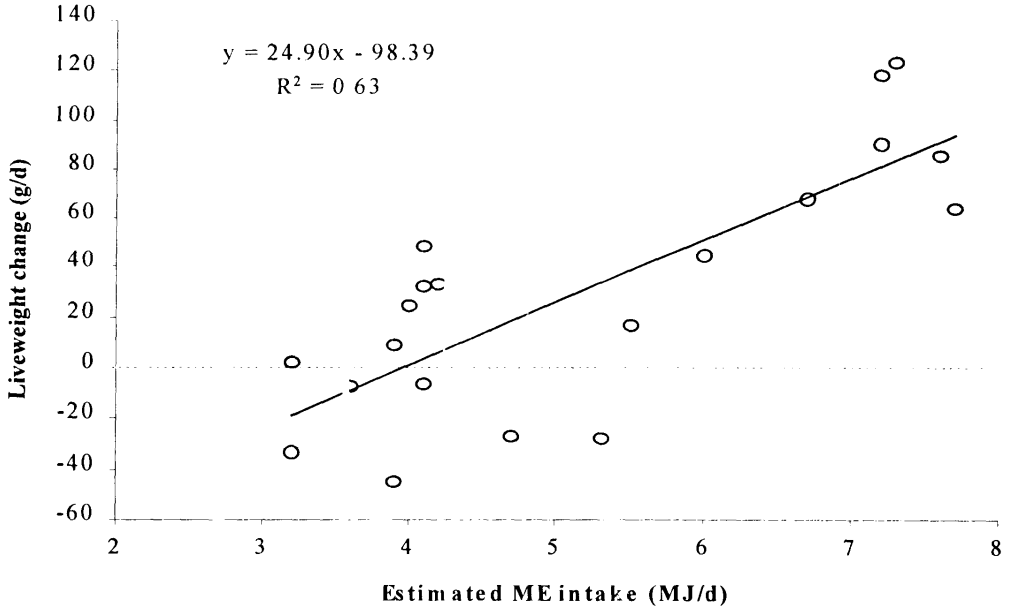

Figure 11.1. The relationship between MEI and liveweight change (based on pooled data from Experiments 3, 5, 6, 7 and 8).

Because of difficulty in accurately measuring carcass gain, the relationship between MEI and liveweight change (Figure 11.1) varied considerably.

11.3 Concluding remarks

The results reported in this thesis do not seem to support the overall hypothesis that bypass protein may increase the intake and utilisation of dietary nutrients by ruminants fed low quality roughage at high ambient temperatures. Improved DMI and LWG in Experiment 6 (Chapter 7), for example, cannot be interpreted as a result of improved efficiency of utilisation of acetogenic substrates because when similar amounts of "Norpro" and barley grain were supplemented to the same basal diet (Experiment 7), the responses in DMI and LWG did not differ significantly. Instead, the improved responses observed were most likely a reflection of improved total OMI. Because the "Norpro" also contained a considerable amount of energy, the improved DMI and LWG may have been due to, in part, increased availability of energy (McLennan *et al.*, 1995), which is required for thermoregulation during heat stress (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brinks and Ames, 1975; Ames and Brinks, 1977; Ames *et al.*, 1980; Beede and Collier, 1986).

The results of Experiment 9 also suggest that the theory that bypass protein supplementation may improve the efficiency of utilisation of acetogenic substrate under tropical conditions (Leng 1990) does not appear to be true under the condition of this study. It seems that the primary limitation to productivity of ruminants in the tropics is more likely to be related to the low total OMI, due to low potential digestibility of available feedstuffs, rather than to inefficient utilisation of acetogenic substrates. The results of Experiment 8, for example, demonstrate that low digestibility of the basal diet (barley straw) limited the extent to which the total DMI may be increased by supplementation with bypass protein. However, when the basal diet was ground, CSM supplementation may have corrected amino acid deficiency, and DMI and LWG were significantly improved.

From the results discussed in Chapters 9 and 10 it can be argued that the conversion of acetate into heat through so called 'futile cycles' is not a significant factor at high ambient temperature. At such low DE intakes, most dietary acetate (about 73%) appeared to be oxidised, probably to meet the high energy requirements of heat stressed animals (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brinks and Ames, 1975; Ames and Brinks, 1977; Ames *et al.*, 1980; Beede and Collier, 1986), leaving a small fraction of it

being available for productive purposes. Thus, as the acetate apparently did not accumulate, there was no need o dissipate acetate through so called "futile cycles". Also, the heat generated from s ich cycles, if any, would contribute very little to the total heat load (see Crabtree *et a'*., 1987. 1990).

To some extent, the sigr ificant response to bypass protein supplementation observed in Experiment 9 appea:s to have been due to reduced energy expenditure on activities associated with eating and digestion (Orskov and McLeod, 1990; Goetsch *et al.*, 1997; Lachica *et al.*, 1997) that outweighed any possible increases in heat production due to "futile cycle" of acetate metabolism.

It can be concluded tha t improvement in feed intake and thus ruminant productivity in the tropics cannot be achieved only by optimising rumen function and bypass protein supplementation. As the digestibility of most tropical forages is low, the rate of digestion should first be increased, for example by grinding, and additional bypass protein may then be needed to improve the balance of the absorbed nutrients. In animals on a very low quality basal diet (e.g. barley straw) the ME intake is most likely to be deficient if a high rate of production is to be achieved. Additional carbohydrate-rich supplements are expected to support higher rates of microbial growth. These combined dietary treatments may improve the P/E ratio (Leng, 1990), reduce energy wastage during digestion (Orskov and McLeod, 1990), increase the availability of energy and thus improve ruminant production.

11.4. Suggestions for further studies

The increasing use of arable lands for food productions in the tropical developing countries, necessitates the use of agricultural by-products such as rice straw as a major component of ruminant diets. Owing to the low N content and digestibility of agricultural by-products, treatments with NPN such as urea and ammonia have been applied. However, improvement in feed intake due to such treatments alone or in combination with bypass protein are so far still lower than the levels required by ruminants for high levels of production. Thus, the potential degradability of such low quality roughages needs to be improved further before strategic supplementations are applied.

Results of the present study have demonstrated that reducing the particle size of low quality roughage, and supplementation with nutrients required by rumen microbes and with dietary proteins that escape rumen fermentation, dramatically increased DMI and LWG. However, the high cost of grinding (and pelleting) may preclude its application in the tropics, where ruminants are mainly raised by small-holder farmers. Thus, to enable an efficient use of abundantly available agricultural by-products in the tropics, cost-effective ways of increasing degradability of low quality roughages need to be developed. The use of a simple hammer-mill (without pelleting) in conjunction with ammoniation, for example, may be worth studying. Where technology is available, microbial/enzymatic treatments such as the use of white-rot fungi to increase degradability (see Berger *et al.*, 1994) may be feasible.

Once degradability of roughages is increased, the next step is to define the optimum balance of nutrients both for the rumen microbes and for the host. This is expected to maximise feed intake and retention of nutrients, and to improve productivity. It should also reduce environmental pollution by minimising the release of excess nutrients, such as N from urea, to the environment.

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