CHAPTER 11

11. EFFECT OF FEEDING FREQUENCY ON FEED INTAKE, FEEDING BEHAVIOUR, DIGESTION AND YIELD OF MICROBIAL NITROGEN FROM THE RUMEN OF MERINO SHEEP GENETICALLY DIFFERENT IN WOOL PRODUCTION.

11.1 Introduction

Provision of feed at regular intervals throughout the day is likely to provide a more stable environment for the ruminal microflora (Faichney 1968; Kaufmann 1976; Cecava *et al.* 1990b). In theory, regular provision of nutrients (as opposed to irregular provision) should increase apparent microbial growth yield (Baldwin and Denham 1979). Whilst there is a general agreement in the literature that increased feeding frequency stabilises ruminal fermentation patterns, much conflict exists as to the benefits of this to the animal.

Increasing the frequency of feeding has been found to either increase (Moir and Somers 1957; Ikhatua *et al.* 1987) or have no effect (Faichney 1968; Ulyatt *et al.* 1984; Bunting *et al.* 1987) on apparent digestion in the whole-tract. Where digestion has been stimulated, a tendency for a greater voluntary feed intake has been observed (Ikhatua *et al.* 1987). Changing the frequency of feeding has a variable effect on both the duodenal flow of microbial-N and the yield of microbial-N per unit DM or OM apparently and truly digested in the rumen (Ulyatt *et al.* 1984; Robinson and Sniffen 1985; Bunting *et al.* 1987; Cecava *et al.* 1990a).

In the previous experiment, when 6 year old ewes were fed an oaten chaff diet once per day, ewes from the F_p and F_m flocks consumed 65 and 57% and 517 and 419 g respectively of their daily intake by 4 h after feeding. Thus by 4 h after feeding, ewes from the F_p flock had eaten 23% more feed than ewes from the F_m flock. The difference in eating behaviour was consistent for 4 measures taken over approximately 90 days (Chapter 10). In the same experiment, it was also observed that the yield of microbial-N per unit dry matter intake was greater for ewes from the F_p flock. If the differences in the rate of feed intake between the selection lines were associated with the greater yield of microbial-N in ewes from the F_p flock,

it seems probable that the effect would have been mediated through changes to dilution rate as increasing dilution rate is known to stimulate microbial growth (Harrison *et al.* 1975; Isaacson *et al.* 1975; Kennedy *et al.* 1976; Kennedy and Milligan 1978).

The current series of experiments were established to determine whether the greater yield of microbial-N per unit feed intake for F_p as compared to F_m ewes, was related to differences between the selection lines in feeding behaviour.

11.2 Materials and Methods

11.2.1 General description of studies

There are 2 total collection periods that are reported in this Chapter. In the first period, 24 Merino ewes, 12 from the fleece plus (F_p)and 12 from the fleece minus (F_m) Trangie selection lines (see Chapter 10), were fed *ad libitum* and once daily a diet of oaten chaff (*Avena sativa*) sprayed with urea (1 g urea / 100g chaff). In the second period, the same diet was fed hourly by means of an automatic overhead belt-feeder to the same animals. A 7 day and 5 day total collection was performed for periods 1 and 2 respectively

11.2.2 Animals and conditions

Twenty four Merino ewes (12 F_p and 12 F_m) were chosen at random from the remaining animals used in the preceding experiment (Chapter 10). Throughout the duration of the experiment, the animals were kept in metabolism crates with continuous lighting and unrestricted access to water.

11.2.3 Experimental design and feeding

Prior to the ewes entering the metabolism crates, the ewes were stratified within selection line on the basis of live weight (before daily feeding) and then randomly allocated to either of 3 metabolism rooms (blocks) used during the experiments. Ewes were then randomly allocated to metabolism crates (8) within each room.

The diet for the 2 experiments consisted of oaten chaff (*Avena sativa*; 90% DM and 0.64% N) sprayed with urea at 1% (w/w) (total N of chaff 1.1%) and minerals (2% w/w of intake) (see Chapter 10 for details of mineral/vitamin mix and spraying of oaten chaff with urea). During period 1, ewes were fed *ad libitum* + 0.1 (based on a 2 day rolling average) and daily at 10.00 am after refusals from the previous day had been collected and weighed. Ewes were kept on this feeding protocol for 13 days during which the voluntary intake (g/d) of each

animal was determined. This intake level was then maintained for a further 5 days prior to the start of the 7 day total collection. At the conclusion of this period, the feeding protocol was changed to *ad libitum* and hourly from automated overhead feeders. This was continued for 9 days to once again ascertain voluntary intake. This level of intake was maintained for a further 5 days prior to the start of the next 5 day total collection (period 2).

11.2.4 Analytical procedures

The procedure for the total collection periods has been described elsewhere (Chapter 10); the only change being collection of urine into 1 l of tap water containing 10 ml 50% v/v sulphuric acid. Determination of percentage dry matter (DM) in feed and faeces, percentage nitrogen (N) in feed, faeces and urine, the concentration of urinary purine derivatives and calculation of the yield of microbial-N from the rumen were as described in Chapter 10

11.2.5 Measured variables

The measured variables during the experimental period were, dry matter intake (DMI) and nitrogen (N) intake, apparent digestibility of dry matter in the whole-tract (ADMD) and subsequently digestible dry matter intake (DDMI), proportion of daily intake consumed by 4 h after feeding, urinary excretion of purine derivatives, nitrogen retention and live weight. The yield of microbial-N (MN) from the rumen was calculated from the measurement of urinary purine excretion (Chen *et al.* 1992a) and the efficiency of microbial-N supply per unit DMI and N intake were calculated from the measured variables.

11.2.6 Statistical analysis

Three ewes from period 1 and 2 ewes from period 2 were omitted from the analysis on the basis of fluctuating intake during the collection period. A generalised linear model was used to assess the significance of selection line and its interactions for the various traits under examination during periods 1 and 2 Least squares means (l.s.mean) are used throughout the results. The same animals were used in both experimental periods, and consequently when the data from the 2 periods were combined, a split-plot design was used to estimate the significance of feeding frequency (sub-plot) and its interaction with genotype (Brown, in an appendix to Dolling and Piper 1963). The computer program SAS (SAS Institute Inc) was used for all analyses. Given the relatively short duration of the experimental periods and the controlled environment of the metabolism rooms any possible confounding with feeding regime are considered to be negligible.

The physiological components of the yield of MN were expressed as:

$$\log(MN) = \log(MN/I) + \log(I/B) + \log(B)$$

where MN = microbial-N yield.

I = dry matter intake.

B = live weight.

The variance of MN was partitioned among its components by using the method of Henderson and Hayman (1960) (see Chapter 10).

11.3 Results

11.3.1 Once per day feeding (Period 1)

The proportion of daily intake consumed by 4 h after feeding did not differ between the selection lines (F_p 68, F_m 65%). However, given that ewes from the F_p flock tended to have a greater daily intake (F_p 883, F_m 773 g/d; NS) the amount of feed consumed by 4 h after feeding was greater for F_p ewes (F_p 593, F_m 496 g/d) and the difference between the selection lines approached statistical significance (P = 0.06). There were no significant differences between the selection lines for any of the other measured variables. However, the yield of MN (F_m 7.6, F_p 8.8 g/d; NS), and nitrogen retention (F_m 1.4, F_p 2.0 g/d; NS) tended to be greater for ewes from the F_p flock.

11.3.2 Hourly feeding (Period 2)

There was a significant difference between the selection lines in the apparent digestibility of dry matter in the whole-tract (ADMD) and in the yield of MN per unit DMI and N intake (Table 11.1). The yield of MN (g/d) tended to be greater in F_p as compared to F_m ewes but the differences were not statistically significant (P = 0.06) (Table 11.1).

Table 11.1. Apparent digestibility of DM in the whole-tract and yield of microbial-N (calculated from the urinary excretion of purine derivatives) of F_m and F_p ewes when fed hourly a diet of oaten chaff and 1% urea.

Variable	F_m		F_p		
	l.s.mean	s.e.	l.s.mean	s.e.	P value
ADMD (%)	55	0.9	51	0.9	P < 0.01
Microbial-N (g/d)	9.0	0.55	10.6	0.55	P = 0.06
MN/DMI (g/kg)	10.4	0.23	11.1	0.23	P < 0.05
MN/N intake (g/g)	0.97	0.021	1.04	0.021	P < 0.05

11.3.3 Combined feeding regimes

When the data from both feeding regimes were combined, significant differences between the selection lines were recorded for ADMD (F_m 0.55, F_p 0.52; $P < 0.01$). Ewes from the F_p flock tended to have a greater yield of MN (F_m 8.2, F_p 9.7 g/d; $P = 0.06$) and there was a suggestion that these ewes also a greater yield of MN per unit DMI (F_m 10.0, F_p 10.6, NS) and N intake (NS). The interaction between feeding regime and selection line (Table 11.2) was significant for ADMD ($P < 0.01$) and DDMI ($P < 0.05$). The interpretation of these interactions was that feeding regime did not statistically alter the ADMD of either selection line however the DDMI of F_m ewes increased with the frequency of feeding. When comparing selection lines within feeding regime, the ADMD of F_m ewes was greater than that of Fp ewes during period 2 but DDMI did not differ between the selection lines for either feeding regime.

Both DMI and DDMI were greater for hourly fed ewes ($P < 0.01$), however the stimulation of voluntary intake was completely explained by the greater live weight of the ewes during period 2. Increasing the frequency of feeding resulted in a greater yield of MN (as estimated from the urinary excretion of purine derivatives) and also improved the yield of microbial-N per unit DMI and N intake ($P < 0.01$) (Table 11.2).

Table 11.2. Effect of feeding frequency and selection line x feeding regime interactions for apparent digestibility of dry matter in the whole-tract, digestible dry matter intake and the yield of microbial-N per unit dry matter intake.

Variable	Period 1			Period 2			Period effect	Interaction
	F_m	F_p	Mean	F_m	F_p	Mean	P value	P value
ADMD (%)	53.7	52.3	53.0	55.3	51.1	53.2	NS	$P < 0.01$
DDMI (g/d)	413	460	437	472	480	476	$P<0.01$	$P < 0.05$
MN/DMI (g/kg)	9.7	10.0	9.9	10.4	11.1	10.8	$P<0.01$	NS

When ewes were fed once daily, the efficiency term, MN/DMI, accounted for 15.6% of the variation between the selection lines in the yield of MN. The remainder was accounted for by DMI which was a composite of DMI/B and B (Table 11.3). When the frequency of feeding was increased to hourly, a greater proportion of the variation between the selection lines in the yield of MN was accounted for by the term MN/DMI (Table 11.3). The negative association between live weight and the yield of MN was evident at both feeding regimes.

Table 11.3. Percentage of the variation (100 b_i) between the selection lines in the yield of microbial-N from the rumen that was attributable to the physiological components when ewes were fed once per day and hourly.

Source of variation	Physiological components		
	MN/DMI	DMI/B	B
Once per day	15.6	104.5	–20.3
Hourly	17.9	82.1	–20.3

11.4 Discussion

Increasing the frequency of feeding failed to stimulate either voluntary feed intake (adjusted for live weight) or apparent whole-tract digestion of DM. These findings agree with those when sheep were fed higher quality diets (> 15% CP) consisting of either ground and pelleted lucerne hay (Faichney 1968), lucerne hay (Ulyatt *et al.* 1984) or tall fescue hay (Bunting *et al.* 1987). In contrast, Ikhatua *et al.* (1987) reported a significant improvement in the apparent whole-tract digestibility of DM and OM in Zebu cattle by increasing the frequency of feeding of a low-quality hay (< 5% CP) from once to three times per day.

Selection of plant parts within the diet on offer may provide a basis for explaining the variable effect of feeding frequency on digestive function. The vast majority of research aimed at determining the effect of feeding frequency on apparent whole-tract digestibility has been conducted with restrictively-fed animals, thus negating the confounding influence of a change in feed intake but also preventing the animal from either selecting more nutritious plant parts within a diet, or increasing feed intake. This may explain the responses of Ikhatua *et al.* (1987) where cattle were essentially fed *ad-libitum* and as a consequence, had the opportunity to select from the material on offer. Unfortunately, it does not explain the lack of effect of feeding frequency on digestion in the current study where animals were fed *ad-libitum.* However, in the studies reported here, the opportunity for animals to select within the chopped oaten chaff ration was minimal.

Increasing the frequency of feeding increased both the yield of microbial-N from the rumen and the yield of microbial-N per unit intake of DM and N. Increasing the frequency of feeding removes much of the diurnal variation in the availability of substrate for ATP production, concentration of ammonia and volatile fatty acids (VFA) and also of pH in ruminal

fluid (Faichney 1968; Bunting *et al.* 1987; Ruiz *et al.* 1989; Cecava *et al.* 1990a) thus providing potentially higher average rumen ammonia levels, a more stable environment for bacterial growth and consequently a greater yield of microbial cells from the rumen. Increasing the frequency of feeding is particularly useful for maintaining higher average rumen ammonia levels when urea forms a part of the crude protein supply to the animal (Tudor and Morris 1971; Romero *et al.* 1976). Within the rumen, urea is rapidly hydrolysed to ammonia by bacterial urease (Tillman and Sidhu 1969) and the liberated NH_3-N is used for microbial protein synthesis (see Chapters 3 and 5). However when urea is provided in excess of microbial-N requirements there is potential for a large fraction of the urea-N to be absorbed from the rumen into the blood as NH_3-N (Romero *et al.* 1976). Blood ammonia is metabolised to urea in the liver and largely excreted in urine. Hence, when urea is fed once per day, incorporation of NH_3-N into microbial protein is potentially restricted and the yield of microbial-N from the rumen may be lower than when urea is fed at regular intervals throughout the day.

It is also plausible that the greater yield of MN from the rumen and yield of MN/DMI with hourly feeding may have been due to an increased digestion of OM in the rumen. The experimental ewes were not cannulated and estimates of OM digestion in the rumen were therefore not made but ADMD was unaffected by the frequency of feeding. Ulyatt *et al.* (1984) suggested that feeding frequency has little effect on the proportion of OM that is apparently digested in the whole-tract, that is digested before the proximal duodenum. Given this, it seems reasonable to assume from the estimates of ADMD that the association between increased frequency of feeding and a greater yield of MN from the rumen and yield of MN/DMI was unrelated to OM digestion in the rumen.

Changing the feeding regime from once per day to hourly was used to provide a basis for investigating the importance of the rate of intake in determining the differences between the selection lines in the yield of MN/DMI. When feed was offered once per day, there were no differences between the selection lines in the proportion of daily intake consumed by 4 h after feeding but the amount of feed consumed by F_p ewes by 4 h after feeding was 20% greater than that of ewes from the F_m flock. In spite of this, there were no differences between the selection lines for the yield of MN/DMI during period 1, and it seems apparent that the rate of feed intake was not a factor in the differences in MN/DMI between the selection lines observed in the studies reported in Chapter 10.

When feed was offered hourly, the yield of MN/DMI of F_p ewes was greater than that of ewes from the F_m flock. When the data from the 2 feeding regimes were combined, the yield of MN/DMI was also greater for F_p ewes, but the selection line differences were not statistically significant ($P = 0.09$). The combined analysis utilised the split-plot design with selection line as the main-plot. The sensitivity of detecting the statistical difference of a main-plot term is reduced because in the split-plot design, the main-plot error term was larger than that for the equivalent two way analysis of variance used to analyse separately the data from periods 1 and 2. Considering this, the data from the current trial provide some support for the suggestion (Chapter 10) that ewes from the F_p flock have a greater yield of MN/DMI than F_m ewes. In the studies reported here, the greater yield of MN/DMI of F_p ewes was most pronounced with hourly feeding. Possible causes for the greater yield of MN/DMI of ewes from the F_p flock were discussed in Chapter 10.

When the feeding regime was daily, the efficiency term MN/DMI accounted for approximately 15% of the variation between the selection lines in the yield of MN from the rumen. This was a much lower contribution than that reported previously (*c.* 45%) (see Chapter 10). Increasing the frequency of feeding from daily to hourly increased the proportional contribution of the efficiency term, MN/DMI, to *c.* 38% of the variation between the selection lines in the yield of MN. Increasing the frequency of feeding increased the yield of MN of both selection lines by the same proportion but ewes from the F_m flock had a greater proportional increase in DMI. As a consequence of this, the difference in the yield of MN/DMI between the selection lines was greatest when the diet was fed hourly and the efficiency term MN/DMI accounted for a greater proportion of the variation in the yield of MN between the selection lines.

In conclusion, it seems that the yield of MN from the rumen is greater in ewes from the F_p as opposed to the F_m flock and that divergent selection for wool growth has also been associated with a change in the yield of MN/DMI. Calculation of the weighted mean for the efficiency term, MN/DMI, from this and the previous trial (see Chapter 10), indicated that MN/DMI accounted for *c.* 35% of the differences between the selection lines in the yield of MN from the rumen.

CHAPTER 12

12. THE USE OF LITHIUM CHLORIDE FOR ESTIMATING SUPPLEMENT INTAKE IN GRAZING SHEEP: ESTIMATES OF HERITABILITY AND REPEATABILITY[1]

12.1 Introduction

One of the aims of the research presented in this thesis was to investigate whether genetic variation existed between fine-wool Merino bloodlines, grazing at pasture, in the response of wool growth rate and average fibre diameter to increasing amino acid intake. Supplementation of grazing sheep with a protein concentrate which is partially resistant to degradation within the rumen but is digestible in the intestines (e.g. cottonseed meal) is a common method for increasing amino acid intake. However, when grazing sheep are fed supplements, it is common for large differences in intake of the supplement to exist between sheep (Nolan *et al.* 1975). The problem of variable supplement intake in grazing ruminants, has discouraged nutritional based research in the field and has made such research difficult to interpret. The existence of a marker of supplement intake would alleviate some of the difficulties of field research. In this regard, tritiated water (Nolan *et al.* 1975; Rocks *et al.* 1982; Dove 1984) and Cr_2O_3 (Corbett *et al.* 1958; Langlands 1975) have been used for the estimation of supplement intake.

Recently, Suharyono and co-workers (1991) proposed that lithium chloride might be used as a marker for estimating supplement intake in grazing animals. Potential problems exist in that lithium chloride is a known emetic agent (Ralphs and Cheney 1993) and has been used to create feed aversions in ruminants (Burritt and Provenza 1989). Fortunately these problems can be avoided by maintaining the level of lithium chloride that an animal consumes below a tolerance threshold of 50 mg LiCl /kg live weight /d (Suharyono 1992).

When sheep consumed feed sprayed with lithium chloride, peak plasma lithium concentration was attained 12 h after lithium ingestion and remained substantially constant for

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[1] Kahn, L.P. (1994). The use of lithium chloride for estimating supplement intake in grazing sheep: Estimates of heritability and repeatability. Australian Journal of Agricultural Research. **45**, pp 1731-1739.

a further 12 h (Suharyono 1992). Plasma lithium concentration decreased slowly from 24 to 30 h after lithium ingestion, thereafter the decline was more rapid. Increasing the level of sodium intake increased the clearance rate of lithium from plasma without significantly changing the length of the plateau.

The studies of Suharyono and co-workers (1991) and Suharyono (1992) were done with penned sheep. To date there have been no reports on the suitability of lithium chloride as a marker of intake in grazing sheep. If the lithium technique is to be used as a marker of supplement intake in grazing sheep then knowledge of the build up and decline of plasma lithium in grazing animals is required. The aim of this experiment was to obtain such kinetic data and to evaluate the use of lithium as a marker for estimating supplement intake in grazing sheep. This research, would then provide the basis for investigating the extent of genetic variation between fine-wool Merino sheep in the response of wool growth rate and average fibre diameter to increasing amino acid intake. In addition, the opportunity was taken to estimate the repeatability and heritability of supplement intake because of the absence of such information in the literature.

12.2 Materials and Methods

12.2.1 General description of studies

There are 2 experiments reported in this Chapter. In the first experiment, 8 Merino weaners (8 months of age) were offered varying levels of lithium-containing cottonseed meal (CSM) pellets. The build up and decline of plasma lithium concentration was measured in these sheep over 29 h. The time of blood sampling after lithium ingestion that resulted in the best correlation between estimated and actual pellet intake was ascertained. This information was used to determine the optimum time between lithium ingestion and blood sampling in order to best predict supplement intake in experiment 2. In experiment 2, CSM pellets (32% CP, 1% urea) were fed daily to 2 mobs (n = 366/mob) of grazing Merino weaners for a period of about 2 months. Pellet intake was estimated on 3 occasions over 62 days to examine the range of intakes within a mob and to estimate the repeatability and heritability of pellet intake.

12.2.2 Preparation of the lithium-containing supplement

Protein pellets (10 kg and 90% DM) were placed in a feed mixer and a weighed amount of lithium chloride was dissolved in one litre of water and sprayed over the pellets. The pellets

were then spread on a tarpaulin and dried (25–30°C) for 48–72 h. Pellets were turned daily to increase drying efficiency and to prevent pellet aggregation.

12.2.3 Kinetic study

Eight fine-wool Merino weaners (8 months of age), comprising four bloodlines, averaging 24.6 kg (s.d. = 2.46) live weight (LWt), were randomly selected from a flock (n = 366) that had been trained to eat CSM pellets (32% CP, 1% urea) for the previous 20 days. These animals were catheterised in the jugular vein 16 h prior to ingestion of lithium-containing CSM pellets (0.83 mg Li /g pellet) and then returned to their flock to continue grazing. At the commencement of the experiment, the catheterised sheep were placed in single pens and offered varying levels of the lithium-containing pellets (25, 50, 100, 200 g) according to a completely randomised design. Feed refusals were collected 30 min later to determine actual intake. These sheep were then returned to the flock to graze. Blood samples were taken from these sheep prior to lithium intake and a further nine times (4, 8, 10, 13, 16, 21, 23, 25, 29 h) thereafter.

Blood samples were centrifuged (3000 *g* for 10 min), and plasma was separated and immediately frozen (–20°C). Plasma lithium concentrations were then determined on diluted (50 fold with Corning 460/405 diluent concentrate) samples using a flame photometer (Corning, 405). A commercial standard which included sodium, potassium and calcium at physiological concentrations and ratios was used to calibrate the photometer. The suitability of this technique was checked by calculating the recovery of known amounts of lithium added to plasma samples.

12.2.4 Grazing study

The results of the kinetic study were then used to establish the protocol for measuring intake of CSM pellets in two mobs (n = 366/mob) of grazing Merino weaners on three occasions over a 62 day period (these studies are reported in Chapter 13). These animals were representative of 9 fine-wool and 2 medium-wool Merino bloodlines and had a 20 day training period to accept the offered supplement before the start of the trial. On the day of measurement each mob was offered its normal ration of CSM pellets (mob A 55 g/hd/d, mob B 110 g/hd/d) except that the pellets had been sprayed with lithium chloride at either 1.66 mg Li /g pellet (mob A) or 0.83 mg Li /g pellet (mob B). The CSM pellets were trailed over about 100 m and were consumed within 40 min. Animals were mustered to yards 5 h later.

Blood was taken from all animals by jugular venipuncture, stored on ice (1–5 h) in the collecting vessel containing sodium heparin and then centrifuged (3000 *g* for 10 min). The plasma was then removed and immediately frozen (–20°C). The whole flock was bled in 4 h so that the maximum time between lithium ingestion and blood sampling was 9 h.

The heritability of supplement intake was estimated by analysis of variance (Harvey 1988). Model (1) included the fixed effects of bloodline (B_i), birth type (T_k), maternal handicap (M_l; progeny of an adult or maiden ewe), sex (X_m), treatment group (G_n) and all two way interactions that were considered to be either statistically or biologically significant. Sire nested within bloodlines (S_{ij}) was fitted as a random variable. There were 60 sires with an average of 17.6 offspring per sire. Heritability (h^2) values were estimated as four times the sire component of variance divided by the phenotypic variance (Becker 1985).

$$Y_{ijklmno} = u + B_i + S_{ij} + T_k + M_l + X_m + G_n + BG_{in} + BX_{im} + e_{ijklmno} \quad (1)$$

Where BG_{in} and BX_{im} are the bloodline by treatment group and bloodline by sex interactions respectively.

The repeatability of pellet intake by individuals was determined using model (2):

$$Y_{ijk} = u + E_i + A_{ij} + O_{ijk} \quad (2)$$

Where Y_{ijk} is an observation on a weaner, E_i is the combined fixed and random effects, A_{ij} is the animal effect and O_{ijk} is the observation for each animal and was used as the error term. From model (2) repeatability (R) was estimated as the sum of the effect and animal components of variance divided by the phenotypic variance (Becker 1985).

12.3 Results

The recovery of known amounts of lithium standard added to plasma samples (n = 5) was on average 95.2% (c.v. = 1.3%).

For the blood samples taken, lithium concentration in plasma was at its maximum level by about 4 h after lithium ingestion, and remained at this level (plateau) for a further 10 h (Figure 12.1). Animals that consumed the lowest levels of lithium tended to have a shorter plateau period.

To calculate estimated pellet intake the method suggested by Suharyono (1992) was used. Suharyono (1992) argued that the effective volume of distribution of lithium would be proportional to live weight. Thus plasma lithium concentration should be scaled according to live weight to correct for this dilution effect. This value (lithium x live weight, Table 12.1) for each animal in the trial is summed to give a flock value (30.72) and each animal's individual value is expressed as a proportion of the summed flock total value (individual proportion, Table 12.1). This proportion is then multiplied by the mass of pellets that the entire flock has consumed (580 g) to give estimated intake (Table 12.1).

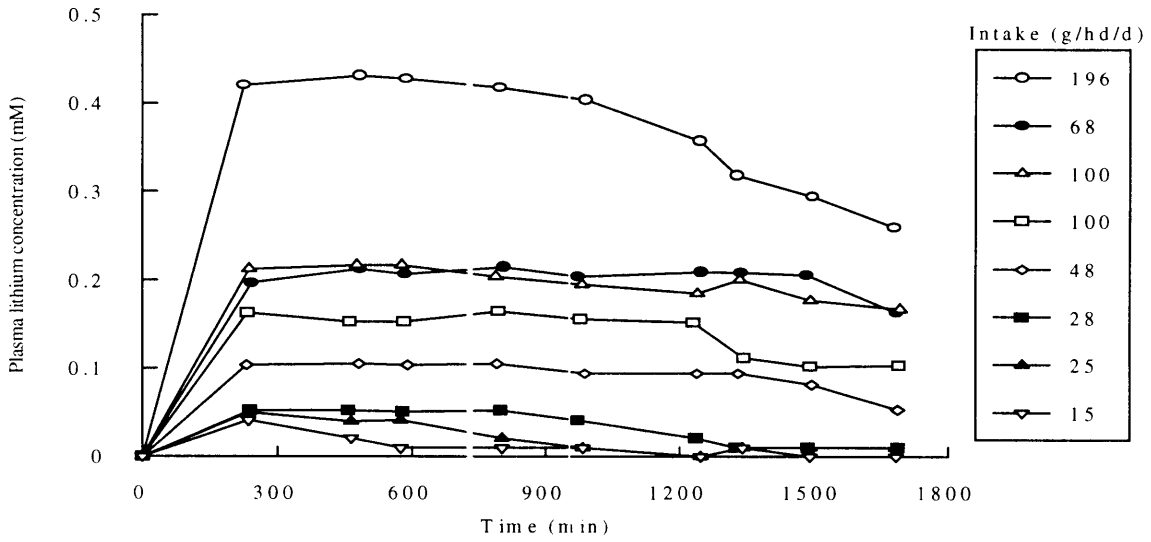

Figure 12.1. Plasma lithium concentration in jugular blood taken from 8 Merino sheep, prior to, and a further 9 times after lithium ingestion.

Plasma lithium concentration 4 h after ingestion of lithium-containing CSM pellets facilitated accurate prediction of pellet intake. The relationship between actual and estimated intake (g/hd/d) was:

$$\text{estimated intake} = 0.30 + 0.996(\text{s.d.} = 0.0586)\ \text{actual intake}\ (r^2 = 0.98)$$

the intercept was non-significantly different from zero. When plasma lithium concentration at 29 h was used in the calculations, the relationship deteriorated (r^2 = 0.91). Of more importance, animals that had consumed 25 g or less of lithium-containing pellets, had plasma lithium concentrations too low for detection on the flame photometer.

In the grazing study (exp 2), estimated pellet intake (g/d) for each animal was expressed as estimated pellet intake category (Table 12.2) because of the small uncertainties that existed in the prediction of actual intake level. Pellet intake was heritable, although the value was quite low (0.168 s.e. = 0.072). The repeatability of supplement intake as determined from three observations per animal (Model 2) was 0.48 which was significantly different from zero ($P < 0.05$). The most noticeable feature of the data was the large variation in intake of the CSM pellets that existed between individuals (Figures 12.2 and 12.3).

Table 12.1. Calculation of pellet intake based on lithium concentration in blood 4 h after sheep ingested lithium-containing CSM pellets.

Sheep number	Plasma lithium (mmol/l)	Live weight[A] (kg)	Lithium x live weight[B]	Individual proportion	Estimated intake (g)	Actual intake (g)
1	0.050	26.8	1.35	0.044	25.6	25.0
2	0.041	21.2	0.86	0.028	16.3	15.1
3	0.104	23.6	2.45	0.080	46.2	47.7
4	0.052	25.0	1.31	0.043	24.7	27.9
5	0.213	23.0	4.91	0.160	92.7	100.1
6	0.163	29.0	4.74	0.154	89.4	100.1
7	0.197	23.0	4.53	0.148	85.6	67.7
8	0.420	25.2	10.57	0.344	199.7	196.4
		Total	30.72			580.0

[A] Live weight estimated after feed and water were unavailable for 18h.

[B] Lithium x live weight is the product of plasma lithium concentration and live weight

Table 12.2. Transformation of pellet intake (g/d) to pellet intake category.

Pellet intake category	Pellet intake (g/d)	Pellet intake category	Pellet intake (g/d)
1	0–10	8	191–220
2	11–40	9	221–250
3	41–70	10	251–280
4	71–100	11	281–310
5	101–130	12	311–340
6	131–160	13	341–370
7	161–190	14	371-->

12.4 Discussion

An important characteristic of any marker technique is that the background level of the marker substance in animals that have not previously been exposed to the marker should be minimal, or more ideally, too low for detection This condition was satisfied by non-detectable levels of LiCl in the plasma of 12 sheep which had not been offered lithium-containing CSM pellets.

The results illustrated in Figure 12.1 indicate that the kinetics of ingested lithium in grazing sheep are different to that which exists in the penned sheep (Suharyono 1992). However, in spite of the change in the time course of lithium clearance, the overall pattern of build-up and decline of plasma lithium concentration was similar to that previously reported for penned sheep (Suharyono 1992). Of more importance was the confirmation that plasma lithium concentrations scaled for live weight allowed accurate prediction of pellet intake.

Lithium associated with feed material entering the rumen (the primary pool) will leave either by digesta outflow, and then become available for absorption across the lower gut wall (Altman and Dittmer 1974) or via absorption across the rumen wall. Passage into the blood and body fluids will occur as the result of both these processes. Once in the blood pool, lithium is available for renal filtration (Ulyatt 1964) incorporation into wool (Mòcsennyi *et al.* 1987) or secretion to sweat and saliva (Ulyatt 1964). Harrison *et al.* (1963) have shown that lithium entering saliva can be recycled to the rumen. The fraction not entering the blood pool, generally small (Suharyono 1992), will appear in the faeces. The concentration of lithium in plasma is likely to be the net effect of transfer of lithium from the primary to the blood pool and clearance of lithium from the blood. The period of constant plasma lithium concentration (plateau) will occur when entry to and excretion from the blood pool is equivalent.

In this series of experiments, the concentration of lithium chloride in the CSM pellets was chosen to keep the daily consumption of lithium chloride below 50 mg/kg LWt. Assuming that the average live weight of the Merino weaners in experiment 2 was 25 kg, animals could have consumed 1250 mg lithium chloride (123 g pellets /hd/d mob A, 245 g pellets /hd/d mob B) before this threshold was reached. Increasing the concentration of lithium chloride in the CSM pellets may have facilitated a longer plateau period of blood lithium for those animals in experiment 2 which consumed small amounts of pellets. This would have enabled more accurate prediction of supplement intake. However, the disadvantage of this, was that those animals which consumed large amounts of lithium-containing CSM pellets would have exceeded the threshold value proposed by Suharyono (1992) and may have developed an aversion to the supplement.

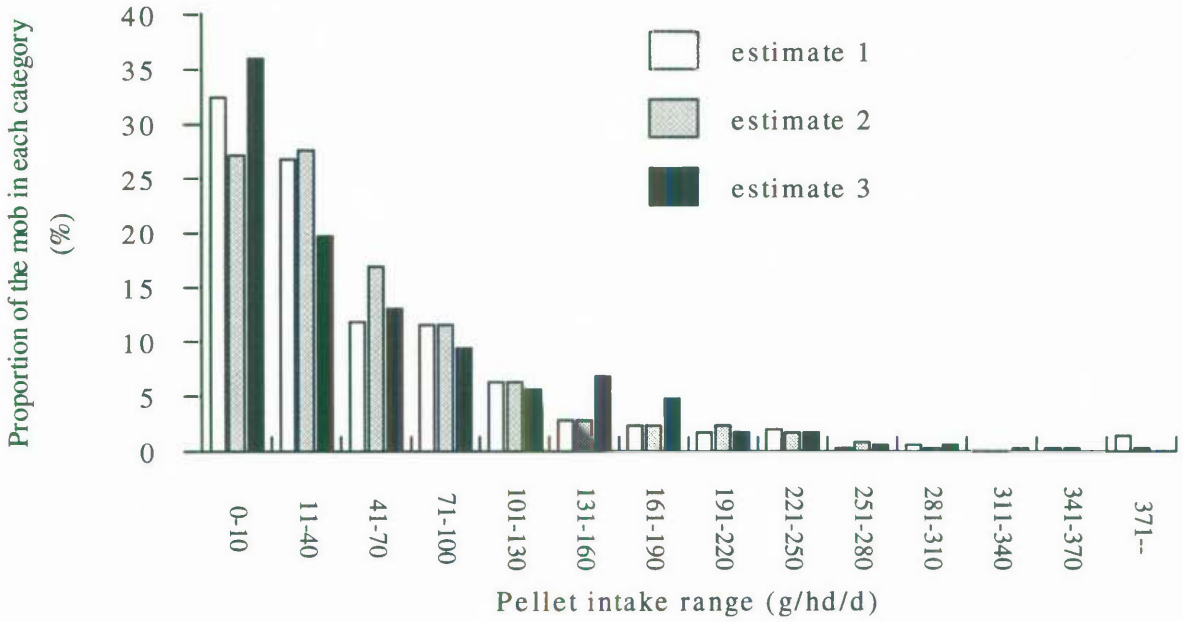

Figure 12.2. Frequency distribution of animals (n = 366) to pellet intake ranges when offered on average 55 g/hd/d.

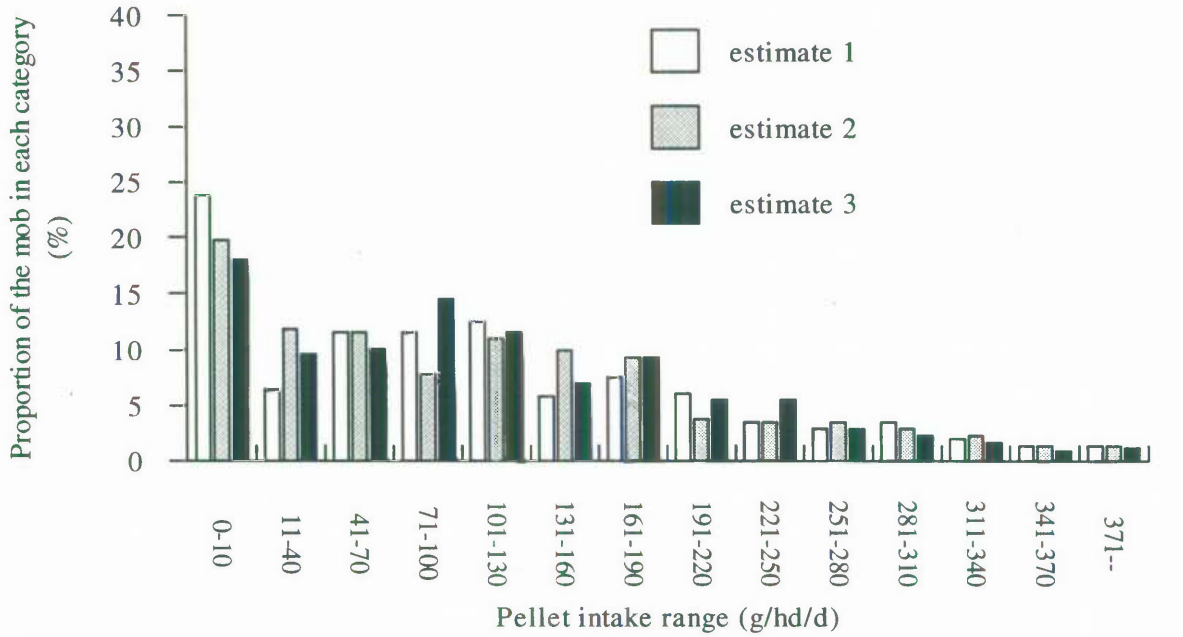

Figure 12.3. Frequency distribution of animals (n = 366) to pellet intake ranges when offered on average 110 g/hd/d.

The notion of creating a feed aversion in animals that consume excess lithium chloride is supported by the study of Burritt and Provenza (1989) who reported that when sheep

consumed 150 mg LiCl /d/kg LWt, intake dropped sharply and was maintained at a low level for a further 9 days. Suharyono (1992) reported that when sheep consumed 12.5–72 mg LiCl /d/kg LWt there were no observed clinical symptoms or aversions. The threshold value of 50 mg LiCl /d/kg LWt appears to allow for variation in intake between animals within a flock. It appears that there is a possibility of a small increase in the concentration of LiCl in feeds without creating any physiological problems.

The reason for the increased clearance rate of lithium in grazing sheep relative to penned animals (Kauter 1992; Suharyono 1992) has yet to be determined. The availability of pasture in this series of experiments was low (< 900 kg DM/ha) with the majority of the herbage being green material. Possibly, an increase in the fractional turnover rate of rumen contents in grazing sheep, associated with a diet with less structural carbohydrates (relative to their penned counterparts) may partly explain the increased rate of lithium clearance seen in this study.

In experiment 2, there was a large amount of variation in supplement intake between sheep within mobs for all 3 estimates of supplement intake. In mob A (animals offered on average 55 g /hd/d) there did not appear to be any further acceptance of the supplement after the 20 day training period. The proportion of animals that consumed 0–10 g/d of supplement did not decrease throughout the trial and was about 30% at all 3 estimates (Figure 12.2). Examination of successive intake estimates for mob A (Figure 12.2) failed to show any migration of individual intakes to an average value.

When animals were offered on average 110 g/hd/d (mob B) the proportion of non-eaters declined from about 25% at the first estimate to about 17% at the final estimate. This proportion of non-eaters was lower than that in mob A and this resulted in the spread of intakes in mob B more closely approximating a normal distribution (Figure 12.3).

The repeatability estimate for supplement intake (0.48) obtained from three observations suggests that more than one estimate of pellet intake needs to be taken over the duration of an experiment in order to maximise the accuracy with which the mean intake value for the experimental period can be estimated. Multiple measurements will lessen the problem of fluctuating intakes when attempting to estimate an average intake value over an experimental period. The heritability estimate of 0.17 was low but does suggest that there is a genetic basis for food acceptance. This notion is supported by the review of Chapple and Lynch (1986).

In conclusion, plasma lithium concentration 4–9 h after lithium ingestion allowed accurate prediction of pellet intake. The wide range in pellet intake that existed in the grazing flocks used in this trial, stresses the importance of being able to estimate individual intakes, when attempting to relate an imposed nutritional treatment to a production response in a field trial. Additionally, this range in intakes makes it difficult to interpret the effect of a supplement when a flock is regarded in statistical terms as an experimental unit.