CHAPTER 13

13. DIFFERENCES BETWEEN MERINO BLOODLINES, GRAZING AT PASTURE, IN PRODUCTION RESPONSES TO PROTEIN SUPPLEMENTATION: ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS.

13.1 Introduction

Studies involving large numbers of animals have been conducted to compare wool and body traits of Merino sheep sampled from different strains and studs (Jackson and Roberts 1970) grazing together in a range of different environments (Dunlop 1962; Dunlop 1963). In these studies, the main effects of strain, stud within strain and environment generally accounted for most of the variation in wool and body traits. Interactions between environment and strain, and environment and stud within strain, accounted for a small proportion of the variation in the measured traits. However, for some traits, these interaction terms are statistically significant and biologically meaningful.

A strain x year interaction for average fibre diameter (AVFD) was reported in the study of Jackson and Roberts (1970). The interaction was observed when the abundance of feed increased significantly in the second year of the trial. In that year, 2 of the 3 strains exhibited an increase in AVFD of 3–4 μm, whereas the AVFD of the remaining strain remained unchanged. The improved nutrition in year 2 also allowed the detection of a stud within strain x year interaction for yield and greasy and clean fleece weight (Jackson and Roberts 1970). Some studs had greater fleece weights during the second year whereas others failed to respond to the improved nutrition.

Dunlop (1962) also found evidence for a strain x year interaction for clean wool production and AVFD. In that study, the clean wool growth of medium and strong-wool strains more closely approached that of the fine-wool strain when the animals were grazed in the most nutritionally adverse environment. That location, Armidale, N.S.W, probably most closely resembled the environment from where the fine-wool animals had been bred. The

interaction for AVFD arose primarily because the fine-wool strain changed least in response to geographical location and presumably, level of nutrition.

The results from these studies indicate that the response of wool growth and AVFD to changes in nutrition can differ between Merino strains and studs. The aim of the present study was to identify whether such variation also existed between fine-wool Merino bloodlines. Thus, the studies reported here investigated whether genetic variation existed between 9 fine-wool and 2 medium wool bloodlines, grazing at pasture near Armidale, N.S.W, in the response of wool growth rate, AVFD and live weight gain to increasing amino acid intake. Amino acid intake was increased by supplementation with cottonseed meal pellets and the supplement intake of individual sheep was estimated using lithium-containing cottonseed meal pellets.

13.2 Materials and Methods

13.2.1 Animals and conditions

Ewes and rams representing a random selection from 9 fine-wool and 2 medium-wool Merino bloodlines (Table 13.1) originating from either Tasmania, Victoria or N.S.W were assembled at the C.S.I.R.O field research station 'Longford' during the period 1990–1991. During the autumn of 1991 the ewes were single sire mated and the progeny were identified with their dams at birth and their birth type and sex were recorded. The progeny (about 1100) were stratified into three groups (n = 356/group) at weaning (*c.* 4 months of age) based on the following effects:

1. Bloodline — eleven bloodlines.
2. Sire identification — sixty sires.
3. Sex — male or female.
4. Birth type — single, single uncertain, twin with same sex, twin with opposite sex, twin uncertain, twin with unknown sex, mixed triplets.
5. Maternal handicap — progeny of an adult or maiden ewe.

Following a period of four months, these animals entered the current experiment. Management of the three groups during this pre-experimental period attempted to ensure that the groups grazed paddocks of similar pasture quantity and quality. This aim appeared to have been achieved because at the start of the experiment the average live weight of the three groups did not differ (mean ± s.e.: 23.0 ±0.93, 22.6 ± 0.93, 23.1 ± 0.93 kg). The animals continued

grazing in the three groups throughout the experimental period of 63 days (17th June–19th August 1992).

Table 13.1. Sources of the Merino bloodlines.

Ewes	Bloodline	Rams
Mirani	Mirani	Mirani
Eurompambela	Eurompambela	Europambela
Lyndhurst	Merryville	Grathlyn/Emu Hill
Emu Creek	Emu Creek	Emu Creek
Kelvin Grove	Hillcreston	Mooroowoolen
Legerton	Legerton	Legerton
Grathlyn	Grathlyn	Grathlyn
Mt Morriston	Mt Morriston	Mt Morriston
Wurrock	Wurrock	Wurrock
Woodside	Woodside	Woodside
Coolringden/Hazeldean	Hazeldean	Hazeldean

13.2.2 Experimental procedure

At the start of the trial, each group was randomly assigned to one of three supplementation levels (0, 55, 110 g/d) of a commercial protein pellet (85% cottonseed meal, 32% crude protein and 1% urea; CSM pellets) which was fed daily. Animals had no prior experience with supplementation of any feed type and were given 23 days to become accustomed to the novel feed prior to the start of the trial. To estimate individual pellet intake of the grazing animal, CSM pellets sprayed with LiCl (Suharyono *et al.* 1991) were fed on days 14, 41 and 62 (see Chapter 12, Kahn 1994). On these days, animals were mustered to yards 5 h after feeding of supplement for blood sampling. The average estimated intake over the 3 days was used in the analyses that follow.

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^{o}C). The entire group was bled in 4 h so that the maximum time between lithium ingestion and blood sampling was 9 h.

To minimise differences between the groups in pasture availability, the groups were rotated around 6 paddocks (average area 13.8 ha sem = 0.82) 11 times during the 63 days of the trial. At the conclusion of the experiment, the number of times group A entered a paddock after group B was equal to the number of times group B entered a paddock after group A and so forth. In addition, each group spent about the same number of days in each of the six paddocks.

Animals were weighed (18 h off feed and water) on days 15, 42 and 63. To determine wool growth, a 15 cm dyeband (Chapman and Wheeler 1963) was placed above the midside position on the left side of the animal on day 1 of the trial. A second dyeband was placed on the animals on day 63 and the dyeband was removed (Oster clippers No 40) 14 days later. Animals were shorn approximately 1 month after the trial concluded and the fleece weights, including belly wool, of individual sheep were recorded.

13.2.3 Analytical techniques

Determination of plasma lithium concentration is described in Chapter 12 (Kahn 1994).

Wool growth rate (g/hd/d) was determined from the dyeband wool samples according to the method of Langlands and Wheeler (1968). Average fibre diameter and its standard deviation were determined on 2000 wool snippets using the Sirolan-Laserscan (Charlton 1995).

The *in sacco* degradability of the commercial protein pellets was determined using the nylon bag technique of Mehrez and Ørskov (1977). Dacron bags (7 x 14 cm internally, pore size 44 µm) were filled with about 6 g of the CSM pellets that had previously been ground so as to pass through a 2 mm sieve. Four bags containing the pellet material and one containing about 5 g of oaten chaff (*Avena sativa*) were placed in the rumen of four sheep (5 bags/sheep) that were consuming a diet of 600 g oaten chaff, 200 g lucerne chaff (*Medicago sativa*) and either 110 or 55 g/d of the pellet material. Duplicate bags were removed at 14 and 24 h (the bag containing oaten chaff was also removed at 24 h and used as a standard reference) and washed to remove external debris. The disappearance of material from the bags due to the washing procedure was determined and referred to as losses of water soluble material at zero time.

To further examine the potential degradability of the protein pellets in the rumen, *in vitro* ammonia production was determined. To gauge the relative rate of ammonia liberation from the CSM pellets, a readily soluble and easily fermented protein grain, lupins (*Lupinus augustifolius* L.), was assayed at the same time. Rumen fluid (1 l) collected from the sheep used in the *in sacco* studies was strained through cheesecloth and then constantly stirred while

50 ml was dispensed into flasks containing 0.5 g of feed material that had been ground so as to pass through a 1 mm sieve. The feed material (duplicates) was incubated in rumen fluid in a shaking water bath (37° C) for either 1, 2, 3, 4, 5 h. Incubations were stopped by the addition of 1 ml conc. H_2SO_4 to a subsample (20 ml) of the rumen fluid.

The subsample of acidified rumen fluid was then centrifuged (1500 *g*) for 5 min and a fraction from the supernatant was diluted (40 fold) with 0.2% sulphuric acid. Ammonia concentrations were then determined using a Technicon Auto-analyser (Technicon Instruments Comp. New Jersey, U.S.A) according to the method of Crook and Simpson (1971) and modified by Beitz (1974).

Total N of the CSM pellets was determined using an automated Organic Nitrogen Determinator (FP-228, Leco Corporation, USA).

13.2.4 Statistical methods

Heritabilities and phenotypic and genetic correlations for the measured traits were estimated by analysis of variance and covariance (Harvey 1988). Model (1) included the fixed effects of bloodline (B_i), birth type (T_k), maternal handicap (M_l), 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. Heritabilities (h^2) were estimated as four times the sire component of variance divided by the phenotypic variance. Genetic and phenotypic correlations were estimated by dividing the genetic and phenotypic covariances by the square root of the product of the appropriate genetic and phenotypic variances (Becker 1985). Appropriate standard errors were evaluated for a single parent design (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.

Analysis of covariance (SAS, SAS Institute Inc.) using a general linear model (GLM) was used to analyse the above effects adjusted for differences in individual pellet consumption and initial live weight. Differences between least squares means were tested using multiple t-tests. To reduce the probability of finding spurious differences, only means where the F statistic was significant ($P < 0.05$) were compared.

13.3 RESULTS

13.3.1 Raw means and standard deviation

The raw means and standard deviation of the measured traits are presented in Table 13.2. The most noticeable feature was the very large standard deviation associated with live weight gain.

Table 13.2. Simple means and standard deviations (s.d.) of measured traits.

	Mean	s.d.
Greasy wool production (g/d)	7.6	1.88
Live weight gain (g/d)	17	19.8
Initial live weight (ILWT; kg)	23.7	3.07
Fibre diameter:		
average (μm)	16.6	1.50
coefficient of variation (%)	15.3	2.41
standard deviation (μm)	2.5	0.52

13.3.2 Feed analysis

The CSM pellets had a dry matter (DM) of 900 g DM/kg air dry and a nitrogen (N) content of 5.6% of which 0.47% was present as urea-N. The *in sacco* disappearance of DM and N over 0, 14 and 24 h was 0.47, 0.47 and 0.62, 0.60 and 0.68, 0.67 respectively, with losses at zero time being due to the loss of water soluble material and particles less than 44 μm in the washing procedure. *In vitro* ammonia production rates indicated that the N fraction of the protein pellets was significantly ($P < 0.01$) less degradable than that associated with lupins (Table 13.3).

13.3.3 Supplement intake

Estimated pellet intake (g/d) for each animal was expressed as an estimated pellet intake category (1–14) (see Chapter 12 in this thesis, and Kahn 1994). The raw data for pellet intake exhibited a correlation between the mean and the variance. To remove this correlation, the data were subjected to log transformation for subsequent analysis.

13.3.4 Response to supplement intake

The data for pellet intake categories 12–14 was omitted from any analysis of dependent variables because the number of animals consuming these levels of supplement were very small and consequently the standard errors were very large. Increasing intake of the CSM pellets increased live weight gain (LGAIN; measured 18 h off feed and water and free from wool) ($P < 0.01$), greasy wool production (GWP) ($P < 0.01$), average fibre diameter (AVFD) ($P < 0.01$) and standard deviation of AVFD (SDFD) ($P < 0.05$). The coefficient of variation of AVFD (CVFD) was unaffected by the level of pellet intake. The increase in the above mentioned variables was adequately explained by the linear term of pellet intake (Figures 13.1, 13.2, 13.3) over the range of intakes observed in the trial (0–310 g/d).

Table 13.3. *In vitro* ammonia production rates.

Feed type	Incubation time	Ammonia production (mg NH_3-N/l)[A]		Fractional ammonia production (mg NH_3-N /mg Feed -N)
	(h)	mean	s.e.	mean (%)
CSM pellets[B]	1	52	0.0	10.1
	2	55	0.0	10.7
	3	55	3.0	10.7
	4	48	5.3	9.4
	5	66	0.0	12.9
Lupins	1	51	0.0	9.7
	2	102	1.5	19.2
	3	150	3.0	28.5
	4	182	5.3	34.3
	5	209	0.0	39.4

[A] Values are adjusted for ammonia production from the rumen fluid alone.

[B] The CSM pellets contained 0.47% urea-N and consequently the ammonia concentration values for the CSM pellets are adjusted for the ammonia contribution that arose from the urea. The calculations for this adjustment assume that 100% of the urea-N will contribute to ammonia production and are as follows; 0.5g dry pellet*0.47% urea-N = 2.35 mg urea-N per 50 ml incubation flask → 2.35*20 = 47mg N/l. Hence ammonia production values for the CSM pellets have 47 mg N/*l* subtracted.

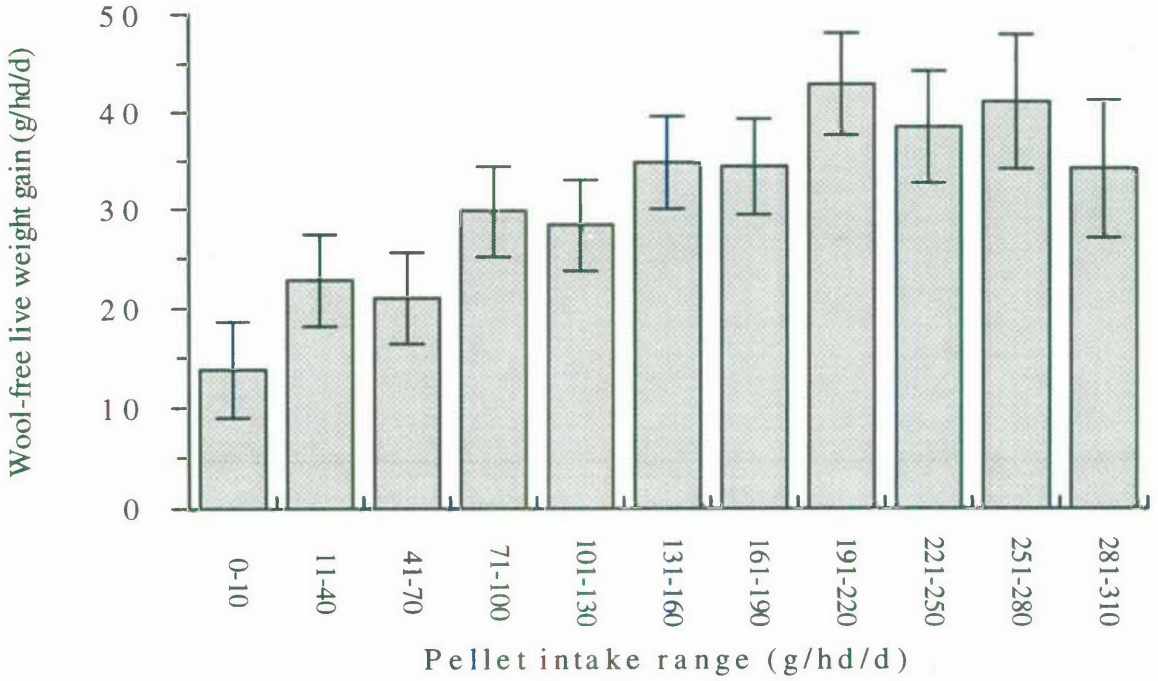

Figure 13.1. Live weight gain (mean ± s.e.) in response to increasing intake of CSM pellets.

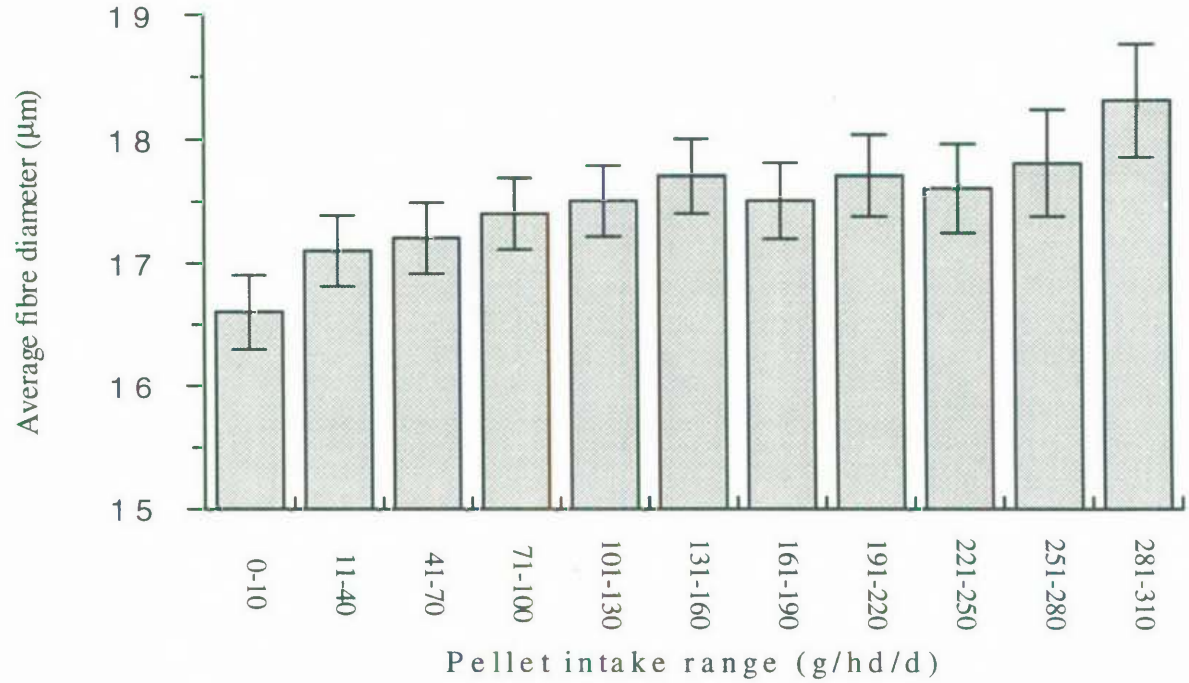

Figure 13.2. Change in average fibre diameter (mean ± s.e.) in response to increasing intake of CSM pellets.

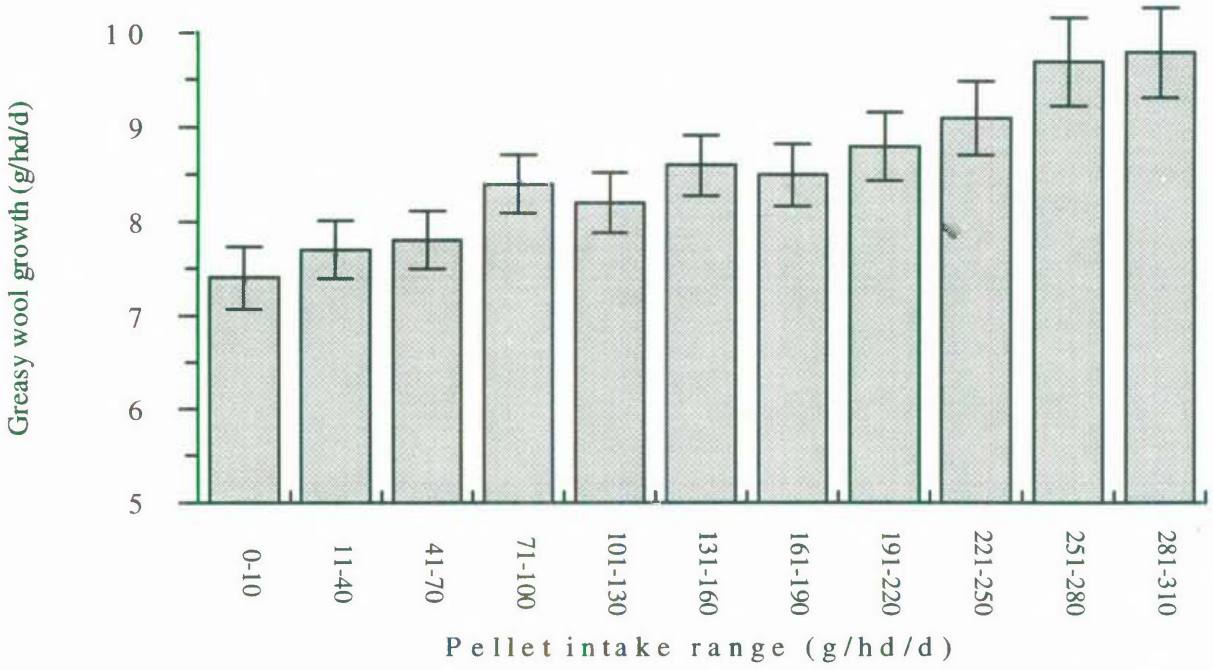

Figure 13.3. Rate of greasy wool growth (mean ± s.e.) in response to increasing intake of CSM pellets.

13.3.5 Results from analysis of variance

The results of the analysis of variance are summarised in Table 13.4 and the least squares means for pellet intake category, GWP, AVFD, CVFD and LGAIN of the Merino bloodlines are outlined in Table 13.5. Differences between sires were evident for all the measured variables ($P < 0.01$). Birth type significantly affected AVFD ($P < 0.01$) with AVFD increasing with the number of lambs per litter. Maternal handicap was only important for AVFD ($P < 0.01$) with progeny from adult ewes having a reduction in AVFD of nearly 0.5 µm. Male weaners grew about 6 g/d faster than their female counterparts ($P < 0.01$). The interaction between the covariate, pellet intake category (Pintake), and bloodline for the traits GWP and LGAIN ($P < 0.05$) suggests that the bloodlines used the CSM supplement for LGAIN and GWP with differing efficiencies.

13.3.6 Differences between bloodlines

There were significant differences ($P < 0.01$) between the eleven bloodlines for GWP, AVFD, SDFD and CVFD; LGAIN failed to reach significance ($P < 0.07$) (Table 13.4). In order to further analyse the genetic variation observed in Table 13.5, regression coefficients for the relationship between pellet intake and LGAIN and GWP for a selection of the bloodlines are

presented in Table 13.6. The results further confirm the apparent differences between the bloodlines in the efficiency of use of CSM for both GWP and LGAIN.

Table 13.4. Sources of variation, degrees of freedom (df) and significance of effects.

Source of variation	d.f.	GWP	LGAIN	AVFD	CVFD	SDFD	ILWT[A]	Pintake[A]
Pintake	1	**	**	**	NS	*		
ILWT	1	**	**	**	**	NS		
Bloodline	10	**	NS	**	**	**	**	**
Sire(Bloodline)	50	**	**	**	**	**	**	**
Birth Type	6	NS	NS	**	NS	**	**	NS
Maternal Handicap	1	NS	NS	**	NS	NS	*	NS
Sex	1	NS	**	**	NS	**	**	NS
Bloodline x sex	10	NS	NS	NS	NS	NS	NS	NS
Pintake x Bloodline	10	*	**	NS	NS	NS		
Group	2	**	NS	NS	NS	NS	NS	**
Bloodline x Group	20	NS	**	NS	NS	NS	NS	NS

[A] When ILWT and Pintake were analysed as dependent variables no covariates were used.

* $P < 0.05$, ** $P < 0.01$, NS: non-significant.

13.3.7 Parameter estimates

Heritabilities and genetic and phenotypic correlations for LGAIN, ILWT, GWP, AVFD, CVFD and Pintake are presented in Table 13.7. All heritabilities were significantly different from zero ($P < 0.05$). Most wool traits exhibited medium to high heritabilities whilst pellet intake exhibited low levels of additive genetic variation. CVFD had negative genetic and phenotypic correlations with GWP, LGAIN, AVFD and Pintake.

13.4 DISCUSSION

13.4.1 The value of protein supplementation to the grazing animal

The value of a protein source to ruminants is largely dependent on the extent that it can resist degradation in the rumen; a high proportion of undegraded digestible dietary protein in digesta outflow from the rumen being optimal. The *in sacco* estimate of DM and N degradability of 0.67 at 24 h suggests that a substantial proportion of the dietary protein would be degraded within the rumen. Proteins that are degraded in the rumen are initially subjected to proteolysis with peptides and amino acids resulting from the hydrolytic process. The resulting peptides

and amino acids are transported into bacterial cells where peptides are degraded by a range of peptidases (see Chapter 3). Amino acids can either be deaminated with the subsequent formation of volatile fatty acids and ammonia or incorporated into bacterial protein (Tamminga 1979). These processes are much less than 100% efficient at metabolising dietary protein to microbial protein, and hence proteins that are more extensively degraded in the rumen will have a lower feeding value for the animal.

Table 13.5. Least squares means (± s.e.) for the eleven Merino bloodlines for Pintake, GWP, AVFD, CVFD and LGAIN. Least squares means are adjusted for differences in initial live weight and pellet consumption.

Bloodline	Pintake[A]		GWP		AVFD		CVFD	LGAIN	
	(g/d)				(μm)		(%)	(g/d)	
	l.s.mean	s.e.	l.s.mean	s.e.	l.s.mean	s.e.		l.s.mean	s.e.
B1	4.1^{a}	0.29	7.2^{ab}	0.30	16.9^{cd}	0.28	13.9^{ab}	24	4.5
B2	4.9^{b}	0.24	8.5^{d}	0.29	17.4^{e}	0.27	14.4^{b}	22	4.3
B3	4.1^{a}	0.30	8.6^{d}	0.28	16.8^{bc}	0.26	15.0^{c}	27	4.2
B4	4.8^{b}	0.23	8.1^{c}	0.28	17.1^{cd}	0.26	15.0^{c}	28	4.2
B5	4.4^{a}	0.25	7.2^{ab}	0.29	16.3^{a}	0.27	13.4^{a}	20	4.3
B6	5.7^{cd}	0.30	10.4^{f}	0.36	18.5^{f}	0.33	16.8^{d}	31	5.3
B7	5.0^{b}	0.30	6.9^{a}	0.30	16.5^{ab}	0.28	14.2^{b}	18	4.4
B8	6.0^{d}	0.28	9.2^{e}	0.30	18.9^{f}	0.27	17.9^{e}	27	4.4
B9	4.4^{a}	0.30	7.5^{b}	0.30	17.2^{de}	0.28	13.4^{a}	16	4.5
B10	4.4^{a}	0.41	7.4^{ab}	0.34	16.8^{bc}	0.31	14.0^{ab}	21	5.1
B11	5.1^{bc}	0.23	7.2^{ab}	0.29	17.1^{cd}	0.27	13.5^{a}	24	4.3

[A] Pintake means are unadjusted

Within columns, means with a common suffix do not differ significantly ($P > 0.05$).

The *in sacco* estimate of crude protein disappearance in the rumen may be an overestimate of the potential degradability of the protein source. Ørskov and McDonald (1979) suggested that the potential degradability of a protein source should be adjusted down to account for the rate of passage (k; proportion/h) of that feed source from the rumen. Ignoring the rate of passage ignores the fact that some particles will flow from the rumen

before they have an opportunity to be degraded. Failure to account for this will lead to an overestimation of the potential degradability of a feed, but can also introduce a bias if different feed types are compared. Substrates that are most slowly degraded will be penalised for the above reasons whilst those that are more rapidly degraded will suffer little from overestimation (Ørskov and McDonald 1979). Nevertheless, the *in sacco* estimate of N disappearance from CSM pellets was similar to the *in vitro* and *in situ* values reported for CSM and CSM pellets by Neutze *et al.* (1993).

Table 13.6. Regression coefficients (± s.e.) between GWP, LGAIN and Pintake for a selection of bloodlines.

Bloodline	GWP	LGAIN
	(g GWP/d) / (intake category)	(g LGAIN/d) / (intake category)
B1	0.10 ± 0.070	1.8 ± 1.24
B3	*0.30 ± 0.077*	*5.5 ± 1.43*
B4	*0.19 ± 0.075*	*2.6 ± 1.04*
B6	*0.40 ± 0.102*	*5.8 ± 1.35*
B8	*0.29 ± 0.115*	*3.0 ± 1.37*
B9	*0.38 ± 0.102*	*3.0 ± 1.39*
B11	*0.15 ± 0.053*	0.7 ± 0.89

Regression coefficients significantly different from zero ($P < 0.05$) are in italics.

The possible overestimation of the degradability of the CSM pellets by the *in sacco* technique is supported by the results from the *in vitro* ammonia production trial. This trial showed that the protein contained in the CSM pellets was substantially more protected than that in lupin grain when incubated in the same system (Table 13.3). After 5 h incubation with CSM pellets, the ammonia concentration of the rumen fluid supernatant was 66 mg NH_3-N/*l*. This concentration of ammonia-N represented only 13% of the feed-N added to the incubation. This does not appear to be congruous with 60% disappearance (in sacco) at 14 h. Before this conflict can be resolved it is necessary to examine the *in vitro* culture method that was used.

The *in vitro* method did not utilise compounds such as hydrazine sulphate or chloramphenicol to give quantitative recovery of protein breakdown products. Accordingly, the quantitative data presented from the *in vitro* study is confounded by microbial uptake of

ammonia and amino acids. This would result in the ammonia production rates being an underestimate of quantitative protein breakdown because utilisation of ammonia and amino acids by micro-organisms would not be accounted for (Broderick 1987). However, the *in vitro* estimate of the potential degradability of the CSM pellets indicated that the protein fraction in CSM pellets was substantially more protected from ruminal degradation than that in lupin grain, which is known to be readily degraded in the rumen.

Table 13.7. Heritabilities (on diagonal) and genetic (above diagonal) and phenotypic (below diagonal) correlations and standard errors (below estimates) for growth and wool traits and pellet intake.

	Live weight		Wool Traits				Supplement Intake
	LGAIN	ILWT	GWP	AVFD	CVFD	SDFD	Pintake
LGAIN	**0.145**	0.269	*0.736*	0.176	*–0.749*	–0.557	*0.661*
	0.069	0.321	*0.236*	0.252	*0.277*	0.270	*0.285*
ILWT	*–0.229*	**0.295**	*0.600*	0.251	0.088	0.250	–0.006
	0.058	**0.090**	*0.192*	0.203	0.253	0.242	0.293
GWP	*0.306*	*0.426*	**0.211**	0.227	–0.166	0.022	0.086
	0.050	*0.080*	**0.078**	0.218	0.274	0.272	0.316
AVFD	*0.212*	0.143	*0.404*	**0.706**	–0.321	*0.502*	0.048
	0.068	0.077	0.068	**0.140**	0.206	*0.162*	0.248
CVFD	*–0.130*	–0.069	*–0.250*	–0.080	**0.288**	*0.654*	–0.192
	0.059	0.065	*0.062*	0.078	**0.089**	*0.130*	0.294
SDFD	0.093	0.005	0.183	*0.433*	*0.858*	**0.305**	–0.144
	0.062	0.061	0.598	*0.062*	*0.427*	**0.092**	0.288
Pintake	*0.223*	*0.125*	*0.302*	*0.156*	–0.047	0.027	**0.168**
	0.052	*0.059*	*0.054*	*0.071*	0.059	0.066	**0.072**

Correlations that are significantly different from zero ($P < 0.05$) are in italics.

In conclusion, it would appear that the provision of CSM pellets to the grazing animal provided some by-pass protein and may have also stimulated microbial growth by the provision of amino acids for bacterial utilisation (Maeng *et al.* 1976; see Chapter 5).

13.4.2 Response to supplement intake

Wool growth is primarily limited by the provision of sulphur amino acids and lysine to the wool follicle (Reis and Schinckel 1963; Reis 1989). The supply of energetic substrates to the animal is relatively unimportant to wool growth (Ball *et al.* 1972; Black *et al.* 1973; Kempton *et al.* 1978; Reis *et al.* 1992; see Chapter 2), however the existence of a protein x energy interaction for wool growth has been reported (Black *et al.* 1973; Reis *et al.* 1992). The form of this interaction is such that when protein supply is limiting, an increase in the absorption of energetic substrates reduces wool growth. In contrast, tissue growth is dependent on the intestinal supply of both protein and energetic substrates (Kempton *et al.* 1978; Preston and Leng 1987).

Increasing intake of the CSM pellets by the grazing animal increased LGAIN and GWP in a linear relationship (Figures 13.1, 13.3). Undoubtedly, the stimulation of wool growth by increasing intake of the CSM pellets was attributable to a greater flow of dietary protein to the intestines, which in turn would have increased the circulating concentrations of cysteine and methionine and other essential amino acids in plasma (Reis *et al.* 1992).

The relationship between PI intake (measure of amino acid intake) and 1) GWP and 2) LGAIN (Table 13.6) differed between the bloodlines. Further examination of the regression coefficients presented in Table 13.6 also indicated that bloodlines with similar efficiencies of GWP did not necessarily have similar efficiencies of LGAIN. A change in the partitioning of nutrients between wool and other tissue groups may account for this observation. Absorbed nutrients are potentially available for metabolism by all body tissues. However, it appears that the partitioning of nutrients between wool and other tissues varies within Merino strains (Hutchinson 1961) and selection lines (Williams and Winston 1965; Piper and Dolling 1969a). Factors that may contribute to differences in nutrient partitioning between tissues have been discussed by Black and Reis (1979) and include:

1. Rate of blood flow to the tissue and the concentration of nutrients in blood.
2. Availability of energy carriers such as adenosine triphosphate (ATP) and reduced cofactors such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) within each tissue group. The availability of these compounds will control many metabolic reactions.
3. The relative metabolism of nutrients within each tissue which will largely be a function of Michaelis-Menton kinetics.
4. Physiological and endocrinal status of the animal.

In addition to this list of possible metabolic controls, nutrient partitioning between tissues is potentially controlled by the ratio of digestible protein to energy (Kempton 1979) in nutrients leaving the rumen. Some of the factors that are responsible for alterations in the protein to energy ratio include microbial protein production and outflow from the rumen, concentration of protozoa and ammonia in rumen fluid, turnover of bacterial protein in the rumen and dilution rate (see Chapter 5).

Differences between the bloodlines in one or a number of the metabolic and nutritional controls, as discussed above, has the potential to alter the partitioning of nutrients between tissues and the wool follicle. Unfortunately, the regression coefficients (Table 13.6) are only indicative of differences in efficiency between the bloodlines. This is so, because pasture intake was not estimated, and hence the total nutrient intake of the animals was unknown. Nevertheless, the notion of competition for nutrients between wool follicles and other tissue groups is supported by the reports that both greasy wool growth (Atkins and Robards 1976; Butler-Hogg 1984) and the efficiency of clean wool production (g wool /kg feed DM) (Williams and Winston 1965) are negatively related to increasing body weight gain.

13.4.3 Results from analysis of variance

The AVFD of animals from twin births was about 0.5 µm greater than that of single births. This observation is supported by the findings of Lax and Brown (1967), who found that animals born in multiple births had reduced crimp frequency and increased AVFD relative to their single contemporaries. The increase in AVFD with increasing litter size is possibly due to a reduction in the number of secondary follicles initiated in the foetus as a result of periods of placental insufficiency. The result of this would be a decrease in the secondary to primary ratio of wool producing follicles and a corresponding increase in AVFD (Short 1955; see Chapter 2).

The AVFD of animals born to adult ewes was about 0.5 µm less than that of animals born to maiden ewes. This is likely to be the result of a better maternal environment provided to lambs by ewes that had previously reared a lamb and is then similar to that discussed above. Maternal handicap did not account for a significant proportion of the variance for the other measured variables (Table 13.4). The relative unimportance of maternal handicap observed in the current study is supported by the findings of Walkley *et al.* (1987), who found that the age of dam was unimportant for 14 mth weaning weight, greasy fleece weight, yield, clean fleece weight, AVFD, crimp frequency and staple length. In contrast, Turner (1961) reported that animals born to maiden ewes or as a twin (clumped together as maternal handicap) had reduced

levels of wool production when compared to animals born as a single to adult ewes. A possible explanation for this discrepancy is that in the studies reported by Turner (1961) the effects of birth type and dam age were pooled.

13.4.4 Parameter estimates

The estimates of heritability and phenotypic and genetic correlations derived from paternal half-sib analysis are generally in close agreement with those that exist in the literature. However, the genetic and phenotypic correlations of ILWT (weight at 8mth) and GWP are higher than other published estimates (Gregory 1982; Mortimer and Atkins 1989). In fact, Gregory (1982) and Mortimer and Atkins (1989) suggested that these traits are genetically independent. A possible explanation for the high correlations estimated from the present study is that initial live weight may be an indirect measure of birth date in the present data set. Mortimer and Atkins (1989) reported that birth date explained a significant proportion of the within-flock variation for hogget greasy fleece weight despite the short (5 week) mating period. Given this, the correlation between initial live weight and GWP may be confounded with the relationship between birth date and greasy fleece weight.

Of the measured variables, the only trait that Pintake was independent of was ILWT (live weight at 8mths). The estimate of the genetic correlation between these traits was not different from zero. However, the phenotypic correlation (0.125 ± 0.059; $P < 0.05$) suggests that animals that were heavier at the start of supplementation tended to consume more of the supplement throughout the experimental period.

The estimates of genetic parameters made from the current study (Table 13.7) are based on a small number of rams and are peripheral to the main purpose of this study. Accordingly this area will not be discussed further.

One perceived disadvantage of CSM supplementation is the resulting increase in AVFD that is intrinsically linked to a stimulation of wool growth. It has been argued that this is financially undesirable because the reduction in dollars per kg of wool sold (as a result of an increase in AVFD) outweighs the increase in kilograms of wool to be sold. There is no definite answer, however: it all depends on the duration of supplementation, the sensitivity of wool price to changes in AVFD, and the ratio of the linear growth rate of the wool fibre (L) to the diameter of that fibre (D). It has been suggested (Kahn and Nolan 1994) that supplementation for short periods of time (e.g. 3–4 months over winter) will generally have little effect on the AVFD of the fleece but may reduce the along-fibre variability of fibre diameter and increase tensile strength.

CHAPTER 14

14. GENERAL DISCUSSION

Ruminants derive their protein supply from two major sources: the diet and rumen microbes. Generally, a large proportion of the dietary protein is degraded by ruminal micro-organisms to yield ammonia via intermediates which include peptides and amino acids. As a consequence, microbial-N is often the major component of the total non ammonia nitrogen (NAN) flowing into the proximal duodenum.

Various methods have been used to estimate the yield of microbial-N in rumen digesta outflow. These methods are imprecise, tedious and require animals that are surgically modified e.g. with rumen cannula. Recently, urinary excretion of purine derivatives (principally allantoin) has been used as a non-invasive indirect estimate of the yield of microbial-N from the rumen. However, some concerns exist about the validity of the technique. In the current research, ^{14}C-allantoin was used to investigate some of these concerns and also to investigate the metabolism of allantoin in sheep.

When exogenous purines are infused into either the abomasum or the duodenum, the molar recovery of purine derivatives excreted in urine is incomplete and ranges from 84–93%. Chen and co-workers (1990c) suggested that purine derivatives enter saliva and this may account for the incomplete urinary recovery of infused purines. These authors reported that the saliva of sheep contained allantoin and uric acid at concentrations greater than that in plasma and that salivary allantoin and uric acid would be degraded in the rumen. In contrast, Surra *et al.* (1993) reported that the salivary concentrations of allantoin and uric acid are insignificant. If the concentrations of allantoin and uric acid in saliva are subject to variation, then the equations which are based on the relationship between purine absorption and urinary excretion may be inappropriate to predict microbial yield.

The studies using ^{14}C-allantoin which are presented in Chapters 8 and 9 clearly indicated that, in sheep, allantoin does not enter saliva to any extent and that degradation of allantoin to carbon dioxide is quantitatively unimportant. These results suggest that loss of allantoin via saliva will not compromise the urinary purine technique.

A further concern about the validity of the urinary purine technique is that the urinary recovery of allantoin infused into the jugular has been reported to be on average 72% (Chen *et*

al. 1991). Of greater concern, in the studies reported by Chen and co-workers (1991), the urinary recovery of allantoin varied between sheep from 62–105%. In sheep, allantoin is the end-product of purine degradation and non-renal routes of loss have not been experimentally verified. However, the data reported by Chen and co-workers (1991) indicate that a variable fraction of the allantoin flux through blood is not excreted in the urine.

In the studies reported in Chapter 8, the daily urinary excretion of allantoin was considerably less than the daily allantoin net flux through blood plasma. This indicates that urinary excretion is not the only route of loss for allantoin passing through the plasma pool. However, end point errors in urine collection may contribute to this difference. Alternatively, the net flux of allantoin during the period of measurement, effectively only the few hours after tracer injection, may not be representative of the true long-term mean flux rate. If this is so, then comparisons between urinary excretion of allantoin and flux rates through blood plasma may not be valid. Nevertheless, if allantoin is lost from the blood via routes other than urine, and if the quantitative importance of that loss varies between animals, the equations which are used to predict purine absorption (and hence the yield of microbial-N from the rumen) from the urinary excretion of purine derivatives may not be appropriate. In light of this potential problem, the possibility of non-renal loss of blood allantoin in sheep warrants further investigation.

Analysis of the allantoin SR versus time curves indicated that, in sheep, allantoin moves between at least 2 kinetically distinct compartments (e.g. blood and extracellular fluid). However, there was visual evidence of a possible third exponential component becoming apparent 9 h after injection with ^{14}C-allantoin. A third component could not be fitted mathematically with confidence and did not significantly change the kinetic data. This component was presumably a result of ^{14}C-allantoin re-entering the primary pool from another, much larger compartment (e.g. body water). If allantoin did enter body water, it would provide an explanation for the appearance of ^{14}C-allantoin in urine for several days following its intravenous injection.

The studies reported in Chapters 8 and 9 raised some uncertainties, at least in sheep, about the suitability of using urinary excretion of purine derivatives to predict the yield of microbial-N from the rumen. Whilst these uncertainties require further investigation there was no direct evidence to preclude the use of this non-invasive technique from further use in the research presented in this thesis. Hence, urinary excretion of purine derivatives was used to

assess the importance of microbial yield from the rumen in accounting for differences in the rate of wool growth between sheep selected either for or against clean fleece weight.

The rate of wool growth is primarily dependent on the supply of sulphur amino acids (Reis and Schinckel 1963) to the intestines, but when a range of essential amino acids (including methionine) is infused into the abomasum, wool growth is further stimulated (Reis *et al.* 1990). Clean fleece weight is a moderately heritable trait and consequently responds to selection. Numerous selection experiments were initiated in the period 1950–60 and ewes from one of these selection experiments, the Trangie fleece plus (F_p) and fleece minus (F_m) Merino selection lines (see Chapter 10 for a full description) were used in the studies reported in this thesis.

Over the last 30 years many studies have been undertaken in an attempt to determine the biological mechanisms that are responsible for the genetic differences in clean fleece weight between the Trangie selection lines This research has been unable to elucidate a metabolic basis for differences in wool growth rate but has revealed the following: The following list refers to differences between sheep from the F_p and F_m flocks.

1. Differences between the selection lines for voluntary feed intake, adjusted for live weight, are usually small but significant differences were evident in the studies reported in Chapter 10.
2. Plasma cysteine concentration and flux rates are less in F_p sheep.
3. The concentration of glutathione in erythrocytes is less in F_p sheep.
4. The levels of thyroxine, cortisol, insulin and growth hormone do not differ consistently between the selection lines.
5. The wool growth rate of F_p sheep is more sensitive to nutrient supply and physiological status.
6. The net portal absorption of α-amino nitrogen tends to be greater in F_p sheep.

Research conducted with other fleece selection lines has clearly indicated that superior rates of wool growth are not related to a greater apparent digestibility of DM in the whole-tract (Piper and Dolling 1965b). This has now been confirmed for the Trangie selection lines (Chapter 10). However, whilst estimates of apparent digestibility give an indication of the proportion of the dietary constituents that are absorbed into the body they do not give any information on the site of digestion nor the quality (e.g proportion of volatile fatty acids and supply of microbial protein) of those nutrients that are available to the animal.

A greater net portal absorption of α-amino nitrogen in F_p sheep has been reported (Lush *et al.* 1991) but the proportional contribution of dietary and microbial amino acids was not determined. As part of the research reported in this thesis, the yield of microbial-N from the rumen of ewes from the F_p and F_m flocks (Chapters 10, 11) was estimated from the urinary excretion of purine derivatives. The yield of microbial-N was greater for ewes from the F_p flock and these ewes also had a greater yield of microbial-N per unit DMI.

Using the approach of Hutchinson (1961) and Henderson and Hayman (1960), a model relating the yield of microbial-N to 1) the yield of microbial-N per unit intake (MN/DMI), 2) intake per unit live weight (DMI/B) and 3) live weight (B) was established (Chapter 10, 11). The results from this model indicated that variation in the yield of microbial-N/DMI and DMI (a composite of DMI/B and B) accounted for *c.* 35 and 65% of the between-selection line differences in the yield of microbial-N from the rumen. Lower concentrations of the branched chain volatile fatty acids in rumen fluid and a lesser contribution of these acids to total volatile fatty acid concentration indicated that the greater yield of microbial-N/DMI of ewes from the F_p flock may have been the result of lower rates of turnover of bacterial protein and fewer protozoa.

Using a similar approach to that discussed above, a model relating wool growth rate to 1) intake, 2) the yield of microbial-N and 3) the efficiency with which microbial-N was used for wool growth was established (Chapter 10). Analysis of this model revealed that variation in the efficiency with which microbial-N was used for wool growth accounted for *c.* 80% of the differences in wool growth rate between the selection lines. The yield of microbial-N/DMI accounted for a further 8% of the between-selection line differences in wool growth rate. The remainder of the variation (11%) was attributable to variation in DMI. Thus, whilst the studies reported in Chapters 10 and 11 indicated that the yield of microbial-N from the rumen was greater in ewes from the F_p as compared to the F_m flock, it was apparent that this had little bearing on the between-selection line difference in wool growth rate.

Lithium chloride has recently been proposed as a marker for estimating the supplement intake of individual penned sheep (Suharyono 1992). However, the use of lithium chloride for estimating the supplement intake of individual sheep when grazing at pasture had not been assessed. Therefore in the studies reported in Chapter 12, 8 sheep, grazing at pasture, were fed known amounts of lithium-containing cottonseed meal pellets. Lithium was cleared from plasma at a rate greater than that reported for penned animals but the concentration of lithium in plasma 4 h after ingestion of lithium provided an accurate means for predicting the intake of

pellets. Lithium chloride was subsequently used to estimate the intake of cottonseed meal pellets by 730 Merino weaners, representative of 9 fine-wool and 2 medium-wool Merino bloodlines, on 3 occasions over a 62 day period. This study provided the basis for investigating whether genetic variation existed between fine-wool Merino bloodlines which were grazing at pasture, in the response of wool growth rate, AVFD and live weight gain to increasing amino acid intake.

Live weight gain, greasy wool production, average fibre diameter and the standard deviation of average fibre diameter increased linearly with intake of the cottonseed meal pellets. However, the interaction between bloodline and supplement intake was statistically significant for live weight gain and greasy wool production. This indicated that the efficiency with which cottonseed meal was used for live weight gain and greasy wool production differed between the bloodlines. To further elucidate the interaction, regression coefficients relating greasy wool production and live weight gain with supplement intake were calculated for a representative sample of bloodlines. With the exception of one of the bloodlines, the efficiency of greasy wool production (g greasy wool/pellet intake category; see Chapter 12) increased linearly with greasy wool production.

The efficiencies with which the cottonseed meal supplement was used for greasy wool production and live weight gain were unrelated. That is, bloodlines that had a similar efficiency of greasy wool production did not necessarily have a similar efficiency of live weight gain. A difference between the bloodlines in the partitioning of nutrients between wool and other tissue groups may account for these observations. However, care needs to be taken when considering the efficiency results because the total intake (i.e. pasture plus supplement) of the various bloodlines was not estimated. Nevertheless, the studies presented in Chapter 13 indicate that further investigation of the genetic variation in wool growth efficiency between fine-wool Merino bloodlines is warranted.