

Chapter 7 Effect of Nutrition on Plasma Metabolites and Hormones in Merino Sheep Selected for Divergent Clean Fleece Weight

7.1 Introduction

Genetic selection has created Merino flocks with superior fleece characteristics and different body traits. It normally takes 3 – 4 years for animals to realise maximum fleece yield (Brown *et al.* 1966), but sheep designated to enter a breeding flock are normally selected at or before 18 months of age. Gene-markers have potential as a method for identifying superior animals at an earlier age (Purvis and Franklin 2005). Although gene-markers for important traits may be potentially an accurate means of identifying genetically superior animals, they are not always cost effective tools (Abbott *et al.* 2006). A number of studies have shown there are associations between different physiological markers (plasma hormones and metabolites) and wool or body traits (Williams 1987; Hough *et al.* 1988; Hegarty *et al.* 2006). As an alternative to gene markers, physiological markers that are related to improved fleece productivity could be used for early identification of genetic merit for fleece production. To be useful, however, any marker has to be easily and quickly determined and relatively inexpensive. It also needs to be highly repeatable, non-invasive and most importantly, highly correlated with genetic merit for the relevant production trait.

In order to identify potentially useful physiological markers, Merino sheep selected for high (F+) and low EBVs for wool growth (F-), but with similar EBVs for fibre diameter and liveweight were screened for differences in plasma concentrations of several metabolites (total protein, glucose and urea N) and hormones (insulin, insulin-like growth factor 1 (IGF-1) and leptin).

7.2 Materials and methods

7.2.1 Animals, diets and treatments

The sheep and feeding regimes are described fully in Chapter 4, Section 4.2.1. In brief, 20 castrated male sheep were selected from a commercial flock, 10 sheep with high estimated breeding values (EBVs) (F+) and 10 sheep with low EBVs for wool

production (F-). Animals were given 1.2 M (1.2 times their metabolisable energy (ME) requirement for maintenance) for 4 wks. After 4 wks acclimatisation, 5 sheep from each EBV group were offered a ration of 0.8 M and the other 5 from each EBV group were offered 1.8 M for 5 wks.

7.2.2 Sampling and measurements

Blood samples were collected before the 5-wk feeding period commenced (Day -2), and again on Day 37. Briefly, jugular blood was obtained by venipuncture and collected into sodium-heparinised tubes immediately before animals were offered their daily ration at 0900 h. All blood samples were centrifuged at 1500 g for 10 min and plasma from these samples was stored at -20 °C until plasma metabolites and hormone concentrations could be determined.

7.2.3 Laboratory analysis

The total protein concentrations in plasma were measured using a Dupont Dimension[®] XL Clinical Chemistry System combined with an Auto-analyser (Dade Behring Diagnostics, QLD) using the Total Protein Flex[®] reagent cartridge kits (Cat. No. DF73). These kits are designed for human diagnostic purposes and have a linear range of 2 – 12 g/dL. Serial dilutions of ovine plasma exhibited parallelism with the standard curve and internal recovery of added standards (6 pooled results) averaged 105 %. Intra-assay coefficients of variation averaged 6.5 % and all samples were run in duplicate in one assay.

Plasma glucose concentrations were determined in duplicate by enzymatic analysis (Kunst *et al.* 1983) using hexokinase and glucose-6-phosphate dehydrogenase, with measurement of the formation of NADH photometrically at 340 nm (Dupont Dimension[®] XL Clinical Chemistry System combined with Auto-analyser-Dade Behring Diagnostics, QLD). The intra- and inter-assay coefficients of variation were both < 5 %.

Urea N concentrations in plasma were determined by the diacetyl monoxime method using an Autoanalyser (Technicon Ireland Ltd) (Marsh *et al.* 1965).

Analyses of plasma insulin and IGF-I concentrations were conducted in outsourced laboratories (insulin: CSIRO Laboratory, Perth, Australia and IGF-I: University of

Western Australia, Perth, Australia). Insulin was measured in duplicate by the double-antibody radioimmunoassay (RIA) method of Bassett and Wallace (1966). Mean concentrations used for low-, medium- and high-quality controls were 4.0, 9.0 and 49.5 $\mu\text{U/ml}$ (micro units per ml), respectively. The intra- and inter-assay coefficients of variation were 10.1 % and 1.02 %, respectively, and the minimum detection limit was 1.09 $\mu\text{U/ml}$.

The assay for insulin-like growth factor-I (IGF-I) was carried out on duplicate 100 μl aliquots as described by Breier *et al.* (1991). The minimum detectable concentration was 0.28 ng/ml. The intra- and inter-assay coefficients of variation were both < 5 %.

To determine plasma leptin concentrations, a competitive Enzyme-Linked Immunosorbent Assay (ELISA), previously validated for sheep plasma (Kauter *et al.* 2000) was used. The sensitivity of the assay was 0.5 ng/ml and the inter- and intra-assay coefficients of variation were both < 10 %.

7.2.4 Statistical analysis

Data were analysed using the generalised linear model (GLM) of GenStat 8.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) for a balanced 2×2 factorial experiment. There were two feeding levels (0.8 M and 1.8 M) and two genotypes (F+ and F-). Data were normally distributed.

Initial measurement of any metabolite or hormone concentration (Day -2) was used as a covariate. The initial fitted model included genotype, feeding level, and the two-way interaction associated with these factors and the covariate effect. In those analyses where the covariate effect was not significant ($P > 0.05$), the above model was refitted with the covariate term omitted. The significance level was set at $P < 0.05$ and a trend or tendency was defined as $0.05 \leq P < 0.10$. Unless stated otherwise, the results (mean \pm standard error of difference of mean) were adjusted by covariance for initial measurements. One of the F- sheep from the 1.8 M treatment became ill during the study, and was treated as a missing value in all analyses.

7.3 Results

7.3.1 Concentrations of total protein, glucose and urea N in plasma

Results of plasma concentrations of total protein, glucose and urea N are summarised in Table 7.1. Plasma total protein ($P < 0.01$) and glucose ($P < 0.001$) concentrations were higher in animals fed 1.8 M than those fed 0.8 M, and F+ sheep had lower plasma total protein ($P < 0.01$) and lower plasma glucose ($P < 0.01$) concentrations than F- sheep. Plasma urea N concentration did not differ ($P < 0.05$) between feeding levels or between genotypes.

7.3.2 Concentrations of insulin, IGF-I and leptin in plasma

Results of plasma concentrations of insulin, IGF-I and leptin are given in Table 7.2. The sheep that were offered the 1.8 M ration had higher plasma insulin, IGF-I and leptin concentrations than those offered 0.8 M ($P < 0.05$), but these concentrations did not differ between genotypes ($P > 0.05$).

The relationship between plasma IGF-I concentration and eye-muscle depth was analysed further by regression analysis. Differences in slopes and intercepts between genotypes or feeding levels were not significant and therefore, a common slope and a common intercept were applied. The linear regression equation of fitted model was:

Eye-muscle depth (mm) = 12.9 (± 1.00) + 0.06 (± 0.010) \times IGF-I (ng/ml), ($n = 19$, $r^2 = 0.68$, $P < 0.001$).

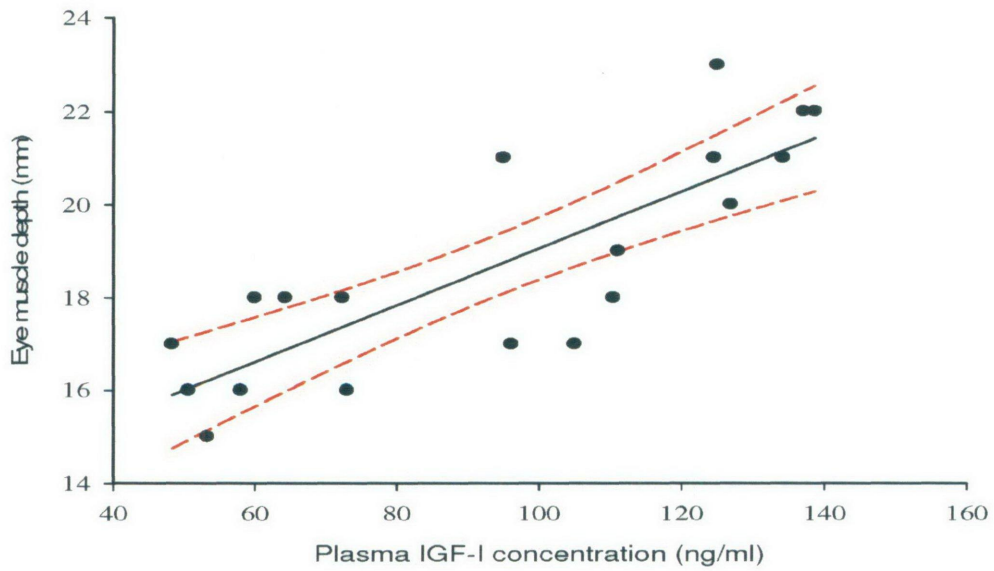


Figure 7.1 The relationship between plasma IGF-I concentration and eye-muscle depth in Merino genotypes with high (F+, n = 10) and low (F-, n = 9) estimated breeding values for clean fleece weight when offered below-maintenance (0.8 M) and above-maintenance (1.8 M) rations for 37 d. The fitted regression line (—) and 95 % confidence intervals (-----) are shown

Table 7.1 Mean (\pm s.e.d.) plasma concentration of metabolites and hormones in Merino genotypes with high (F+, n = 10) or low (F-, n = 9) estimated breeding values for clean fleece weight when offered below-maintenance (0.8 M) and above-maintenance (1.8 M) rations for 37 d

Feeding level	0.8 M				1.8 M				P values		
	F +	F -	F +	F -	F +	F -	F +	F -	s.e.d.	Genotype	Feeding level \times Genotype
Total protein (g/dL)	6.36	6.76	6.75	7.2	0.181	0.004	0.006	0.826			
Glucose (mmol/L)	3.50	3.66	4.03	4.26	0.082	<0.001	0.005	0.508			
Urea N (mg/100 ml)	19.2	19.1	27.0	19.1	6.42	0.417	0.504	0.389			

Table 7.2 Mean (\pm s.e.d.) plasma concentration of hormones in Merino genotypes with high (F+, n = 10) or low (F-, n = 9) estimated breeding values for clean fleece weight when offered below-maintenance (0.8 M) and above-maintenance (1.8 M) rations for 37 d

Feeding level	0.8 M				1.8 M				P values		
	F +	F -	F +	F -	F +	F -	F +	F -	s.e.d.	Genotype	Feeding level \times Genotype
Insulin (μ U/ml)	6.0	6.4	11.8	8.3	2.60	0.033	0.597	0.215			
IGF-1 (ng/ml)	80.6	69.2	117.0	91.7	16.93	0.021	0.204	0.572			
Leptin (ng/ml)	8.6	6.9	13.5	12.7	3.39	0.037	0.576	0.858			

7.4 Discussion

The plasma concentration of several metabolites (total protein, glucose and urea N) and hormones (insulin, insulin-like growth factor 1 (IGF-1) and leptin) were analysed to identify physiological markers that might be correlated with higher fleece productivity. Concentrations of protein and glucose in plasma might have potential to identify sheep with high genetic merit for fleece production at an early age.

7.4.1 Plasma total protein and glucose

The F+ sheep had significantly lower plasma concentrations of protein than F- sheep at both feeding levels. Plasma contains three major proteins: albumen, globulin and fibrinogen. Amino acids absorbed in excess of immediate needs will be proportionally stored as plasma proteins (Riis 1983). Plasma proteins have important functions as sources of amino acids for tissue protein synthesis (Connell *et al.* 1997). It is not surprising to find that animals offered a 0.8 M ration had lower plasma concentrations of protein than animals offered a 1.8 M ration, but the reason for the differences in concentrations between genotypes is not known. The genotypic differences in concentrations of plasma protein may be due to: 1) a different rate of uptake and utilisation of free amino acids from the plasma by a range of body tissues, for instance, muscle and skin (see Chapter 5); and 2) different proportions of plasma proteins being used to release some amino acids directly into the circulation as opposed to uptake by adjacent tissues. Hough *et al.* (1988) found non-pregnant, non-lactating Merino ewes selectively bred for high clean fleece weight maintained 15 % higher levels of α -amino N in their plasma than did sheep selected for low clean fleece weight, but it is not known which amino acids contributed to the higher concentration of α -amino N (Williams 1987). Plasma total protein concentrations might be used for identification of genetic merit for fleece production, only if the results presented here were to be confirmed in a much larger sample of animals.

In the current study, F+ sheep had significantly lower plasma glucose concentrations than F- sheep at both feeding levels. Hough *et al.* (1988) examined the concentration of glucose in plasma collected from Merino sheep genetically selected for high or low wool production and found similar average levels of plasma glucose from the two groups across three feeding levels (0.8 M, 1.0 M and 1.3 M). Harris *et al.* (1993a) also

found there were no differences in the concentration of plasma glucose ($\mu\text{g/g}$ blood) between selected high fleece-producing Romney rams and unselected control rams. These workers also found there was no difference in the uptake of glucose by the skin ($\mu\text{g}/\text{min}$ per cm^2); however, the arterial glucose concentration was negatively correlated ($P = 0.06$) with wool production. Bermingham *et al.* (2004) studied the glucose production rate in Merino ewes from the Katanning flocks selected for or against fleece weight. They also reported that plasma glucose concentrations did not differ significantly between the two groups, but the high fleece-producing sheep had 28 % lower glucose production rate than the low fleece-producing sheep. In a subsequent study on the same animals, Adams *et al.* (2006a) found plasma glucose concentration was lower in the high wool-producing sheep. Even though genotypic differences in concentrations of plasma glucose have not been consistently observed (Hough *et al.* 1988; Bermingham *et al.* 2004; Adams *et al.* 2006a), the concentrations of plasma glucose combined with the glucose production rate might be a suitable marker for identification of superior genotypes for fleece production.

7.4.2 Plasma urea N, insulin and leptin

In the current study, we found plasma urea concentrations did not differ significantly in the F+ and F- sheep. Hough *et al.* (1988) and Williams *et al.* (1991) also found that Merino sheep genetically selected for and against high wool growth had similar concentrations of urea in their plasma and similar rates of clearance of urea and creatinine by the kidneys. McCutcheon *et al.* (1987) suggested similar concentrations of urea in the plasma of F+ and F- sheep might indicate that F+ and F- sheep had similar kidney function. Studies on Romney sheep, however, have consistently shown that sheep selected for high fleece weight have lower concentrations of urea in plasma than sheep from a randomly bred control flock (McCutcheon *et al.* 1987; Thompson *et al.* 1989a). The different responses to selection for fleece weight in Merino and Romney sheep indicate that the metabolism of urea varies between the two breeds (Williams *et al.* 1991). Therefore, plasma urea N concentration is not an ideal marker for identification of superior Merino genotypes for fleece production.

No differences in plasma insulin concentrations between F+ and F- animals were observed in the current study. In contrast, Adams *et al.* (2004; 2006a) observed that plasma concentrations of insulin were lower in sheep selected for high fleece weight.

They argued that the lower level of insulin was related to a lower availability of glucose, and suggested that animals with high wool-growth potential had smaller metabolic energy reserves to support their maintenance and production through harsh feed conditions. Secretion rates of insulin are sensitive to variations in nutritional level (Trenkle 1981; Prior and Smith 1982; Vandehaar 2005). Evidence for this was found in this study, i.e. animals offered the 1.8 M ration had higher concentrations of insulin than those given the 0.8 M ration.

In our study, the plane of nutrition affected plasma leptin concentrations, although we did not find any difference between F+ and F- genotypes. Leptin acts on the central nervous system to prioritise the use of available energy among tissues during periods of nutritional deficiency (Boisclair *et al.* 2006). Previous studies in sheep have also shown plasma leptin concentration was affected by fatness and to a lesser extent by plane of nutrition (Blache *et al.* 2000; Delavaud *et al.* 2000; Daniel *et al.* 2002). Our study confirmed leptin is involved in mediating the animal's responses to the level of nutrition (Zhang *et al.* 1994; Keisler *et al.* 1999), but suggest leptin is unlikely to be a marker for identifying F+ sheep.

7.4.3 Role of IGF-I

There was no difference in plasma IGF-I concentration between F+ and F- sheep. Previous studies have shown that IGF-I has no effect on wool growth (Cottam *et al.* 1992; Adams *et al.* 1996a; Adams *et al.* 1996b). The lack of effect of IGF-I on fibre production is further supported by the non-significant relationships between wool growth rate and plasma IGF-I concentration observed in this experiment ($r^2 = 0.15$, $P = 0.09$). It has been shown, however, the local administration of IGF-I to skin increased uptake of cysteine and the rate of utilisation of cysteine for protein synthesis (Harris *et al.* 1993b).

Nutritional status affects plasma concentration of IGF-I in ruminants (McGuire *et al.* 1992). For example, reducing feed intake decreased plasma concentrations of IGF-I in lambs (Pell *et al.* 1991; Oddy and Owens 1996). This is consistent with the results obtained from the current study.

Statistical analysis of the results from the current study indicated plasma IGF-I concentrations were significantly correlated with eye-muscle depth ($r^2 = 0.68$,

$P < 0.001$, Figure 7.1). There are reports that plasma IGF-I concentration is positively correlated with liveweight (Speck 1991; Mears 1995) and carcass lean weight (Cameron and Cienfuegos Rivas 1994; Hegarty *et al.* 2006). A possible explanation for the correlation between eye-muscle depth and plasma IGF-I concentration is as follows: when animals were offered the 1.8 M ration, circulating IGF-I concentrations were increased, promoting higher rates of muscle protein synthesis and consequently muscle protein gain; when animals were restricted to the 0.8 M ration, circulating concentrations of IGF-I were reduced and this led to greater reductions in muscle protein synthesis rate than in degradation rate and, as a result, muscle protein loss (Breier *et al.* 1986; Bass *et al.* 1991; Wylie 1995).

Our study suggests plasma IGF-I concentration was not correlated with higher fleece productivity, and so it is unlikely to be a useful physiological marker of superior fleece producing sheep. However, IGF-I may have potential value as a physiological marker to identify genotypes with potential for higher meat production.

7.5 Conclusion

No differences in plasma concentration of insulin, IGF-I, leptin and urea N were observed between F+ and F- sheep, but plasma leptin, insulin and glucose were altered in response to the level of nutrition. Genotype differences were observed in plasma concentrations of glucose and total protein. The lower concentrations of plasma glucose and total protein in F+ sheep probably reflect a higher efficiency of utilisation of circulating amino acids leading to higher genetic capacity for wool growth. Total protein and glucose concentrations in plasma might be used as physiological markers to enable genetically superior wool producing sheep to be identified. The IGF-I concentration was significantly related to eye-muscle depth, and so plasma IGF-I concentration might be useful as a selection marker of muscularity. The results indicate that selection for clean fleece weight does alter various physiological processes in the body.

Chapter 8 General Discussion

The Australian Merino industry is being continuously challenged to produce more high quality wool and at the same time to increase meat production. In response to this challenge, sheep breeders are starting to place more effort into trying to produce dual-purpose sheep by using selection indices which contain contributions from many traits weighted according to genetic parameters and economic value (Sheep Genetics Australia 2007).

Experiments conducted on Merino genotypes developed using single-trait selections for superior wool production (Williams 1987) or liveweight gain (Oddy *et al.* 1998) have provided excellent information on the physiological bases for genetic differences in wool or meat production (Section 2.1), but the physiological consequences of practical multi-trait selection of sheep are less clear. To provide information to help sheep breeders and producers to improve wool and meat turnoff simultaneously, the work reported in this thesis was undertaken to provide information on the processes by which sheep partition digested protein and energy between the competing needs for wool production and body growth at the whole-body and individual tissue level.

8.1 Implications of the first study

The first study reported in Chapter 3 was undertaken to test the suitability of two tracers for determining whole-body protein turnover in sheep. A plant protein source (duckweed), with its entire array of amino acids uniformly labelled with ^{15}N , and ^{15}N -glycine were compared as tracers. The results clearly demonstrated that both tracers were useful for comparative estimates of whole-body protein metabolism. It was decided to use ^{15}N -glycine for the second study on whole-body protein metabolism because of its lower cost and simplicity of application. Therefore, the initial aim of selecting a suitable tracer was achieved.

Before this study was commenced, some animal nutritionists had argued strongly that wool (skin) and muscle compete for the same amino acids in the blood pool; increases in the requirements of one tissue (e.g. skin) would inevitably decrease the availability of amino acids for other tissues (e.g. muscle) (Adams *et al.* 2000b). The results obtained from the current study do not support this. The ewes from the HHL group (selected for

high wool production and low liveweight) partitioned a greater fraction of abomasally administered ^{15}N to both skin tissue and wool, but partitioned a similar proportion of the injected ^{15}N to skeletal muscle as did the LLH group (selected for low wool production and high liveweight). There was no evidence of competition between skin and muscle tissues for absorbed amino acids. The results of the first study suggested that selecting sheep for traits (wool growth and body growth) that apparently compete for the absorbed amino acids resulted in different proportions of absorbed amino acids being directed to skin (wool) and muscle in the different selection lines.

The animals used for the experiment reported in Chapter 3 were derived from the Trangie QPLU\$ selection lines that were established to evaluate the consequences of long-term within-flock selection to simultaneously improve fleece weight and fibre diameter using a range of selection indices in a range of Merino strains (Taylor and Atkins 1997; Taylor *et al.* 2006). The sheep were selected for us by workers at Trangie and sent to us as distinct genotypes. However, when their genetic history was examined, the sheep turned out to be distinct phenotypic groups from a mixed genetic base, rather than distinctly different genotypes. They were therefore not ideal candidates for a study of how the partitioning of amino acids between wool and meat is affected by genotype.

Building on the experience gained in the first study, we chose two groups of wethers differing in Estimated Breeding Values (EBVs) for wool growth, but with similar EBVs for liveweight and fibre diameter for the second study described in Chapters 4 – 7. EBVs are calculated from a range of genetic parameters, derived from the animal itself for the measured trait, from other related traits, and from relatives, and are adjusted for non-genetic, i.e. environmental influences, across all measured animals in the industry (MGS 2004). EBVs make it feasible to select suitable genotypes that have combined genetic merit for traits such as high wool growth and liveweight. Sheep Genetics Australia has recently renamed EBVs as Australian Sheep Breeding Values (ASBVs) (Sheep Genetics Australia 2007) but the term EBVs is retained in this thesis.

8.2 Physiological consequences of multi-trait selection of sheep for high and low wool growth

There have been many F+ and F- comparisons published (see Section 2.1.1) where the terms 'F+' and 'F-' are used to denote animals with genetically high and low fleece production. However, in the many different studies conducted in NSW and Western

Australia, F+ and F- genotypes are not likely to be identical. The F+ and F- sheep have been selected using different criteria, and for different generation intervals. For instance, F+ and F- animals used in studies by Kahn (1996) and Williams (1987) were derived from single-character-selection flocks at the Trangie Research Station, NSW. The Trangie flocks were established in 1951 using medium-wool Merinos of the Peppin strain (Dun 1958) and have undergone many generations of selection. On the other hand, the F+ and F- sheep used in studies by Adams *et al.* (2005; 2006a) were obtained from the Katanning Merino resource flocks. The Katanning flocks were formed in 1981 using three strains of Merinos (Peppin, Collinsville and Bungaree) and were selected for traits such as meat, staple strength and fibre diameter (Lewer *et al.* 1992). Furthermore, none of these selection studies at Trangie or Katanning (Dun 1958; Lewer *et al.* 1992) have included replicated lines. Studies with mice selected for body weight have shown that the variability of response in replicated selection lines results from different underlying physiology (Falconer 1973).

Genetic selection for high fleece weight using different approaches over many generations would have increased the frequency of the major gene(s) responsible for higher wool production within the different F+ populations. However, the genes affecting wool production in one 'F+' population might well be different from those in another 'F+' population. Thus, it is possible that the improvements in wool growth resulting from different genetic selection approaches (Williams 1987; Adams *et al.* 2000b; Adams *et al.* 2006b) are controlled by different genes or combinations of genes under different environmental conditions and are achieved via different physiological mechanisms.

The F+ Merino sheep used in the experiments described in Chapters 4 – 7 were selected for superior wool production using a multi-trait EBVs selection. This approach combined phenotypic and genetic information but also applied selection pressure to maintain similar liveweight and fibre diameter. Results showed these F+ and F- sheep had the following physiological and metabolic differences:

1. More ingested protein and energy was retained in wool and body tissues in the F+ sheep.

2. Energy expenditure rate (MJ/d) was higher in F+ sheep, partly because more body protein was synthesised and degraded in F+ sheep; but F+ sheep had a lower EE per unit of metabolic liveweight (MJ/kg LW^{0.75} per d).
3. Both skin and muscle protein FSR (%/d) were higher in F+ sheep, but F+ sheep had a higher efficiency of protein deposition (protein retained/protein synthesised).
4. The efficiency of use of microbial amino acids for wool protein accretion (wool N accretion/flow of microbial N) was higher for F+ sheep.
5. Concentrations of glucose and total protein in plasma were lower in F+ sheep.
6. The F+ sheep had a higher S/P follicle ratio and straighter, deeper, and more even follicles; higher skin and protein mass per unit area of skin; lower skin collagen concentration; lower sebaceous gland scores; and a higher efficiency of methionine utilisation in the skin tissues.

In this study, the F+ sheep were superior to F- sheep for a number of production traits when fed below or above maintenance (Chapters 4 – 5). This superiority is illustrated by the composite traits (higher wool production), correlated traits (better body condition) and causative traits (higher protein synthesis rates in the epidermis, dermis and muscle). Superiority in wool production was also associated with a higher efficiency of conversion of protein and ME into wool and body tissues. There was no evidence of a ‘trade off’ between deposition of ingested protein and ME in the body and wool in the F+ sheep, which supports the view of Hatcher *et al.* (2004) that although ‘theoretical relationship between clean fleece weight and liveweight should exist, this does not occur in practice’. Fogarty *et al.* (2006) have argued that improvements in wool and meat production can be achieved concurrently by using appropriate selection indices – estimated breeding values (EBVs). The results obtained here provide evidence that gains in both traits are feasible through more efficient use of absorbed energy and protein.

8.3 Quantitative aspects of protein metabolism in high and low fleece EBVs sheep

The variations in tissue protein metabolism, the capacity of sheep to produce microbial protein and differences in wool protein accretion between genotypes under different nutritional conditions are illustrated in Figure 8.1 (data were obtained from Chapters 4

and 5). These data show the highly plastic nature of protein metabolism and provide vital information about how the animals synthesise and process proteins in different tissues in response to nutrient supply.

There were significant differences in protein synthesis and retention between genotypes. The F+ sheep deposited more of their whole-body protein synthesis in their body tissues than F- sheep, but the F+ and F- sheep used a similar proportion of the protein synthesised in the body for wool N accretion (approximately 6.1 %). These results indicate that F+ sheep were more efficient in depositing protein in body tissues but did not differ from F- sheep in their efficiency of wool protein accretion.

At the tissue level, the skin of F+ sheep accounted for 30 % of whole-body protein synthesis, whereas muscle represented about 26 %. In contrast, the skin of F- sheep represented about 25 % of whole-body protein synthesis and for muscle the value was about 23 %. Selection for wool growth not only increased the rate of protein turnover in the skin, but also increased protein turnover in skeletal muscle.

These results suggest that the relationships between amino acids supply and protein deposition in different tissues are not fixed because this deposition is modulated by variable rates of protein synthesis and degradation, and differences in protein turnover are also the reason for the variable energy costs of net protein deposition. The genotype-dependent differences in protein metabolism indicate the capacity of different genotypes to respond to different nutritional conditions for body and wool growth, as illustrated above. Practically, it might be difficult to measure protein metabolism in the field; however, one way to generate outcomes directly applicable to industry could be to study tissue protein metabolism in large numbers of sheep and combine the results with whole-genome screens using high-density single nucleotide polymorphism (SNP) analysis (Craig and Stephan 2005). These outcomes could be eventually used to guide breeding programs.

The wide range of potential mechanisms described in Sections 8.2 and 8.3 indicate that it should be possible to breed animals with the physiological capacity to utilise absorbed amino acids and energy substrates more efficiently for both wool and body growth.

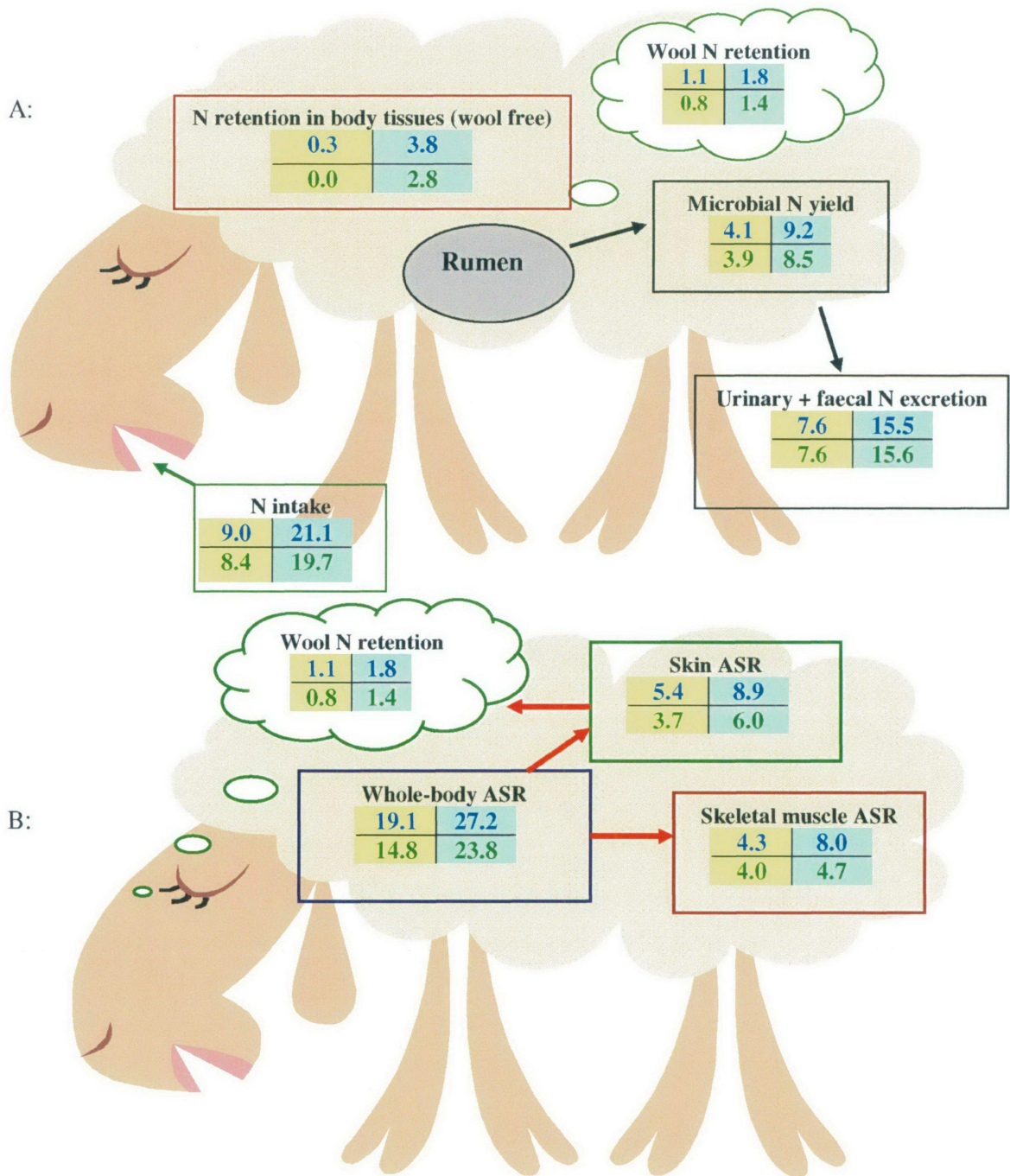


Figure 8.1 A) Whole-body protein intake and protein utilisation (g N/d) and B) absolute protein synthesis rates (ASR, g N/d) in different body tissues of Merino genotypes with high (F+) and low estimated breeding values for wool growth (F-) when offered below-maintenance (0.8 M) and above-maintenance (1.8 M) rations. Values listed in the order of:

0.8M	1.8M
F+	F+
F-	F-

8.4 Physiological traits related to the gross selection criteria, such as the annual wool production or liveweight

Annual wool production and liveweight have been used as single-trait selection criteria for decades. These are traits that producers are paid for, but they are only output variables; they do not include the efficiency terms that contribute to profit on farm. Physiological markers that include efficiency terms, i.e. efficiency of energy and protein utilisation for wool and body growth, need to be identified and included in selection indices. Williams (1987) claimed that no single physiological trait had been identified that could replace gross selection criteria such as annual fleece weight and liveweight. It is not surprising that Williams (1987) made such a statement because wool and body growth are regulated by genetic factors that are modified by environmental (nutritional, physiological, climatic) factors. If one factor changes, the others react to a greater or lesser extent and, as a consequence, wool and body growth rates will be affected.

Based on the results obtained from multiple regression analyses, we found that whole-body energy expenditure rate (Chapter 4), efficiency of utilisation of microbial N supply for wool N accretion (Chapter 6) and plasma IGF-I (Chapter 7) are genotype-dependent and could be used to increase the power of selection traits such as annual wool production or liveweight.

First, the strong relationship ($r^2 = 0.82$) between protein synthesis and energy expenditure rate at the whole-body level (see Section 4.3.4) could be used to identify genotypes that differ in protein synthesis and associated heat production, and would therefore achieve a particular liveweight and body composition with less feed intake.

Second, there were positive relationships between wool N production and rumen microbial N outflow rate, with a steeper slope for F+ than for F- sheep (see Section 5.3.1). These relationships suggest that if microbial N outflow rate increases by one unit, say, 1 g N/d, the F+ sheep will produce 0.06 g more wool N/d than F- sheep. Kahn (1996) found the yield of microbial-N from the rumen of sheep from the Trangie fleece selection lines was greater in F+ sheep than in F- sheep, and a higher efficiency of utilisation of microbial-N for wool growth accounted for approximately 80 % of the differences in wool growth rate between the selection lines. Taken together, our results and those of Kahn (1996) suggest that both single- and multi-trait selection for high

wool growth can produce genotypes that utilise absorbed amino acids more efficiently for wool production. Thus, it appears to be possible to breed lines of sheep with higher microbial yield per unit of feed intake (Kahn 1996) and higher efficiency of use of absorbed amino acids by both skin and muscle tissues (the current study).

Third, plasma IGF-I concentration explains 68 % of variation in eye-muscle depth (see Section 7.3.2). Results from our study and others (Cameron and Cienfuegos Rivas 1994; Hegarty *et al.* 2006) suggest that plasma IGF-I concentration could be used as a potential physiological marker to identify more productive meat-producing animals.

If quantitative measures of these physiological traits could be added to gross selection criteria such as the annual wool production or liveweight, then more selection pressure could be applied to alter the mechanisms responsible for superior wool- and meat-producing sheep.

8.5 Future studies

Further studies are needed to provide a better understanding of how physiological processes differ in different sheep selection lines. Selection tools using direct knowledge of genomic differences and their effects on dependent biological mechanisms need to be developed to enable the physiological knowledge gained in the course of studies, such as those reported in this thesis, to be used to improve selection indices. A particular opportunity exists to combine the detailed physiological measures of difficult-to-measure 'efficiency' traits, e.g. for wool and meat production, with whole-genome, high-density, single nucleotide polymorphism (SNP) analysis and thereby to generate associations between genes and their function that can be used for direct selection of breeding animals. Studies of physiological differences between genotypes possessing economically valuable traits such as those discussed in this thesis would then have a direct route for delivery to industry.

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