Understanding physiological drivers of resilience in sheep

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Abstract

Grazing sheep undergo seasonal variation in body condition due to changes in pasture growth and nutrient availability. Anecdotal evidence from producers suggests that during these times, individual variation exists in ‘resilience’ to these sub-optimal conditions. These ‘resilient’ individuals are able to maintain health and productivity whilst flock mates are unable to. There is little empirical evidence to verify these claims, yet they resound from producers in a wide range of production environments. The aim of this doctorate study was to define ‘resilience’ in sheep under sub-optimal nutrition, and investigate the physiological causes of variation in the trait.

Three mechanisms to facilitate ‘resilience’ were proposed, namely that resilient sheep a) commenced the period of nutritional stress in better condition, B) have greater energy intake, or C) have lower energy expenditure when under sub-optimal nutrition. Two separate experiments were conducted to investigate these hypotheses under indoor and outdoor environments. From the results of both experiments, average daily weight gain (ADG) was the most appropriate measure of ‘resilience’, as it encapsulated hard to measure changes in adipose tissue depots, muscle and organ mass.

Investigation of the effect of body condition and weight on subsequent response to sub-optimal nutrition (Mechanism A), revealed that a sheep’s initial body condition did not dictate ‘resilience’ to the nutritional treatment. This implies that although body reserves can serve as a useful energy reservoir, differences in efficiency do exist.

Investigating the role of feed intake (Mechanism B), revealed a 1.4kg range in voluntary feed intake, which was associated with a 25% range in energy digestibility. As a result of these two factors, daily digestible energy intake accounted for 48.3% of the variance in ADG measured.
In the evaluation of Mechanism B, the use of heart rate (HR) and oxygen pulse (Oxygen uptake pet heartbeat) measures to calculate energy expenditure was evaluated. Sixty eight and 92% of the variance in oxygen consumption could be accounted for in experiments 1 and 2 respectively. Body weight, individual sheep differences and the measurement environment all contributed significantly to the variance but most importantly, HR only had a small effect, thus implying that it can be used to oxygen consumption in the field for a given individual. The use of external logging devices to record HR of sheep whilst grazing was then evaluated in experiment 2. A combination of animal movement, rubbing activity, dust accumulation and lanolin and suint content of the fleece and skin between the electrodes and skin contributed to difficulties in measurement. As a result no continuous long term measures of HR were possible. These difficulties may explain the lack of published literature on the use of the monitors in sheep when compared to those using cattle and goats as experimental subjects. Future attempts to use HR as a proxy for energy expenditure in sheep will require novel methods of logging HR which avoid these constraints. No measures of energy expenditure or thyroid hormone activity were correlated with resilience in this study.

Under the *ad libitum* low quality feeding regime implemented in experiment 2, the gross digestible energy intake consumed had the greatest effect on average daily weight gain (ADG) or ‘resilience’ to nutritional stress. Whether these differences were due to differences in appetite control or rumen microbe populations could not be determined in this study. Further investigation into the causes of individual variation in digestible energy intake and appropriate means of selection is warranted.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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1.1 Introduction

As one of the world’s leading producers of sheep meat, Australia’s sheep meat industry is valued at $3.8 billion (Meat and livestock Australia 2014). The global trend towards increased consumption of animal products is predicted to increase the demand for sheep meat by 2.2 million tonnes (carcase weight) by 2022 (OECD/FAO 2013). In order to meet this demand, Australian production needs to increase from 350,000 tonnes (carcase weight) per annum. The ability for this demand to be met is limited by the fact that the Australian sheep flock has decreased from 170 million head in 1990, to 72 million head in 2014 (Meat and Livestock Australia. 2012). As a result, the breeding ewe flock has significantly diminished. The demand for lamb has seen prices remain high and lamb slaughtering favoured over growth of the national flock. This has set a trend where ewes are being kept in production for longer and fewer ewe lambs being kept to replace them. Thus, in order to attempt to meet the demand for sheep meat products within the constraints of low ewe numbers, the efficiency of the animals used in production needs to be increased (Brosh et al. 2007).

Optimising the production from ewes involves the maintenance of a suitable body condition from year to year throughout the production cycle (Vatankhah et al. 2012). One of the major constraints on the ability to maintain body condition is variability in the production environment as a result of seasonal fluctuations and uncharacteristic weather events such as drought (Chemman et al. 2009).
Variability in energy availability throughout the year produces a seasonal pattern of changes in the body composition of sheep in these systems (Ball et al. 1996; Azevedo et al. 2009). During periods of high feed availability, sheep will have an excess of energy and thus be able to deposit muscle and fat. During times of feed restriction sheep will have to mobilise their body reserves as an energy source. This ‘phenotypic plasticity’ is commonly observed in wild mammals when faced with seasonal variation in feed availability, with differences reflected in subsequent reproductive performance (Pelletier et al. 2007). Thus, fat reserves are particularly important during times of feed shortage to provide sufficient energy for pregnancy and lactation, even when seasonal reproduction is matched to best meet those requirements (Wylie et al. 2011).

Poor nutrition during late pregnancy and/or in early post-natal periods can have a permanent effect on the reproductive potential of a ewe for the rest of her life (Gunn et al. 1995; Morgan et al. 2007; Martin et al. 2010; Seijan et al. 2010). Poor nutrition increases the incidence of anoestrous (Seijan et al. 2010), reduces oocyte quality, conception rates (Vatankah et al. 2012) and embryo survival (Boland et al. 2001). Poor peri-conception nutrition can also reduce the number and quality of oocytes in their female offspring at sexual maturity (Abecia et al. 2013). Increased perinephric and retroperitoneal fat deposition may also be invoked in the offspring (Munoz et al. 2013). This increase in adipose deposition will affect carcase yields of slaughter lambs and may have a flow on effect on the reproductive potential of ewes retained in the breeding flock (Bocquier et al. 1993; Seijan et al. 2010). Thus to ensure maximal production and the longevity of ewes within the flock it is imperative that the condition of sheep is managed to ensure they have sufficient condition to remain productive, yet not so much that they become impaired by excessive body fat stores.

Anecdotal evidence from producers suggests that during “tough” times, there is variation between individuals in their ability to maintain condition and thus productivity, often
colloquially termed ‘do-ability’ or ‘resilience’. There is however, little empirical evidence to verify these claims. Whilst supplementary feeding practices alleviate the pressure of reduced feed availability, differences between individuals in their body composition are often still evident (Chemman et al. 2009). If these observed differences are due to genuine differences in efficiency, selection for more “resilient” individuals will not only simplify flock management, but increase the efficiency of the production system as a whole. Determining the degree of variation in ability to maintain body composition during times of feed restriction will be conducive to improving efficiency of production systems.

Whilst the simple solution would seem to be select animals which have proportionally lower weight loss, studies by Rauw et al. (2010), Rose et al. (2013), Walkom et al. (2014) reported variable, and generally low heritability estimates (0.04-0.3) for the weight loss. The solution posed by Walkom et al. (2014) is to select for greater weight and fat within the ewe flock, capitalise on feed supplies when they are there and then utilize body reserves during ‘tough’ times. Although this recommendation is readily applicable, the underlying physiology which allows the greater fatness and weight during good times is not understood. Similarly, Rose et al. (2010) conceded that the animals used in their study were fed ad libitum, and thus differences in intake and grazing behavior were not accounted for when looking at the range in weight loss. Although there is scope for a change in genetic propensity for weight change by selecting on weight differential alone, the underlying physiology facilitating this variation is still not well understood. Furthermore, the isolation of pathways enabling greater efficiency may allow more targeted selection and greater genetic progress to be made in the specific traits that will ultimately affect the degree of body weight loss.

This doctorate study was proposed in the context of Mediterranean production systems of Southern Australia, where pasture growth is minimal from December to April/May. Although
residual feed stocks extend the grazing period, animals face periods of time from 2-4 months where feed supply is less than required for maintenance of body condition. This literature review investigates the potential sources of variation in physiology and metabolism that may lead to differences in animal performance during these periods of sub-optimal nutrition. Existing evidence for variation within and between genotypes in the components of energy pathways are discussed and conclusions drawn about potential mechanisms facilitating individual variation in resilience.

1.2 Energy metabolism in the sheep

In order to determine the potential for selecting on resilience as a trait, the underlying physiological mechanisms that may cause individual variation need to be investigated. The main driver of growth and production is energy availability, and thus, the various pathways of energy flow in the animal must be addressed. An animal’s total energy balance can be encapsulated simply by the following equation:

\[
\text{ENERGY RETAINED} = \text{ENERGY INTAKE} - \text{ENERGY EXPENDITURE}
\]

For the purposes of this study, the primary trait of focus is energy retained as adipose and muscle tissue. If energy intake and expenditure can be quantified, any differences observed in the ability to maintain condition, or retain energy, will be able to be explained.

Considerable research has gone into the formulation of feeding standards for sheep, with the National Research Council published ‘Nutrient Requirements of Sheep’ (1985) being one such
example. These guidelines will form the basis of review and highlight the avenues for differences in energy requirement.

The gross energy intake consumed by an animal is not indicative of the actual energy availability to the animal (NRC 1985). As feed is digested, a proportion of the gross energy intake is lost in faeces, urine, and as methane to give the metabolisable energy (ME) intake (Figure 1.1). ME is the most commonly used value for expressing the true feed value and requirements of ruminants. However, further losses are also incurred in the digestion processes, after which the net energy (NE) content can be inferred. This provides the most refined feed value for a given feedstuff.

Figure 1.1: Partitioning of food energy in animals. Energy losses shown in orange (McDonald et al. 2006)
1.2.1 Variation in gross energy intake

The rate of gross feed (and thus energy) intake is largely determined by the physical capacity of the gastrointestinal tract, particularly the rumen. Although rumen size/weight is thought to increase proportionally with the size of the animal (Clauss et al. 2007), variation in rumen volume between sheep of a similar body weight has been reported (Purser and Moir 1966). This may be genetic, or an effect of diet composition during development (Abou Ward 2008). Feed composition also affects intake, with neutral detergent fibre (NDF) content having the greatest affect, impairing intake by filling the gut (Pralomkarn et al. 1995). Bulky roughages high in NDF result in reductions in dry matter, organic matter, crude protein, total carbohydrate and energy intake (McDonald et al. 2002; Silva et al. 2004). The relationship between total feed capacity (rumen size) and diet composition are two main determinants of the rate, passage and total mass of ruminant intakes.

In addition to physical feed-holding capacity, differences in appetite can also lead to differences in gross feed intake. Appetite control is a complex process involving both hormonal and neuronal stimulation and inhibition of the hypothalamus (Sartin et al. 2010). Differences in the endocrine control of intake between individual sheep may allow them to respond differently during times of nutritional stress. Although the endocrine control of intake and metabolism is highly complex and involves the interaction of many hormones, leptin and ghrelin are two major hormonal peptides that have been identified as being determinants of the regulation of feed intake. As a result these hormones have been extensively studied in the field of ruminant research over the last 15 years.

Leptin is a 146-amino acid cytokine-like peptide primarily produced by white adipose tissue cells, brown fat, foetal tissues, mammary gland, the rumen and pituitary gland (Bado et al. 1998; Wylie 2011; Onda et al. 2013). Although involved in the reproductive axis (Miller et al.
Leptin expression increases 3-5 hours post-prandially, following the 0.5-1 hour post-prandial increase in plasma glucose level (Houseknecht et al. 1998; Marie et al. 2001). The presence of external adrenergic stimulation can also decrease leptin secretion (Houseknecht et al. 1998). Thus, the role of leptin in a range of stress and intake/digestion relation pathways, coupled with its response to insulin, Non-Esterified Fatty Acids and adrenergic stimulation highlight its significance in intake relationships, particularly on restricted or low quality feeds.

Differences in leptin levels and response to feeding have been observed in small populations of sheep, with certain individuals exhibiting higher leptin expression despite a similar level of total feed intake (Ban-Tokuda et al. 2008). Single Nucleotide Polymorphisms (SNPs) exist in both the leptin gene, and leptin receptor gene in both man, wildlife and livestock species (Nkrumah et al. 2004a; Schenkel et al. 2005; Suzuki et al. 2009; Anton et al. 2011; de Carvalho et al. 2012; Perez-Montarelo et al. 2013; Jing et al. 2013; McEvers et al. 2013). Positive associations between leptin SNPs and growth rate in sheep have been reported by Barzehkar et al. (2009); Tahmoorespur et al. (2010); Hashemi et al. (2011) and Hajihosseinlo et al. (2012). Evidence also exists for differences in metabolic activity of the longissimus dorsi muscle in sheep with different leptin SNP’s (Boucher et al. 2006). Thus, the relationship between leptin and intake, growth and nutritional status make it a useful tool for understanding differences in physiological state under sub-optimal nutrition.

Another hormone reported to be involved in the regulation of gross intake is ghrelin. An acylated peptide, Ghrelin is produced by the oxytic glands of the abomasum in ruminants
(Ozfiliz et al., 2011; Udom et al., 2012; Steinert et al., 2013). Additional production of ghrelin, albeit small, occurs in the immune cells of the kidney, heart and the hypothalamus (Cummings et al., 2002; Hosoda et al., 2003; Anderson et al., 2005). Serving as the endogenous ligand for the growth hormone secretagogue (GHS) receptor, ghrelin activates mitogen-activated protein kinase, nitric oxide synthase, AMPK and Akt pathways (Kojima et al., 1999; Udom et al., 2012). As a result, the main functions of ghrelin are stimulation of Growth Hormone (GH) secretion, stimulation of feed intake and depression of lipolysis (Takahashi et al., 2008; Tschop et al., 2000; Nakazato et al., 2001).

Ghrelin is the initial food intake signal, with circulating levels rising during a fast, and decreasing following re-feeding (Anderson et al., 2005). It is also believed to be involved in gastric motility along with gastrin and motilin (Ozturk et al., 2013). Evidence also suggests that ghrelin stimulates cortisol production and increases NEFA and phospholipid levels (Udom and Tanriverdi, 2013). Thus, given its intricate involvement in intake control, growth hormone secretion and lipolysis, ghrelin is likely to play a role in explaining variation in these parameters observed in sheep under sub-optimal nutritional conditions.

Variation in ghrelin secretion and action has the potential to be a cause of individual variation in inputs (feed intake) and outputs (growth rate) (Tahmoorespur et al., 2010). Associations have been found between ghrelin polymorphisms and milk fat and protein synthesis in buffalo (Gil et al., 2013), skeletal growth (Zhang et al., 2012) and feed efficiency in cattle (Sherman et al., 2008; Zhang et al., 2009) and wool growth in merino sheep (Ingham et al., 2011).

Ghrelin is an important hormone to consider in the control of growth of ruminants as it not only influences feed intake, but also the secretion of growth hormone and the resultant anabolism of skeletal and muscle tissue (ThanThan et al., 2010). Its role in gastric motility may also have implications on digestive efficiency and flow of digesta through the abomasum. Differences in
ghrelin production and/or sensitivity may have large impacts on the feed conversion efficiency of livestock and is thus a useful measure to employ in metabolic studies of growth and efficiency.

1.2.2 Variation in Digestible energy intake

As shown in Figure 1.1, energy losses in faeces are subtracted from the gross energy intake to provide the DE value for a given feed. Greater faecal energy losses are incurred with lower digestibility feed, and thus the digestive efficiency of an animal is a major determinant in the ultimate energy availability (Wilkes et al. 2012). Intake/digestibility relationships are largely determined by feed composition, rumen morphology, and the extent of physical and microbial particle breakdown. The total flow rate of digesta through the tract affects the degree of fermentation within the rumen and degree of nutrient extraction (Hegarty et al. 2004). The extent of mastication of particles not only affects rate of intake, but also the surface area of particles available for digestion and the rate of saliva production. Lower intakes result in greater mastication, providing microbes a larger surface area for digestion, and increased buffering capacity due to a higher saliva output. Generally, an increase in intake for a given sheep will result in a decrease in particle mastication, increase in digesta flow rate and a decrease in microbial digestion, and thus overall energy digestibility (Hegarty et al. 2004; Kelly et al. 1993; Lourenco et al. 2010).

Genetic variation in gross intake and the resultant digestive efficiency is well documented. When consuming a low quality fibrous diet, studies by Mann et al. (1987), Lourenco et al. (2010) and Silva et al. (2004) showed that certain breeds increase overall intake at the expense of flow rate and digestibility to access sufficient energy. Conversely, Givens and Moss (1994)
Wildeus et al. (2007) and Wilkes et al. (2012) reported similar gross energy intakes, but greater digestive efficiency in comparison between breeds (Givens and Moss 1994). Thus, there is good evidence for differences in relationship between intake and digestibility between genotypes however the mechanism to achieve greater DE intake may not always be the same.

1.2.3 Variation in Metabolisable energy intake

Once the energy losses in faeces are accounted for, further energy losses occur in urine and via methane to provide the metabolisable energy intake value for an animal. NRC (1985) guidelines state that the combined energy losses to these two sinks can be estimated as DE x 0.82.

Energy losses in urine are dependent on the protein, roughage and oil content of the feed, but commonly expressed as 3-5% of the GE value of the diet consumed (NRC 1985). Thus, for conventional diets this value is often assumed to be constant across individuals. Little work has been published addressing the variation within and between breeds in the energy losses in urine.

Methane is produced by the micro-organisms in the digestive tract of ruminants, particularly the rumen and caecum (Blaxter and Clapperton, 1965). Energy loss from methane is of the order of 3-10% of the gross energy intake of an individual (Okine et al. 2004; Cottle et al. 2011). The total methane output is affected by the composition and volume of diet consumed, and the resultant microbial population colonising the rumen. During ad libitum feeding methane production increases with dry matter intake (Cottle et al. 2011), whilst at a maintenance level of feeding, higher digestibility feeds results in a proportionally higher level of methane production (Blaxter and Clapperton. 1965). Despite this, higher fibre, ‘low’ quality diets will also result in greater methane production per unit feed consumed when fed ad libitum (Benchaar et al. 2001; Cottle et al. 2011). In addition to the digestibility of the feed, unsaturated fat content and the presence of inhibitory components such as tannins or saponins can also reduce methane
energy losses (Sun et al. 2012). Thus, feeding level, frequency and composition all have an effect on the methane emissions of ruminants, hence the need to account for it in calculations of MEI.

Even when adjusted for intake level and composition, repeatable genetic variation exists in methane production (Blaxter 1962; Pinares-Patino et al. 2003). Chandramoni et al. (1998) reported breed differences between sheep in methane output, whilst lactating Holstein cows were found to have a CV of 18.9% between individuals and 11.5% within individuals for methane output, with a range of 278-456g/day (Garnsworthy et al. 2012). This highlights the potential between and within individual differences in energy loss to methane day-to-day. This is further validated by the inconsistent ranking of individuals in daily methane output, despite successful efforts to selective breed for reduced average levels (Pinares-Patino et al. 2003; Vlaming et al. 2008). The physiological explanation for individual variation in methane production is still not well understood (Pinares-Patino et al. 2003) and efforts are being made to develop accurate methods for identifying low methane producing individuals (Dehareng et al. 2012; Garnsworthy et al. 2012). Although direct selection for low methane production may be difficult, Hegarty and Waghorn (2011) suggested selection for low residual feed intake (RFI) individuals will simultaneously select for lower methane production per unit of gain. Thus in trials where methane production isn’t measured directly, measurement of RFI will aid in the identification of high methane production (per unit of production) animals.

Despite dietary composition and level of intake playing a significant role in methane production, there is potential for inherent genetic differences in methane output to occur, which may contribute to differences in animal efficiency via improvements in MEI.
1.2.4 Variation in heat increment of feeding

The final energy loss to be accounted for before the net energy value of a feed can be determined is the heat increment of feeding (HIF). The HIF is otherwise known as the energy cost of digestion and assimilation, with the heat produced from the oxidation of substrates in the digestive system to form the high energy phosphate bonds in Adenosine-tri-phosphate (ATP) (Baldwin et al. 1995). Typical values for the HIF of feeding in ruminants are approximately 9% of the total MEI (SCA 1990; Labussiere et al. 2009). As a general rule, HIF, and thus total HP increases with increasing level of energy intake (Labussiere et al. 2011; Lopez and Fernandez 2013). Thus, in studies of intake and energetic efficiency, HIF needs to be understood as it has the potential to have a significant impact on total HP.

Different nutrients have different heat increments, and thus when conducting experiments the relative components of the feed being consumed by animals need to be known in order to predict HIF accurately. In general, the HIF decreases from protein to carbohydrate to fat (Cock et al. 1967). The heat increment of feeding in ruminants is more complex than that of monogastrics in that it is affected by the relative proportions of volatile fatty acids produced by the fermentation in the rumen (Armstrong and Blaxter 1957). The efficiency with which energy is captured from acetate is somewhat lower than for butyrate and propionate, and thus there is greater amount of heat produced. Thus high-fibre forage diets incur a greater heat increment than high starch diets. Despite this, Orskov et al. (1991) showed that as acetate concentration increased, the total heat production decreased. This increase in acetate caused an increase in the time spent lying down by Friesian steers, thereby decreasing their overall energy expenditure, but not necessarily the HIF (Orskov et al. 1991) so care must be taken when interpreting heat production results. Little evidence exists for individual variation in HIF over and above differences in dietary composition. For animals at pasture, selective grazing behaviour has the
potential to alter the composition of intake and potentially the HIF and must be kept in mind when interpreting results.

1.2.3 Variation in net energy utilisation

Net energy consumed is directed into maintenance of body tissues and avenues of production (Figure 1.1) which together are both classed as forms of ‘energy expenditure’. There are a number of factors determining the extent of both maintenance and production avenues of energy expenditure which have potential to give rise to differences in energy retention, particularly under sub-optimal energy intakes.

1.2.3.1 Maintenance energy requirements of sheep

The maintenance energy requirement is defined as the energy required to maintain a steady physiological state with no change in the bodily energy content (Baldwin et al. 1995; McDonald et al. 2002). Although in production animals individuals are not usually in metabolic stasis as they are producing a ‘product’ of some form or other, the quantification of a maintenance component is useful to determine variation in metabolic efficiency (Moe 1992). Fifty percent of the total energy inputs for sheep production as a whole, and 60% of the energy inputs required for lamb production by the ewe is utilised to meet maintenance requirements (Dickerson 1978; Li et al. 2008). In some instances, the HIF is included with the fasting heat production (FHP) as part of the maintenance requirement. As HIF has been discussed in relation to NE intake, FHP will be discussed alone and treated as the predominant maintenance requirement.
FHP refers to the heat produced by an animal in a fasting state, in a thermo-neutral environment, at rest (Baldwin et al. 1995). As muscular movement and thus heat production cannot be eliminated during measurements in live animals, the term fasting metabolism is used (Blaxter et al. 1962). The energy requirements for FHP can be classified into energy required for service functions and cell maintenance functions (Church et al. 1988). Service functions include respiratory and circulatory work, nervous functions and the work of the liver and kidney (Church et al. 1988). These processes account for around 35-50% of the fasting heat production requirements (Baldwin et al. 1980). Cellular maintenance is comprised of ion transport, protein and lipid turnover, glycogen turnover, RNA and DNA synthesis, ketogenesis and urea synthesis (Church et al. 1988; Giles and Gooden 1993). Breed, sex, age, physiological state, plane of nutrition and body composition all affect the fasting heat production and contribute to variance in FHP (Baldwin et al. 1995). Thus, understanding of the FHP and potential for variation between individuals is crucial in studies of energy metabolism.

Increasing age results in a decrease in the FHP of animals (Freetly et al. 1995). As Merino wethers aged from one to six years, a 20% decrease in metabolic heat production was observed (Blaxter et al. 1962). Similar reductions in FHP from age 12-24 months in Water Buffalo were reported by Qin et al. (2011). When FHP of Texel and Suffolk ewes was adjusted for age, the HP: BW ratio was lower for Suffolk ewes, despite Texel ewes appearing to have a lower FHP when only weight was accounted for (Freetly et al. 1995). It is likely that this variation may be explained by differences in the rate of maturity, i.e. differences in the rate of maintenance functions as changes occur in muscular and skeletal growth and production (Church et al. 1988). Therefore when comparing FHP of animals their age must be taken into account before conclusions can be drawn regarding differences in FHP.
Level of nutrition has a major effect on the FHP of animals and needs to be considered when measuring FHP. Higher planes of nutrition resulted in in a 24-50% increase in FHP of pigs (Koong et al. 1982a; Koong et al. 1983), and 32% higher in lambs (Koong et al. 1982b) when compared to similar size and genotype individuals on a lower plane of nutrition. This increase in FHP is due to increased size and activity of metabolically active tissues, namely the stomach, intestines, kidney, liver and pancreas (Koong et al. 1982a; Koong et al. 1985; Rompala et al. 1991). These organs form a combined mass that amount to less than 10% of the body mass of most mammals, yet they receive 55% of cardiac output and thus account for around 50-60% of the fasting heat production (Church et al. 1988). Increased energy intake also correlates with an increase in blood flow through the portal vein in response to the increased level of nutrients from the portal drained viscera (PDV) (Kelly et al. 1993). Rompala et al. (1988) and Ferrel et al. (1986) fed iso-energetic diets of differing bulk volume to lambs, recording a significant increase in rumenoreticulum, omasum, abomasum, large intestine, kidney, heart and lung weight. However, this increase in organ weight didn’t correlate with a greater FHP (Ferrel et al. (1986); Rompala et al. 1988). Conversely, Noziere et al. (1999) refed previously restricted Lacaune ewes with a high energy concentrate diet, observing a 102 and 59% increases in liver and small intestine mass as a result of liver cell hypertrophy and small intestinal cell hyperplasia respectively. This dietary energy derived increase in organ mass also incurred increases in FHP. Thus it would appear changes in organ weight as a result of greater energy will increase FHP, whereas increase due to physical volume of feed ingested alone will not (Mcleod and Baldwin 2000).

Differences in the proportions of fat and protein in the body also have the potential to affect the fasting heat production of individuals and need to be taken into account when comparing individual FHP (Church et al. 1988). In adult animals, body weight change is generally a change in the amount of fat tissue in the body, whereas in growing animals, protein accounts for a
greater proportion of weight change (Olthoff and Dickerson 1989). Although fat is thought to be less metabolically active than lean tissue (Chesters et al. 1975), contradictory reports exist (Graham 1967; Blaxter et al. 1982). McNiven (1984) investigated the FHP of sheep at three levels of fatness (Fat, Medium and Thin), concluding that although fatness didn’t affect the efficiency of ME utilisation, the FHP production was 22% greater for fat than lean individuals. Conversely, Olthoff and Dickerson (1989) found that lean sheep had a higher FHP than their fat counterparts. Changes in body composition with weight loss due to restricted feeding will result in an initial decline in maintenance requirement, which will then stabilise as the ratio of fat:muscle decreases (Ball et al. 1998). Excessive adiposity may act to increase the FHP of sheep, but in general, increased muscle mass will incur a greater level of energy consumption.

When comparing FHP of sheep, particularly when they are under nutritional stress and mobilising body reserves, the relative proportions of fat and muscle need to be considered when explaining differences in FHP.

The presence of breed differences in FHP and the effect that has on total energy requirement are hard to define quantitatively, due to the other variables which may affect their energy consumption such as age, level of production, body composition, current and previous level of nutrition and environment (Koong et al. 1985). Nonetheless, studies have identified breed differences in FHP. Differences in FHP have been found between a range of beef and dairy cattle crosses (Ferrell and Jenkins 1984), British (Blaxter et al. 1966; Freetly et al. 1995) and Indian sheep breeds (Lopez and Fernandez 2011). Within breed differences may also be present, with Blaxter et al. (1962) observing differences of up to 10% in the fasting heat production of Merino wethers over several repeated trials and Rompala et al. (1991) reporting a 7.8% increase in fasting heat production of a performance selected Targhee sheep line compared to their unselected counterparts. The presence of such differences within breeds of up to 10% (Blaxter et al. 1962) and between breeds of up to 18% (Lopez and Fernandez 2011) may be sufficient.
to allow weight and condition advantages when feed supply is restricted. When measuring FHP, accounting for body composition and production differences is necessary to ensure differences are accurately realised. Nonetheless there is good evidence to suggest that between and within breed variation exists in FHP.

The FHP of animals is often thought of as a constant value of ME for maintenance. Evidence now points to numerous factors which can affect this value, and thus it must be treated as a dynamic variable dependant on genotype, body size, age, level of production, body composition and recent feeding level (Labussiere et al. 2011). In order to identify variation in FHP between and within genotypes, these factors need to be accounted for in calculations of FHP.

The energy cost of activity through feeding, locomotion and rumination all contribute to the total heat production of an individual (Herd et al. 2004). Activity level and its associated energy costs can be influenced breed (Beker et al. 2010), temperature (Bueno and Ruckebusch 1974), the time of day (Tovar-Luna et al. 2011) and season (Beker et al. 2009). The energy cost of rumination, standing and lying are considerably less than eating and walking, hence animals in a grazing situation have been shown to have a 8-16% higher EE than those indoors (Blaxter et al. 1966; Osuji et al. 1974; Lachica et al. 1997; Brosh et al. 2006a; Aharoni et al. 2009; Labussiere et al. 2009; Kaufmann et al. 2010). In grazing situations, feed supply and available grazing area will affect activity level and when conditions are sub optimal, differences in grazing activity have great potential to affect total EE.

Within and between breed differences in activity level may contribute to significant differences in EE, and then ultimately retained energy. Activity level differences have been reported to account for 5-10% of the variation in RFI of cattle, with number of strides taken per day the predominant source of variation (Richardson et al. 2000; Herd et al. 2004). Variation in stride length has also been attributed to a 20% lower energy cost due to activity between breeds.
(Aharoni et al. 2009; Aharoni et al. 2013). Even if speed of travel and stride length are similar, the actual time spent foraging can differ between genotypes and result in differing levels of energy intake (Beker et al. 2009; Beker et al. 2010). In grazing situations, particularly when feed supply is restricted or animals may have to travel to harvest feed, there is potential for differences between breeds, and between individuals within breeds to vary in time spent foraging exist and need to be considered when assessing differences in efficiency.

1.2.3.2 Net energy utilisation for production

Net energy available to the animal after the maintenance requirements have been met are able to be utilised for growth of the animal itself, reproduction, lactation and fibre growth. With the focus of this research being adult ewes, primary changes in growth of the animal will be through changes in the proportion of adipose and muscle tissue rather than skeletal muscle. In the case of hogget ewes, or production systems where lambs are mated at <12 months of age then the energy utilisation for growth is an additional consideration. In young sheep there are several phases of growth, during which the relative deposition of fat, muscle and bone change (Searle et al. 1972). However, during the post-pubertal phases of growth, the energy requirements for gain were constant irrespective of age or body weight (Searle et al. 1972; Blaxter et al. 1982). This finding has been shown in other studies of sheep (Rompala et al. 1991), goats (Tovar-Luna et al. 2007a) and cattle (Gomes et al. 2012; Schiavon and Bittante 2012). Thus in the context of adult ewes, differences in efficiency of gain are unlikely to contribute to differences in the maintenance of weight.

The relative proportions of fat and muscle deposition can differ in the adult sheep and incur different energy costs. The energy required to deposit protein is $190.9 \pm 36.3$ MJ ME/kg protein,
whilst for fat is 42.7 ± 14.9 MJ ME/kg fat (Rattray et al. 1974). Efficiencies for protein gain are generally in the region of k=0.15-0.2, whilst for fat around k=0.6-0.8 (Olthoff et al. 1989). Despite the higher calorific requirement for protein deposition, lean tissue is only about 20-22% protein, thereby reducing the energy requirement/kg lean tissue gain considerably to 45.8 ± 8.7 MJ ME/kg muscle (Olthoff et al. 1989). Identifying the relative changes in adipose and muscle tissue allows an approximation of the amount of energy a given individual has retained.

The energy requirements for reproduction in sheep relate mainly to energy required for growth and development of the foetus (McDonald et al. 2002). Provided ewes are in good body condition at mating, energy requirements during mating and the first 70-80 days of gestation remain similar to the maintenance requirement of the ewe (NRC 1985). During the second half of gestation the energy requirements of the foetus significantly increase, and thus the requirements of the ewe increase to 1.5 to 2 times maintenance (NRC 1985). The increase in energy deposition related to foetal growth at days 100, 120 and 140 of pregnancy has been shown to be 0.27, 0.55 0.92 MJ/day for singles and 0.57, 0.93 and 0.93 MJ/day for twins (Rattray et al. 1974). Although multiple births increase the energy requirement considerably, they do not seem to affect the efficiency of energy utilisation (Tovar-Luna et al. 2007c).

Breed differences in energy retention for reproduction appear to arise based on the previous selection environment of the breed. For example, Scottish Blackface ewes (a hill breed selected for general resilience) exhibited no reduction in lamb birth weight compared to Suffolk ewes (a lowland breed selected for lean tissue growth) when nutrition during gestation was restricted (Rooke et al. 2010). Ewes selected for production in rangeland conditions exhibited a similar resistance to decreased lamb birth weight when compared with ewes that were from conditions where nutrition was always adequate (Vonnahme et al. 2006). A subsequent investigation of breed differences in nutrient partitioning by Ashworth et al. (2011), revealed no difference in
the placental efficiency between these breeds. These results indicate that the partitioning of energy towards foetal growth is prioritised in some breeds, at the expense of the ewes body condition, to ensure that lamb growth and development isn’t compromised (Rooke et al. 2010). Therefore, when studying the energy metabolism of reproducing ewes, the relationship between energy retention in the foetus, and that by the mother is not always the same, and thus needs to be quantified in order to accurately determine RE. In production systems where the number and size of lambs weaned can be accounted for, the relative change in ewe condition can be adjusted for based on their additional nutritional load.

The energy requirements for lactation incorporate milk, fat and protein requirements during the course of lactation period post-partum. The ewe lactation is relatively short (peak lactation is 1-2 weeks post-partum), but energy requirements often far exceed the physical intake capability of the ewe (Alvarez-Rodriguez et al. 2012). The inability of the ewe is to meet her energy requirement through nutrient intake alone during early lactation invokes a period of negative energy balance where body fat reserves are primarily mobilised to provide energy (Weber et al. 2013a). Thus, body condition pre-lamning has a direct effect on the energy availability for lactation, and determines the extent to which body condition is lost (Weber et al. 2013b). Variation in energy use for lactation is driven by lactation length, total milk yield, and milk composition, and as such is largely dependent on factors such as genotype, litter size, nutrient availability and body condition (Bizelis et al. 2000; Charismiadou et al. 2000; Alvarez-Rodriguez et al. 2012; Rocco and McNamara 2013).

Between-and-within-breed variation in level of milk production, and thus variation in energy requirements for lactation exist. Sheep breeds such as the Lacaune, East Friesian, Assaf and Awassi have been selectively bred for milk production, and as such have a higher lactational output than other breeds which have been selected for their meat and/or fibre production.
Length of lactation can also vary from 2-8 months, depending on genotype and suckling load (Komprej et al. 2012). Litter size increases the lactation output, via increased suckling load. Thus when assessing variation in the energy requirement for lactation and its resultant impact on body tissue reserves, the genetic merit for milk production, litter size and pre-partum body condition, and metabolic adaptations to negative energy balance need to be taken into account (Rocco and McNamara 2013).

The energy requirements for fibre (wool) growth in sheep are reported to be low, with estimates ranging from 4-20% of the maintenance requirement (McDonald et al. 2002). Wool growth is still responsive to nutrition level, generally exhibiting a linear response to digestible organic matter intake and thus energy level (Whiting and Slen 1958; Walker and Norton 1971; NRC 1985; Thomas et al. 2007). Dietary protein level, and particularly the availability of sulphur containing amino acids also constrains wool growth (Reis 1967; Reis and Sahlu 1994; Hynd and Masters 2002). When sheep are fed above maintenance, the increase in wool growth does not necessarily continue to increase with energy intake, and is constrained by the genetic potential of the individual (LiWei et al. 2009). The efficiency of energy utilisation for wool growth has been shown to vary within and between strains of Merinos with high-fleece weight estimated breeding value sheep exhibiting a higher efficiency of energy utilisation for wool growth and lean tissue accretion (Li et al. 2008). Differences in efficiency of energy utilisation seem to be unaffected by diet quality, and arise as a result of the individuals genetic potential for wool growth (Dunlop et al. 1966). Thus, when accounting for the energy requirement of wool growth in comparative studies, level of intake, dietary protein level and day length are all factors which may contribute to variance in wool growth, and thus the energy retained.
1.3 Conclusion

In order to understand differences in sheep resilience under sub-optimal nutrition, all avenues of energy metabolism need to be investigated. This will allow the parameters which vary between individuals to be isolated and their effect on resilience determined. Using the NRC feeding guidelines as a basis to partition the various components of energy metabolism, the extent of variation within each component, and the evidence for within and between breed differences has been discussed. The greatest variation in performance under sub-optimal nutrition is likely to be derived from an increased energy intake, either by greater gross energy intake or greater digestive efficiency. Alternatively, differences in energy expenditure, either via a lower metabolic rate and/or activity level, may also facilitate greater levels of retained energy.

From the findings of this review, three testable hypotheses have been formulated to explain variation in resilience of sheep under sub-optimal nutrition:

1. Resilient sheep commence the period of nutritional stress in better condition
2. Resilient sheep have greater energy intake when under sub optimal nutrition
3. Resilient sheep have lower energy expenditure when under sub optimal nutrition
2. Review of methods for measuring energy utilisation in grazing sheep

2.1 Introduction

Having reviewed the components of physiology which may facilitate differences in resilience of sheep under nutritional stress, this chapter will investigate the various options for measuring each component of energy metabolism.

Most nutritional and metabolic studies of the past have been undertaken indoors in a controlled environment. While this provides controlled conditions that allow experimental procedures to be carried out with ease, it appears that once animals are placed outdoors in a production environment any differences observed between individuals indoors often disappear, or animals will re-rank in their performance (Lawrence et al. 2012). Thus, when researching variation in animal performance (phenotype, P), it is not only the genetic potential of an individual (G), but also the way they respond to their environment (G x E) that needs to be addressed. It would therefore appear that conducting trials in field situations, using techniques that allow measurement of physiological parameters outdoors is the only way that will enable accurate estimation of variation in performance (Moule 1965).

This review utilises the equation: ER= EI-EE, along with the hypotheses proposed in the previous chapter as a basis for addressing the available techniques for measuring energy metabolism in the field. Firstly, methods for measurement of energy retention are discussed, as this is the primary measure of robustness and the trait of interest. Accurate measurement of energy retention also allows the first hypothesis, that sheep that commence a period of sub-optimal nutrition in greater condition simply utilise those reserves to remain productive, to be evaluated. Secondly, the techniques available to measure energy intake are evaluated,
addressing the second hypothesis, that greater energy intake facilitates greater energy retention. Thirdly, the available techniques to measure the energy expenditure by sheep are discussed, thereby allowing the third hypothesis of lower energy expenditure facilitating greater energy retention to be tested.

2.2 Estimation of energy retention in sheep

When conducting studies of variation in efficiency and the change in body composition throughout the year, determining the ratio of fat: protein gain is crucial in understanding changes in body composition of sheep (Graham et al. 1991). Understanding the change in composition change with age is also crucial, for example, when body weight exceeds 25kg, weight loss in adult sheep comprises seven times as much fat as protein (Searle et al. 1972; Landau et al. 2006). Once the total gross change in mass of fat and muscle is predicted, the literature values for lean tissue and fat gain (45.8 ± 8.7 and 42.7 ± 14.9 MJ/ME/kg respectively) can be used to calculate the total energy retained by an individual (Rattray et al. 1974; Olthoff et al.1989).

2.2.1 Use of the serial slaughter technique to measure changes in body composition of sheep

The serial slaughter technique involves slaughtering animals at various ages throughout the experimental period, examining their composition and then taking a regression of this data to examine relationships (Searle et al. 1988; Giles et al. 2009). The main disadvantage to this method is that it doesn’t allow the growth of an individual to be measured over time as they mature or face different nutritional treatments. This doesn’t allow the variation between individuals, nor their change in composition throughout the production cycle to be quantified
accurately. Giles et al. 2009 quoted this method as also labour intensive, expensive, involves the use of large numbers of animals. Thus, the serial slaughter technique is not suitable for quantifying changes in body composition of grazing sheep throughout seasonal fluctuations in feed availability.

2.2.2 Use of real time ultrasound scanning to measure changes in body composition of sheep

Ultrasound scanning works by converting electrical pulses to high-frequency sound waves which travel through tissues and reflect from the boundaries of tissues which differ in their density (Stanford et al. 1998). Ultrasound can be used to measure the fat and muscle depth/area in specific locations of the body to give an overall indication of the fat: muscle ratio of an individual in a non-invasive manner. Ultrasound scanning in sheep is usually undertaken at the 12\textsuperscript{th}/13\textsuperscript{th} rib to the right hand side of the spine over the longissimus dorsi muscle, with fat depth and depth of the eye muscle or longissimus dorsii muscle the two primary measurements (Orman et al. 2010). In order to gain a better idea of whole body composition, Ripoll et al. (2010) measured longissimus dorsi width and depth as well as subcutaneous fat at the 10\textsuperscript{th} to 11\textsuperscript{th}, 12-13\textsuperscript{th} thoracic vertebrae and the first to second and third to fourth lumbar vertebrae. The results of this study did not show any one of these sites being the optimum for prediction of body composition (Ripoll et al. 2010). Similarly, Kelly et al. (2010) measured at the third lumbar vertebrae and P8 rump site in cattle to gain an idea of subcutaneous fat and muscling, whilst the measurement between the 13\textsuperscript{th} rib and 1\textsuperscript{st} lumbar vertebrae gave an indication of the kidney fat and thus the internal body fat levels (Kelly et al. 2010). Numerous other studies have used ultrasound to measure backfat depth and eye muscle area with ease and precision (Emenheiser et al. 2010a; Emenheiser et al. 2010b; Lambe et al. 2010; Moreno et al. 2010; Orman et al. 2010).
Ultrasound does have a tendency to underestimate *longissimus dorsi* depth and overestimate subcutaneous fat depth (Leeds *et al.* 2008; Ripoll *et al.* 2010). Lambe *et al.* (2010) found poor correlations between ultrasound measure and carcass characteristics in beef cattle throughout the finishing period, potentially attributed to this. The frequency of the ultrasound may need to be adjusted to get a clear image and increase the accuracy of measurements. Ripoll *et al.* (2010) altered the frequency from 8 to 10 MHz for fat depth measurements and lowered the frequency to 7 MHz for muscle depth, although other studies have used frequencies as low as 3.5MHz to good affect (Orman *et al.* 2010). Emenheiser *et al.* (2010b) conducted a study to validate the ultrasound technique and concluded that the method is appropriate for estimating carcass composition provided trained, consistent technicians carry out the measurements. A review of body composition measurement techniques by Stanford *et al.* (1998) stated that for quick and easy results, ultrasound was the most preferable method for estimating lamb body composition. The portability and comparatively low cost also makes this method appealing (Emeheiser *et al.* 2010a).

2.3.3 *The use of X-ray computed tomography (CT) scanning to measure changes in body composition of sheep*

CT scanning involves the firing of radiation pulse through the subject and measuring the rate of attenuation of X-rays, which allows calculation of the cross sectional density (Stanford *et al.* 1998; Giles *et al.* 2009). The use of CT scanning allows accurate measures of the total body composition of sheep to be made whilst the sheep remains alive. CT scanning has been used as a diagnostic tool in human medicine for decades (Hollo *et al.* 2007). However it has also been used to accurately predict the total body composition in pigs (Giles *et al.* 2009), sheep (Macfarlance *et al.* 2009) and calves (Hollo *et al.* 2007).
Despite its apparent accuracy, Hollo et al. (2007) stated that CT scanning was too involved and difficult to apply in commercial breeding selection practices, primarily due to the size of the machine and the time taken to process individual animals. However, the authors did concede that measurement of carcasses could aid in progeny testing and thus selection methods (Hollo et al. 2007). The New Zealand sheep industry has been employing just such tactics to aid in selecting for increased muscling and leanness with considerably greater affect than ultrasound scan predictions (Jopson et al. 1995; Young et al. 1996; Young et al. 1999; Kvame et al. 2004). Despite logistical difficulties for measurement of commercial animals, the CT scanning technique is a viable option for application to scientific research as it gives an accurate picture of the body composition and allows animals to remain alive so that repeated measures over time can be made. Purchasing a scanner does incur considerable cost, although second hand units are often able to be sourced from hospitals.

2.3.4 The use of dual energy X-ray absorptiometry (DEXA) scanning for measuring differences in body composition of sheep

Dual energy X-ray absorptiometry is a whole body scanning system based upon a three compartment model that divides the body into total fat tissue mass, fat-free soft mass and total body mineral content (Makkar 2008). Thus it is a useful tool for the measurement of body composition of livestock. This technique has been used successfully in the research evaluation of body composition of pigs (Scholz and Forster 2006; Collins et al. 2010; Losel et al. 2010), broiler chickens (Talaty et al. 2010), and sheep (Clarke et al. 1999; Dunshea et al. 2007; Ponnampalam et al. 2007).
Although accurate and relatively simple to undertake, the use of DEXA requires careful evaluation for use in pregnant animals due to the small amount of radiation involved (Makkar 2008). DEXA does have a tendency to underestimate abdominal and visceral fat when compared to CT scanning methods (Jensen et al. 1995) and provides lower resolution images of bone (Bansal et al. 2011). As for CT scanning, there are logistical and financial issues surrounding access to scanners, which may make it an unsuitable option for some research.

2.2.5 *The use of the tritiated water space technique to measure changes in body composition of sheep*

The tritiated water (TOH) space technique involves the administration of a radioactive isotope, tritiated water to sheep, allowing it to circulate within the body water pool after which time a single blood sample is taken. The specific activity of the serum provides the tritiated water space from the following equation:

\[
\text{Tritiated water space (L)} = \frac{\text{Dose TOH (µc) injected}}{\text{Specific activity of serum water (µc per L)}}
\]

This allows the total body water content to be determined by using the regression equation developed by Searle (1970a):

\[
\text{Total Body Water (kg)} = -0.01 + 0.92 (\text{TOH space}) \pm 0.40
\]

Once the TOH space has been calculated, the total body water, protein and fat contents can be calculated by using regression equations. There are a number of different equations that have been developed by different authors. Panaretto and Till (1963) developed equations for total body protein and % fat, which were then revised by Panaretto (1963) to increase the accuracy of estimates in extremely fat or thin animals.
Total body protein (kg) = 0.32(TOH space (l)) − 1.78 ± 0.009 litres

Fat (% LW) = 97.3 − 1.26(TOH space (% LW)) ± 1.32%

Searle (1970a) investigated the effect of measuring body composition with TOH space in growing lambs and published the following regression equations to predict body components for sheep of all ages from 3 days to maturity using TOH space and body weight:

Total body water = 0.01 + 0.92X ± 0.40

Fat = 0.16 − 1.14X + 0.95Y ± 0.64

Protein = 0.154 + 0.117X + 0.056Y ±0.28

Lean= -0.4 + 1.24X

Where X= TOH space (kg)

Y= body weight (kg)

These equations were then used in a subsequent study and found to accurately predict the body composition of sheep of varying genotype, age and parasite burden level (Searle 1970b).

This method allows non-invasive measurements of body composition based on their total body water content to be made repeatedly on an individual over time (Searle et al. 1988). The technique has been used to examine growth and body composition differences between breeds (Searle et al. 1982; Searle et al. 1988) and physiology of growing lambs before and during fattening when they reach maturity (Graham et al. 1991).

In order to obtain a measure of total body water, there are a number of procedural variations of this technique. Volumes of TOH injected into subjects vary from 200µc (Searle 1970a, 1970b).
to 400µc Panaretto (1963). The TOH extraction from serum is conducted by vacuum sublimation/lyophilisation and the specific activity of the serum water measured using a scintillation counter (Panaretto and Till 1963; Searle 1970a, 1970b). When conducting measurements of tritiated water space, adult animals must be deprived of feed and water for 24-48hr and young animals 15 hours prior to injection so as to minimise the diluting effects of gut water content (Panaretto and Till 1963; Searle 1970a). It is to be noted that TOH space does overestimate total body water due to exchange of labile hydrogen atoms in the animal (i.e. incorporation of atoms into body tissues) and water vapour loss during the equilibration period (Panaretto and Till 1963). This was overcome by Panaretto and Till (1963) by reducing TOH space by 3% live weight and by Searle (1970a) by 5.7 ± 0.3% bodyweight. Despite these slight overestimations, studies validating the technique with simultaneous serial slaughter techniques have shown that TOH space is highly correlated with total body water (0.4-0.8% difference) (Panaretto 1963; Panaretto and Till 1963). When calculating the TOH space, the length of a plucked staple of wool needs to be determined at the same time as the TOH infusion so that the fleece-free fasted live weight of the sheep can be calculated, increasing the accuracy of body composition predictions (Graham et al. 1991).

Once an appropriate method for administration of infusate is used, and appropriate regression equations developed, the tritiated water space method is suitable for the measurement of changes in body composition. Reservations exist around the procedure is very involved, labour intensive and prone to error via tritium losses in faeces and urine. The application of this technique for field trials, particularly on commercial properties may be limited due to the need for the use of radioactive isotopes. However, in a pure research context the technique has validity.
2.2.6 Summary of techniques to measure changes in body composition and energy retention in sheep

Measuring the energy retained by individuals can prove quite difficult, due to variation in ratio of muscle: fat gain and differences in tissue distribution throughout the body. The tritiated water space technique allows quantification of the total body water and thus fat content, which negotiates difficulties with measuring internal fat stores. There are however concerns regarding the use of radioactive substances in animals and the environment, which need to be considered when using this technique. Serial slaughter methods are not useful when looking at individuals body composition change over time as animals must be kept alive. Whole body scanning techniques (CT and DEXA) also allow differences in fat distribution to be highlighted, though there are logistical and financial issues surrounding the use of the scanners and transport of sheep to and from scanning equipment. The use of a ‘mobile’ scanning unit which can be used on-farm would make this a far more viable approach. Ultrasound scanning of live animals is much easier than whole body scanning methods, as the equipment is portable, easy to use and comparatively cheaper. Although total body tissue distributions are not attainable with this technique, it does allow subcutaneous fat reservoirs and loin muscle depth to be measured accurately, providing an indication of the change in body composition. For long term studies this is the most viable option. Ideally the inclusion of less frequent whole body scanning and slaughter at the end of trials will support and validate the results from regular ultrasound scans.
2.3 Methods for the estimation of energy intake in sheep

2.3.1. The insensible weight loss technique of measuring feed intake

The insensible weight loss (IWL) technique is based around frequent weighing of sheep before and after grazing periods with the measurement of urine and faeces produced, in conjunction with the calculation of an ‘insensible weight loss’ value. This IWL is the weight lost by means other than in faeces and urine, which is most likely due to water loss via respiratory evaporative cooling or panting (Penning and Hooper 1985). The difference between two live weights once faeces, urine and IWL are taken account of is said to be equal to mass of feed consumed Erizian (1932).

IWL may be affected by activity (walking>lying) and in particular the environmental temperature (Dumont et al. 1994). Thus if this technique is to be employed accurately measures need to be taken to adjust for these factors. To do this, Penning and Hooper (1985) developed a more complex IWL technique based on investigations of IWL in sheep housed individually indoors and grazing. In order to measure IWL in grazing sheep, ewes were fitted with equipment to record time spent grazing, harnessed so that faeces and urine could be collected and fitted with udder cloths so lambs could not access milk. Ewes were weighed (W1), placed in individual pens with their lambs and restricted access to feed. One hour later ewes were weighed again (W2) and returned to their paddock for 1h. No drinking water was available whilst grazing. Ewes were weighed again and harnesses, bags and udder cloths removed. Herbage intake rate was calculated using the equation:
\[ \text{Intake rate (g (min grazing))}^{-1} = \left( \frac{W_2 - W_1}{t_2 - t_1} - \frac{W_3 - W_2}{t_3 - t_2} \right) \frac{t_3 - t_2}{t_4} \]

Where:

\( t_{1,2,3} = \) times of weighings 1, 2 and 3

And \( t_4 = \) total time grazing recorded

This method produced results which were comparable to those obtained from the chromic oxide dilution technique, indicating that the technique should give a reasonable estimate of the herbage intake (Penning and Hooper 1985). Another advantage of the technique is that suitable estimates can be determined from 2-3 days of measurements. This short time enables the intake to be accurately measured on pasture that is growing quickly and thus rapidly changing in composition (Penning and Hooper 1985; Dumont et al. 1994). The fact that the measurements required for this technique are carried out on each individual, and not assumed to be the same as a subset of caged sheep means that quantifying the individual variation in intake is possible.

Although multiple measures of weight and grazing time can increase accuracy, the need to fit sheep with harnesses and equipment to record grazing time is a major disadvantage to this technique. The fitment and upkeep of these devices is time consuming and laborious, and interrupts the normal grazing behaviour of animals. In addition, extreme variation in ambient temperature leads to difficulties in correcting the IWL, which remains a source of error. This technique also doesn’t allow for composition of intake to be measured, and thus selective grazing behaviour will likely lead to differences in energy intake which will not be captured. Another disadvantage is that the procedure may only be carried out during dry weather, as rain
or heavy dew will soak the wool of sheep, increase their weight and thus overestimate the intake (Penning and Hooper 1985).

Although simple to implement, and doesn’t involve the administration of any substrate to the subjects, the effects of temperature on IWL and the inability to quantify composition of intake are the two main shortcomings of this technique. Simultaneous use of other techniques described herein, and adjustment of data for ambient temperature would reduce the effects of these inaccuracies. Regular disruption of sheep to remove faecal collection bags etc. may not be viable for implementation in extensive grazing systems research, though is of less concern.

**2.3.2 Using feed on offer (FOO) before and after grazing to estimate feed intake of sheep**

Total mob estimations of feed intake may be determined by measuring the feed on offer (FOO) within an area before and after a period of grazing. FOO is calculated by taking numerous pasture samples and determining the dry matter content in conjunction with measurement of sward height. The difference between these measurements can be deemed the total feed intake, which can be divided by the number of sheep in the mob to obtain an average individual feed intake value (Reeves et al. 1996). Another alternative method to this would be graze individual sheep small paddocks, perhaps with the use of Technograzing™, or other strip grazing techniques.

Although FOO removal may be applied on an individual basis, the considerable infrastructure and labour costs required are likely to render it impractical. Individual grazing may also prevent sheep from exhibiting normal grazing behaviour by imposing the competitive stress of not being in close proximity to flock mates. In addition, the accuracy of pasture removal estimation from a singular sheep may not be sufficiently accurate. Although, this value is relatively accurate on
a whole mob basis, it does not allow differences in intake between individuals to be determined. Thus, if individual variation in feed intake is to be determined, this technique is not suitable.

2.3.3 Measurement of faecal output to infer feed/energy intake

Total collection of faecal output of sheep can be undertaken over short periods of time with the use of collection devices. Collection devices may be a harness apparatus supporting a canvas bag around the posterior of the sheep, or a bag attached to a PVC ring glued around the base of the tail (Figure 2.1). In order to determine intake, average digestibility (from pasture samples) may be used to infer intake based on faecal output, or measurements need to be made on each individual sheep when fed a representative sample of pasture. Individual digestibility may also be determined in the field by the oesophageal fistulation or Nitrogen index method which will be addressed in sections 2.3.2.4 and 2.3.2.5 respectively (Cordova et al. 1978).

Figure 2.1: PVC ring apparatus for total faecal collection of grazing sheep (Hegarty 2012, Pers. Comm.)
Regular monitoring of sheep is required with the use of collection bags/harnesses in free ranging animals, as the risk of them rubbing or removing the apparatus on trees, fences etc. is high. (Cordova et al. 1978). Collection devices need to be emptied at least once, if not twice daily, so the potential for interruption of normal grazing behaviour is also great. The process of fitting of collection devices takes considerable time, particularly if larger numbers of animals are being measured (Cordova et al. 1978). Concerns have been raised over the collection apparatus affecting animal behaviour and physiology, yet many studies have not found such effects (Raymond et al. 1953; Greenhalgh et al. 1960; Arroquy et al. 2012). Despite its shortcomings, this technique is beneficial as it allows accurate estimates of faecal output and easy sampling of faeces for determination of individual digestive efficiency. Despite being a time-consuming process, with potential for disruption of normal animal behaviour, a well-monitored total faecal output measuring regime remains a suitable means of determining the throughput of feed material in grazing ruminants, which then allows the inference of individual feed and energy intake.

2.3.4 Visual observation of grazing behaviour to determine feed intake

Surveying the eating behaviour of individual animals and recording the amount of time spent feeding, ruminating and resting within a certain period of time is another method that may be used to infer level of intake (Pereira et al. 2013). This method serves an important role in studies where there is a large proportion of browse material of which the FOO cannot be easily determined (Gonzalez-Pech and Agreil 2012). In trials where supplements are being offered,
observation is a useful tool for characterising the proportion of time spent eating the particular feedstuff of interest (Pinheiro et al. 2012; Pereira et al. 2013).

This method is extremely labour intensive, requiring sampling over at least a 24 hour period (Figueiredo et al. 2013). It is difficult to get accurate measures on multiple individuals within a mob at the same time so the method is better suited to smaller subsets of animals. Determining the exact composition of intake may prove difficult in a grazing situation, especially if animals are to be left undisturbed. Although time consuming, visual observations do allow time budgets of grazing animals to be determined, and inferences made about the time and amount of feed consumed. Determining the exact composition and volume of intake is not easily undertaken with this technique, and thus it must be employed with other measures of intake to be valid.

2.3.5 Chromic oxide (Cr₂O₂) dilution technique to measure feed intake

The Chromic oxide dilution technique involves the daily dosing of animals with chromic oxide (Cr₂O₂), an inert and indigestible product that passes through the sheep unaltered (Lambourne 1957a). Animals are dosed at 9 and 15 hours of each day for 5-10 days until the concentration of marker within the body is consistent (Lambourne et al. 1957b; Lima et al. 2008; Silva et al. 2010). Grab faecal samples can then be collected daily and bulked over longer periods for each individual. (Lambourne 1957a). The ratio of Cr₂O₂ concentration in faecal samples relative to the known weight administered daily to the sheep allows calculation of the total faecal output (Coop and Hill 1962).

Once the total faecal output has been calculated, the energy content of the faeces, and feed consumed needs to be determined in order to calculate the MEI. The digestibility of herbage available to sheep can be determined in vitro from plucked herbage samples (Tilley and Terry
Including a period where total faecal collections are carried out on the sheep in the trial does allow for the accuracy of estimates to be validated (Coop and Hill 1962). This technique appears fairly versatile in that it has been used with success in cattle (Lima et al. 2008; Silva et al. 2010), sheep (Lambourne 1957a; Lambourne 1957b), poultry (Palander et al. 2010) and wildlife species such as turtles (Wang et al. 2011).

The most significant issue with this technique is the twice-daily administration of marker disrupting normal behaviour (Lambourne 1957a). The utilisation of long-release capsules to administer the $Cr_2O_2$ alleviates this problem (Nia and Wittenberg 2002). Compared to total faecal collection techniques, the grab-sampling of faeces is relatively un-invasive and of less concern than the administration of marker. Potential issues arise with feed quality affecting the rate at which the marker first appears in the faeces, as well as the rate at which it is excreted (Lambourne 1957a). This poses a risk in grazing trials where feed available will vary in physical form and chemical composition. In addition, selective grazing behaviour will lead to a different composition of intake compared to what would be expected from grab sampling (Lambourne 1957a; Lambourne 1957b). Regurgitation of the capsules containing marker poses another potential source of error and will result in overestimation of faecal output (Carter et al. 1960). Despite this, it has been said that the technique is resistant to inaccuracies as a result of variation in dry matter intake and is said to be more precise than other markers for estimating dry matter apparent digestibility (Rodrigues et al. 2010). Provided that the experimental infrastructure can easily facilitate regular, low stress handling of sheep, the chromic oxide solution is a potential option for measuring feed and energy intake.
2.3.6 Oesophageal fistulation to measure feed intake

First implemented by Claude Bernard in 1855 (Woji and Iji 1996). The oesophageal fistula is installed by surgically transecting the oesophagus and inserting a cannula (Harker et al. 1964; Soest 1994). When sampling occurs the cannula is replaced by a collecting bag so that the feed consumed by the sheep enters the bag, allowing a sample of ingested feed to be taken and analysed for composition (Soest 1994; Woji and Iji 1996). If several samples are taken throughout the day in conjunction with total faecal collection, the dry matter digestibility of the feed consumed and the energy content of the faces can be then used to calculate the intake of energy. Sampling times for grazing animals published range from 30 minutes (Bath et al. 1956) to 2–4 hours (Cook et al. 1958). It is imperative that sheep are trained to halters so that samples may be easily taken with minimal stress (Mee et al. 1996).

There are many issues that may arise using this technique, namely wound infection (Cook et al. 1958), shock, aspiration pneumonia, blockage of the oesophagus and leakage of digesta and saliva (Van Dyne et al. 1964; Mee et al. 1996). In addition, animals may not eat whilst the fistula is installed (Penning and Hooper 1985). To minimise disruption to grazing behaviour, samples may be taken either once or twice a day, although the extrapolation of such few samples to be representative of the feed intake during an entire day is has been questioned (Henley et al. 2001). The inherent risks associated with animals not eating and the potential for infection places too high a risk, particularly for experiments conducted over extended periods of time, as the likelihood of losing animals is relatively high. As a result, this technique is not suitable for extended period grazing trials.
N-alkanes are long-chain carbon molecules which are components of plant cuticular wax. The most common plant species are in the range of C_{25}-C_{35}, with nonacosane (C29), hentriacontane (C31) and tri-triacontane (C33) most prevalent (Mayes et al. 1986). N-alkanes are largely indigestible, thus their ratio in faeces relative to that in the ingested pasture allows the intake and digestibility of individual animals to be calculated. The rates of faecal recovery vary between the different alkanes, with the proportion of ingested alkane recovered in faeces increasing as carbon chain length increases (Mayes and Lamb 1984; Mayes et al. 1986). The proportions of different n-alkanes vary with plant species (Mayes et al. 1986) and stage of plant growth (Bugalho et al. 2001).

The technique has been widely used in grazing ruminants, and remains a popular method for estimating voluntary intake and apparent digestibility of free ranging animals (Brosh et al. 2006a; Chavez et al. 2011; Lawrence et al. 2012; Narvaez et al. 2012; De-Stefani Aguiar et al. 2013). In order to eliminate discrepancies in recovery between individuals, animals themselves can be dosed with n-alkanes of a similar length to those found in the herbage. This may be undertaken via daily dosing with pellets containing alkanes (Mayes et al. 1986), providing feed or lick-blocks containing alkanes (Elwert and Dove 2005; Charmley and Dove 2007) treating drinking water (Chavez et al. 2011) or pasture (Giraldez et al. 2006) with alkanes or by administering intra-ruminal controlled release devices (CRD) (Dove et al. 2002; Ferreira et al. 2004; Charmley and Dove 2007; Chavez et al. 2011). CRD devices are advantageous as they remove the need to handle animals on a daily basis and interfere with their normal behaviour and grazing patterns. Suitable n-alkanes are octacosane (C28) or dotriacontane (C32) as they are present in herbage at low levels and are readily available in pure form. Dosing with these alkanes allows the determination of herbage intake without the need for estimation of total
faecal output, making it an advantageous technique when compared to other marker methods (Mayes et al. 1986; De-Stefani Aguiar et al. 2013). Although ‘spot sampling’ of faeces may be undertaken when using dosed alkanes, optimum timing and frequency of sampling needs to be determined. De-Stefani Aguiar et al. (2013) determined that twice-daily samples, at 7:00 and 19:00 hours, over a five-day period was sufficient to enable accurate predictions of DMI.

Despite its extensive use, and efforts to reduce inaccuracies with supplemental dosing of alkanes, some concerns surrounding the accuracy of this technique remain (Lawrence et al. 2012). The main concerns surrounding the use of this technique are difficulties obtaining representative samples of the feed consumed, and ensuring that animals are dosed accurately (Charmley and Dove 2007). Thorough pasture sampling will reduce the inaccuracy of estimation, but the potential for selective grazing remains. Although daily dosing ensures accurate alkane administration, it interferes with normal grazing behaviour and activity. Administration through lick blocks, feed, water or pasture treatment all are limited by the fact that level of alkane ingestion isn’t accurately determined. CRD devices reduce the need for handling, and the accuracy of alkane intake determination is greater. The use of alkanes to measure feed intake does remain a feasible option for modern day studies of voluntary intake and digestibility in grazing ruminants.

2.3.8 Use of the nitrogen index method to estimate feed intake of sheep

The Nitrogen Index method was developed following the discovery by Lancaster (1949b) that faecal nitrogen excretion per unit feed intake of ruminants over a range of forages is relatively constant at 0.83 ± 0.102 g N/100g organic matter consumed (Arnold and Dudzinski 1963; Lancaster 1949a). The method is based on establishing regression equations between the dry matter digestibility of feed and the Nitrogen content of faeces once it has been consumed
(Wallace and Vandyne 1970). This is conducted by harvesting pasture and feeding it to sheep in metabolism crates so that total intake and faecal output can be measured (Benjamin et al. 1977). Once these equations have been established, a single faecal sample is all that is required from a given individual. The faecal nitrogen content can then be measured and intake calculated based on the previously determined relationship between faecal N and intake (Wallace and Vandyne 1970).

Subsequent studies have shown that seasonal and animal variations have significant effects on the faecal N/feed intake relationship (Arnold and Dudzinski 1963). Up to 90% of the variation and error associated with the faecal N/feed intake relationship was due to variation in the food consumed as a result of species differences, plant maturity and soil fertility (Minson 1958; Arnold and Dudzinski 1963). Thus, when the nitrogen index method is used, faecal N/feed intake relationships must be determined for each specific pasture to ensure accuracy (Odonovan et al. 1967). Other sources of error with this technique are the assumptions that the feed intake by grazing sheep is of the same composition as those of the penned sheep from which the regression equations are derived (Marten and Jordan 1967; Wallace and Vandyne 1970; Benjamin et al. 1977). The use of digestibility estimates derived for a small subset of the flock also removes the ability to identify individual variation in digestibility and intake. Similarly, assuming the digestibility of feed by penned and grazing sheep is the same may incur a degree of error to feed intake estimates (Wallace and Vandyne 1970). However, one of the major advantages to the technique is that there is little interference with sheep whilst they are grazing, thus allowing them to exhibit normal feeding behaviour. As a result, the nitrogen index method may be a useful tool in conjunction with other techniques to allow individual differences in digestibility to be determined, and thus measure feed and energy intake of grazing sheep.
2.3.9 The use of the body water turnover technique to estimate feed intake of sheep

The body water turnover technique is based on the discovery by Benjamin et al. (1977) that there is a relationship of 3.02 (±0.06) litres of water consumed to every 1 kilogram of dry matter consumed by sheep. In order to measure the flow of water into and around the animal, radioactive tritiated water (TOH) is used to determine the size of the total body water pool, the body water turnover and water drunk (Hyder et al. 1968; Benjamin et al. 1975). The protocol for measuring total body water content is exactly as described in section 2.2.5. The calculated weight of body water is then subtracted from the fasted live weight of the sheep to determine total body solids (Searle 1970a).

The total body water turnover of an individual is said to be equal to water obtained from feed in both preformed and metabolic forms, plus the water drunk. In order to calculate the feed intake of grazing sheep, this technique involves having some sheep in metabolism crates, fed picked samples pasture at the same time as sheep are grazing. The TOH turnover/24h needs to be calculated for four periods of 5 days in conjunction with water drunk/24h for the caged sheep. These measurements need to be carried out for two periods of ten days for the grazing sheep (Benjamin et al. 1977). Calculation of the difference between total water turnover and water drunk provides the water obtained from the consumption of fresh herbage, known as the ‘food water’ (Wallace and van Dyne 1970). The calculated food water of grazing sheep is used to estimate herbage intake using a regression equation developed from data for the caged sheep. Benjamin et al. (1977) found a high correlation (r²= 0.92) between food water and herbage intake in penned sheep, indicating the accuracy of the TOH turnover technique in estimating DMI.

There are a number of inherent inaccuracies associated with the application of herbage digestibility and water intake of penned sheep to those that are grazing. Selective grazing of
pastures will affect the water obtained from feed and lead to inaccurate estimates (Wallace and van Dyne 1970). This can be reduced with the use of a mono-species pasture over the summer period to reduce the effect of selective grazing and ensure relatively stable herbage moisture content (Benjamin et al. 1977). Temperature also has the potential to affect the water retention of sheep, and thus lead to error. As for measurements of body composition, the use of tritiated water in extensive grazing trials the use of the radioactive substance, and the implications of radioactive waste disposal and subsequent land use may be an issue. These can be overcome with the use of the stable isotope deuterium, however the cost of administration (>2000/test/head) will likely prove to be prohibitive.

2.3.10 Summary of techniques to measure energy intake

Estimation of feed intake at pasture is difficult, as the selective grazing nature of sheep makes difficult to determine the composition of intake. This issue pertains to the insensible weight loss, FOO before and after, visual observation, Cr₂O₂, Alkane and Nitrogen Index methods. Oesophageal fistulation negates this problem however the concerns for animal health and welfare, coupled with difficulties keeping fistula patent take it out of contention. When conducting trials with free-ranging animals, regular disruption of behaviour to administer measurement procedures can hinder the expression of normal behavioural patterns. The need for frequent weighing with the insensible weight loss technique and daily dosing of animals with Cr₂O₂ or Alkanes raises concern regarding the use of these techniques. The body water turnover technique also requires frequent handling, coupled with potential concerns over the use of radioactive substances, however yields accurate predictions of gross intake. If radioactive substance use isn’t of concern then this technique is extremely useful. Total faecal collection also requires frequent handling, and may affect the behaviour of sheep via
disruption/discomfort, however allows the total faecal output to be determined and a more accurate prediction of intake to be made. If the body water turnover technique is not a viable option then total faecal collection is the most suitable method for measuring intake.

2.4 Methods for estimating the energy expenditure (EE) of sheep

EE is conventionally calculated by measuring gaseous exchange in animals and relating it to the utilisation of ATP in the body. This is based upon the relationship between the consumption of oxygen and heat production, as described by Krebs during the 1950s (Blaxter 1989). Oxidation of nutrients within the body via the tricarboxylic cycle results in the production of CO₂ and Hydrogen ions. The hydrogen ions are involved in the transfer of energy to ATP within the cells mitochondria, and will finally combine with oxygen to form water. The heat produced is directly related to the amount of ATP produced during the breakdown of individual nutrients (Blaxter 1962; Giles and Gooden 1993). Thus, measurement of oxygen consumption and/or carbon dioxide production can thus be used to calculate the energy expenditure of animals. There are a number of methods which can be used to measure the respiration and gaseous exchange of animals and thus infer their energy expenditure.

2.4.1 Calorimetry chambers to measure energy expenditure

The conventional method of determining gaseous exchange and resultant energy expenditure is using the calorimetry chamber system (Brockway and Reid 1972; Lachica and Aguilera 2005). This involves measurement of the heat production (direct calorimetry) or gaseous exchange (indirect calorimetry) or animals whilst housed in a chamber. To ensure a stable, measurement
of EE, measurements should be carried out 4-5 hours after feeding to avoid effects of the heat increment of feeding (HIF) (Lachica and Aguilera 2005). This system provides as near an absolute ‘basal’ value of metabolic energy expenditure possible for an individual as the effects of environmental conditions and physical activity are minimised.

Although providing an indication of the true basal energy expenditure, this system has some disadvantages. Firstly, the lack of control over the animal when in the chamber has the potential for movement to lead to inaccurate measurement of EE. Secondly, variation in the length of time taken for the system to reach a steady state may affect accuracy of measurement of the gas exchange in the system may contribute to error (Lachica and Aguilera 2005). If intending to use calorimeter derived data for grazing studies of total EE, inaccuracies are often encountered as animals are in a restricted environment with stable environmental conditions (Lachica and Aguilera 2010). Temperature, humidity, solar radiation, feed quality and feed availability are all factors that will lead to an under or over estimation of the energy expenditure of sheep derived from calorimetry chambers (Shinde and Karim 2007). Whilst the use of calorimetry chambers provides an accurate measure of the ‘basal’ energy expenditure of sheep indoors in controlled situations, there are numerous factors encountered outdoors that will affect the energy expenditure of grazing sheep, and thus the accuracy of any estimates made. Whilst it may highlight differences in basal metabolism, the consequences of any differences may not be extrapolated accurately to field situations.

2.4.2 Tracheal fistulation for measurement of energy expenditure of sheep

The use of a fistula inserted into the trachea of sheep allows expired air to be sub-sampled at any given time, providing a CO₂ expiration rate, and thus inferring the oxidation of nutrients
and determination of the energy expenditure (Flatt et al. 1958; Young and Webster 1963; Whitelaw 1974). There are a number of different methods of fistulation that have been employed including those which occlude the cranial section of the trachea, preventing rumen CO₂ contamination Blaxter and Joyce (1963) to the use of re-entrant cannula with inspiratory and expiratory valves (Young and Webster 1963). Sampling is taken by bringing sheep in and attaching the fistula to a collection bag for a period of time or with remote gas meters. The use of remote gas meters is far more applicable to grazing sheep studies as it minimises interference with the normal behaviour of the sheep. The Max Planck gas meter has been used in numerous studies, either strapped to the animals back or pulled along behind it on a cart system (Whitelaw 1974). Regardless of the exact methodology, tracheal fistulation allows gas exchange to be measured in free ranging animals and the samples used to calculate EE.

Despite the ability to capture respired gas on an individual basis, and infer expenditure whilst in the environment, there are a number of potential issues surrounding the application of tracheal fistula. Simple fistulas are difficult to keep patent and invariably survival rates of sheep are low over extended periods of time (Blaxter et al. 1963; Brockway and Reid 1972; Whitelaw 1974). The use of re-entrant cannula does cause less respiratory distress and fistulas are more easily kept patent for extended periods of time (Young and Webster 1963; Whitelaw 1974). In addition, the use of remote gas meters have inaccurate accuracies of estimation as they are having to pull or carry the apparatus around, which undoubtedly interferes with normal behaviour (Whitelaw 1974). Although fistulation techniques exist whereby the health and comfort of the animal is optimised, there still is risk of infection and illness as a result of the fistula. Whilst the air collected at any time will give an accurate estimation of the energy expenditure at that time, the inaccuracies in energy expenditure brought about by having to bring sheep in to measure expired air or have them carry/drag gas meters around behind them reduced the effectiveness of this technique for use in grazing trials.
2.4.3 Measurement of VO$_2$/VCO$_2$ and heart rate to calculate energy expenditure of sheep

The oxygen consumed by mammals is transported around the body by the blood, and thus the rate at which it travels around the body is a function of the rate at which the heart beats. As a result, the relationship between heart rate and oxygen consumption for a given individual is strong. Fick’s convection equation summarises this relationship as follows:

\[ VO_2 = HR \times [Vs(\text{CaO}_2 - \text{CvO}_2)] \]

Where:

VO$_2$= Volume of oxygen consumed

Vs= Heart stroke volume/beat

\( \text{CaO}_2 \) = O$_2$ contents of arterial blood

\( \text{CvO}_2 \) = O$_2$ contents of venous blood

(Fick 1870)

As the rate of O$_2$ entry to the body is used to infer the rate of nutrient oxidation, the heart rate of an individual over time may also be used to infer oxygen consumption and thus calculate energy expenditure (Blaxter 1989). Variability in stroke volume of the heart (Vs), and the difference in extraction of O$_2$ by tissues (\( \text{CaO}_2-\text{CvO}_2 \)) may affect the relationship between HR and VO$_2$ (Brockway and McEwan 1969; Green 2011). However, provided these parameters are unique to each individual, and do not vary greatly within an individual, a suitable relationship between HR and VO$_2$ can still be determined (Webster 1967).
Once the measurement of the heart rate: \( O_2 \) intake relationship for an individual is determined under controlled conditions, measurement of heart rate alone is all that need be undertaken (Brosh 2007). These heart rate measurements can be used to estimate the \( VO_2 \) and therefore the energy expenditure of an animal whilst free ranging. Once the total oxygen consumption within a certain time period has been determined the energy expenditure can be calculated using the value of 20.47 kJ/L \( O_2 \) consumed (Nicol and Young 1990; Brosh et al. 1998; Aharoni et al. 1999; Barkai et al. 2002). Whilst this value is well established across a range of studies, Blaxter (1989) used the values of 19.7 -21.5 kJ/L \( O_2 \) depending on the animal’s fasted state or predisposition to deposit large amounts of body fat.

Numerous methods and theories to calculate and define the relationship between HR and EE have been published. These include simple linear or logarithmic (Webster 1967; Yamamoto et al. 1979; Richards and Lawrence 1984; Renecker and Hudson 1985; Purwanto et al. 1990) and pair-wise or ‘flex’ relationships, whereby the relationship differs for resting and exercising states (Brosh et al. 1998; Spurr et al. 1988; Achten and Jeukendrup 2003; Leonard 2003). To address the change in relationship as HR increases from resting to exercising states, polynomial equations (Beghin et al. 2000; Beghin et al. 2002) or the use of HR at a given time as a ratio of basal HR (Yamamoto 1989) have been used to explain the \( VO_2 : \) HR relationship. In grazing animal trials, the rare and infrequent occurrence of intense physical activity leading to an increase in HR means that these methods of calculation are not applicable to calculations of EE (Brosh et al. 2006a).

As the change in \( VO_2 : \) HR as animals enter an exercising state is not of concern in grazing animals, the ‘oxygen pulse’ (\( O_2P \)) method is an alternative for deriving the HR and \( VO_2 \) relationship. Proposed by Brosh et al. (1998), this method is based upon measuring the \( O_2 \) uptake per heartbeat (\( Vs(C_aO_2-C_vO_2) \) from Fick’s convection equation). \( O_2P \) has been shown...
to deviate between 1.8-5% from the whole day average in lambs, calves and goats, indicating short term measures may be indicative of each individuals O₂P value (Aharoni et al. 2003; Puchala et al. 2006). Once the O₂P value is measured over short intervals, it is then multiplied by the HR measured throughout over long term to estimate total oxygen consumption, and thus EE. When measured well, the O₂ pulse method delivers estimates of energy expenditure that are equal to, or exceed the accuracy of predictions based on regression equations (Brosh et al. 1998; Barkai et al. 2002).

Although not affected by exercise, there are a number of other factors that need to be considered when calculating O₂ pulse. When measuring the O₂P, measurement periods should be for 10-15 minutes (Brosh 2007) and multiple times throughout the day (Aharoni et al. 2003). To avoid effects of time of feeding or diurnal patterns of HR, the HR must be measured for 24 hours a day over at least 3 consecutive days (Brosh 2007). Data from excited or stressed individuals may be biased as HR increases at a proportionately greater rate than VO₂, and thus their O₂P and EE may be underestimated (Brosh et al. 2002; Brosh 2007). In addition, the productive/physiological state of the animal can alter the O₂P, hence the need for regular, individual calibration (Brosh 2007). The effect of temperature and thus heat load on O₂ pulse is varied, with Brosh et al. 1998, Aharoni et al. 2003 and Landau et al. 2006) reporting that ambient temperature did not affect the O₂P, whilst (Brosh 2007) found that increased blood flow to provide sufficient cooling under heat load caused a reduction in O₂P. Barkai et al. (2002) and Landau et al. (2006) reported differences in O₂P of 13% between indoor-and outdoor-housed sheep, with the lower value for those indoors attributed to less environmental variation. Dietary induced heat load does not seem to affect O₂P with Brosh et al. (1998) and Berhan et al. (2006) showing diet energy concentration had no effect. Conversely, Barkai et al. (2002) reported differences in O₂P attributed to differences in ME intake. Due to all of these potential effects, ensuring accuracy of the O₂P is critical for accuracy (Brosh 2007). Repeated
measures of $O_2P$ throughout the experimental period will further investigate these affects and allow for any adjustments required.

Assuming that $O_2P$ is a relatively stable parameter, differences in EE by animals are therefore largely a reflection of differences in HR. The behaviour of animals and the different activities performed by the animal incur different heart rates and thus energy expenditure, generally in the order of grazing $>$ idling $>$ ruminating (Palestrini et al. 1998; Barkai et al. 2002; Brosh et al. 2006; Landau et al. 2006). HR increases universally after feeding events and is also higher following consumption of higher quality feeds. The availability of pasture can also affects EE by affecting the time spent foraging (Lambourne and Reardon 1963; Corbett et al. 1971; Arnold and Dudzinski 1978; diMarco et al. 1996; Lachica and Aguilera 2005). Thus, as time spent grazing/ unit energy intake increases, it is possible for the heat increment to decline whilst the energy cost increases (Lachica and Aguilera 2005). However, Brosh et al. (2006) found that grazing time was at its greatest when feed availability was highest.

There are three main methods to measure $VO_2$ of animals, namely, respiration chambers, head hoods and face masks (Brosh 2007). Respired gas is collected and sent off for analysis or run directly from the collection vessel to a real time gas analyser. These techniques have been successfully used with cattle (Matsui et al. 1990; Brosh et al. 1998), goats (Matsui et al. 1990) and sheep (Palestrini et al. 1998) and are relatively straight forward in operation.

HR can be measured using a wide array of HR data logging devices. These may be surgically-implanted devices allowing long range wireless transmission of distances up to 800m (Wild et al. 1998), or remotely attached units with logging capability (Alive Technologies, Queensland). Remote measurement of heart rate is a useful tool as it allows normal physiological responses to be measured in real life conditions without interference from physical or chemical restraint (Wild et al. 1998). It also allows for 24 hour a day monitoring EE (Brosh et al. 2006).
work in this field was undertaken by Henderson and Prince (1914); Boothby (1915) and Krogh and Lindhard (1917), all of whom concluded that for large subjects, the HR method was superior to all other alternatives for measuring the EE of unconfined animals. To ensure the accuracy of EE experiments, it must be ensured that animals are comfortable with the experimental conditions (Boyne et al. 1981; Lachica and Aguilera 2005).

Whilst the calibration of $O_2P$ in conjunction with long term HR measurements allows the EE of free ranging animals to be calculated, it is useful to record the locomotion of animals simultaneously as measuring EE, so that EE for different activities and different times of the day can be applied. Direct observation has been the traditional method for determining the locomotion of grazing animals. Hughes and Reid (1951), Prieto et al. (1991) and Lachica et al. (1997) stated that the activity of a mob of animals could be inferred from close study of one individual within that group. However, if individual differences in grazing behaviour are desired, this method bares little use. With the advent of modern Global Positioning System (GPS) technology, the distances travelled by free ranging animals can be measured with ease and an EE value of locomotion calculated (Brosh et al. 2006; Buerkert and Schlecht 2009).

The HR: $\text{VO}_2$ relationship is well documented and based upon a sound physiological background. It has been widely used in a number of species to measure the energy expenditure over a wide range of terrains and climates (Brosh 2007). With the advancements in modern technology, namely the availability of compact HR monitoring systems and GPS, this technique is non- invasive and relatively simply to apply. As measurements are undertaken on an individual basis, it allows the variation between animals to be observed, which is crucial for studies concerned with variation in animal performance.
2.4.4 The use of the CO\(_2\) entry rate to estimate energy expenditure

Based upon the same principles as the O\(_2\)P technique, the CO\(_2\) entry rate method involves measuring the CO\(_2\) output of animals and then using it to calculate their EE. The CO\(_2\) entry method was suggested by Young (1969) and involves the use of an isotope dilution procedure to measure the rate of formation of CO\(_2\) in the body, rather than the rate of elimination by the lungs (Brockway and Reid 1972). Young et al. (1969) found that in grazing sheep there was a high correlation between the actual CO\(_2\) production and thus their energy expenditure.

It must be noted that feeding rate does have an effect on the CO\(_2\) production of animals (Brockway and Reid 1972; Sahlu et al. 1988) and because of this, they must be in a steady metabolic state for this technique to be successful, which this means that it cannot be used for measuring short term changes or variability in heat production (Giles and Gooden 1993). This technique does not provide an absolute measure of the CO\(_2\) production rate of an animal, rather it allows the determination of the approximate energy expenditure. (Brockway and Reid 1972).

There are numerous means by which this method can be applied in experiments. Brockway and Reid (1972) carried out this procedure by continuously infusing 14C labelled sodium bicarbonate in a sterile 0.9% NaCl solution containing 3-5mg and 3-5 µCi of labelled material/ml. Infusions occur under the skin near the transverse processes of the 5\(^{th}\) and 6\(^{th}\) lumbar vertebrae via a peristaltic pump at a rate of 0.03ml/min for 8-36hrs. Urinary catheters are inserted to drain urine continuously. Blood samples are taken via a jugular catheter when required. The specific activity of both blood and urine samples are measured and the CO\(_2\) entry rate calculated from the following equation:
\[
\text{CO}_2\text{ entry rate (mmole/min)} = \frac{\text{rate of infusion of activity (nCi per min)}}{\text{specific activity of CO}_2 \text{ (nCi per mmole)}}
\]

(Brockway and Reid 1972; Young and Corbett 1972)

Sahlu et al. (1988) used similar techniques, but developed the following equation to accurately predict the HP of fed sheep:

\[
\text{Heat Production (MJ per kg BW}^{0.75} \cdot d) = (4.39 \pm 0.13) \times (\text{Litres CO}_2 \text{ per kg BW}^{0.75} \cdot d + (13.91 \pm 2.86) \pm SE = 2.91
\]

Sahlu et al. (1988)

Sahlu et al. (1988) also used saliva to measure the CO\textsubscript{2} expenditure with success, providing another option for measurement. Young and Corbett (1972) stated that provided sufficient time is given to allow the infusate to equilibrate, it is not necessary to take multiple samples at exact times, as the specific activity of a blood sample at any given point will be representative of the CO\textsubscript{2} production of the tissues between the sites of infusion and sampling. Despite this, taking multiple samples over a period of time will give a more accurate estimate.

The different methods of employing this technique all incur their own margins of error or aspects of experimental difficulty. The subcutaneous infusion technique employed by Brockway and Reid (1972) lead to an accumulation of infusate under the skin and thus impaired dosage of labelled isotope. To avoid this, intra-peritoneal infusion was used by Corbett et al. (1971) and Sahlu et al. (1988), but issues ensuring catheters stay within the intra-peritoneal cavity exist. The consensus seems that jugular infusion methods are most appropriate (Brockway 1972). Infusion times reported vary, with Brockway and Reid (1972) reporting 8 hours of infusion was necessary to allow the radioactive sodium bicarbonate to equilibrate in
the body pool whilst Young and Corbett (1972) and Corbett et al. (1971) found that only 3hrs was necessary.

With the availability of small, portable peristaltic pumps for infusion the CO₂ entry rate technique appears an attractive method for measuring the energy expenditure of sheep in a relatively non-intrusive manner. The potential for variable intake to affect the CO₂ measured, coupled with potential risks associated with the infusion process do complicate the application of the technique. When measuring EE in a grazing situation, the accuracy of results and welfare of animals may become compromised by these issues.

2.4.5 Double labelled water technique to measure the energy expenditure of grazing sheep

The double labelled water (DLW) technique is a two isotope dilution method proposed by Lifson et al. (1955) and involves dosing the animal of interest with {\textsuperscript}{2}H\textsubscript{2}{\textsuperscript{18}}O. The relative elimination rates of {\textsuperscript}{2}H and {\textsuperscript}{18}O as H\textsubscript{2}O and CO₂ respectively are measured by sampling a body fluid (typically blood or urine), and the rate of CO₂ production determined (Lachica and Aguilera 2008). DLW is widely used in human studies (Jones et al. 1987; Gonseth et al. 2014), and has been successfully implemented in a range of other species such as fruit flies (Piper et al. 2014), birds (Buttemer et al. 1986), horses (Fuller et al. 2004), Caribou (Gotaas et al. 2000) and goats (Lachica and Aguilera 2008; Junghans et al. 1997 and Toerien et al. 1999.

Considerations when using the DLW technique include establishing a baseline measurement of {\textsuperscript}{2}H and {\textsuperscript}{18}O in the body, and considering the potential losses of hydrogen if bound to carbon and sequestered in fat, protein, carbohydrate (Lachica and Aguilera 2008) or products of digestion such as methane (Gotaas et al. 2000). Evaporative water loss in hot conditions may also lead to error (Junghans et al. 1997; Lachica and Aguilera 2008).
The DLW technique is best suited to long term studies, where various sources of hydrogen and oxygen loss can be accounted for and multiple samples taken to measure the loss of $^{2}$H and $^{18}$O. As such, it is a technique that would be perfectly suited to long term energy expenditure measures of sheep under sub-optimal nutrition. However, as reported by Lachica and Aguilera (2008) and Toerien et al. (1999), the cost of labelling small ruminants such as sheep and goats is rather large, with the author validating these claims by finding a cost of around $AU 3000 per sheep.

2.4.6 Summary of techniques to measure energy expenditure

Of the methods to measure energy expenditure, calorimetry chambers are undoubtedly the most accurate. They are however, only useful for measuring the ‘resting’ energy expenditure, as animals are unable to move and exhibit normal grazing behaviour. The use of tracheal fistula allows accurate sampling outside the confines of a calorimetry chamber, yet the welfare and patency issues remain. Measurement of the gaseous exchange without confinement or surgical interference is possible with the CO$_2$ entry rate technique. The need to fit animals with peristaltic pumps does make this a somewhat cumbersome technique with potential for logistical issues in a free-ranging flock of sheep. The use of radioactive isotopes may prohibitive due to subsequent land use concerns or inability for subjects to enter the food chain.

Measuring gaseous exchange in relation to an easy to measure physiological parameter such as heart rate is based on sound physiology and is relatively non-invasive. Despite requiring initial basic training of subjects, and fitment/adjustment of small HR monitoring devices regularly, the technique appears far less disruptive to normal behaviour. The concept of O$_2$ pulse being a fairly constant parameter for each individual means makes for easy calculations of energy
expenditure based on long term HR data. Simultaneous measurement of movement via GPS will allow characterisation of differences in foraging pattern and activity, which in conjunction with HR derived EE data may allow estimation of daily EE patterns and the EE for different grazing behaviours.

2.5 Conclusion

When evaluating the potential options for measuring energy metabolism, the ease of administration to sheep in a free-ranging mob, overall cost, accuracy and environmental implications need to be taken into account. Having reviewed the literature, the following table (Table 2.1) has been formulated to rank the available techniques for each parameter based on their suitability for the research of this doctorate study.

Table 2.1: Ranking of available techniques based on applicability for measuring energy expenditure of grazing sheep

<table>
<thead>
<tr>
<th>ENERGY RETENTION</th>
<th>ENERGY INTAKE</th>
<th>ENERGY EXPENDITURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Whole body scanning (DEXA or CT)</td>
<td>1. Total faecal collection</td>
<td>1. VO₂:HR measurements</td>
</tr>
<tr>
<td>5. Nitrogen Index</td>
<td>5. Tracheal fistulation</td>
<td></td>
</tr>
<tr>
<td>6. Oesophageal fistulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Individual observation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. FOO before and after grazing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. Validation of the oxygen pulse method for estimation of the energy expenditure of sheep

3.1 Abstract

Calculation of the energy expenditure of animals by gaseous exchange allows the energy requirements and differences in efficiency to be determined. Typical methodologies include restraint of the animal in calorimetry chambers or surgically interfering with the subject. Measurement of energy expenditure in free-ranging animals based on heart rate (HR) and oxygen consumption (VO$_2$) and in particular the ratio between these parameters (oxygen pulse) has been reported to work successfully in cattle and goats (Brosh et al. 1998; Brosh 2007). However, little published data exist around the effect of physical activity, ambient temperature and feeding pattern on oxygen pulse for sheep (Brosh 2007). The experiment reported here aimed to determine the between-and-within-individual variation in HR and VO$_2$, as well as the consistency of O$_2$ pulse. Ten Merino and Border Leicester ewes were individually housed indoors and fed a pelleted diet of low quality (~7MJ ME/kg dry matter, 8% crude protein). Three measurements of HR and VO$_2$ were conducted over a 70 day period using ADInstruments physiograph machine technology. Overall, 68-76% of the total variance in VO$_2$ could be explained by bodyweight, feed intake, back-fat depth, breed, individual sheep differences in VO$_2$ and differences in O$_2$ pulse between sheep. Within normal grazing HR ranges, the relationship between HR and VO$_2$ did not change. The results indicate that the O$_2$ pulse theory as proposed by Brosh et al. (1998) holds true, and that an individual’s O$_2$ pulse can be used to calculate energy expenditure from long term measures of HR.
3.2 Introduction

Techniques for estimating energy expenditure (EE) predominately revolve around the measurement of gaseous exchange (Blaxter 1962). Some require surgical intervention (Whitelaw 1974), the use of radioactive substances (Brockway and Reid 1972) or considerable physical and behavioural restriction (Lachica and Aguilera 2008). As a result the application of these techniques to study the energy expenditure of free-ranging animals is limited.

The use of HR as a predictor of VO$_2$ and hence energy expenditure, is based on the assumption that there is a constant relationship between HR and VO$_2$. This constant, referred to the oxygen pulse, reflects the amount of O$_2$ consumed per heartbeat (Blaxter 1989; Brosh 2007). Brosh et al. (1998) provided data from cattle that indicated the O$_2$ pulse is “constant” for an individual but varies between animals. However, no such studies have been conducted with sheep.

The experiment reported herein was developed to investigate the measurement of HR and VO$_2$ simultaneously in sheep. The aim was to determine the most accurate method for applying measurement procedures and validate the previously reported relationship between the two parameters. In addition, characterisation of the factors contributing to variance between these two traits is warranted. This chapter will report the development of appropriate measurement protocol and determine the factors which contribute to within or between variance in VO$_2$ and affect its relationship with HR.

3.3 Materials and Methods

Animals and their management

A summary of the experimental timeline is presented in Figure 3.1. Ten Merino ewes and ten Border Leicester ewes, approximately 18-20 months of age were sourced from Martindale Holdings, Roseworthy South Australia Inverbrackie Stud, Strathalbyn South Australia.
respectively. On day 0, sheep were placed in a small holding paddock at Roseworthy Agricultural Campus, South Australia, where they were drenched (Ivermectin, at 0.2mg/kg bodyweight) and introduced to oaten hay. After a one week acclimatisation period, a ‘low’ quality pellet diet of ~7MJ ME/kg dry matter, 8% crude protein (Appendix 1) was introduced between days 7 and 14. Sheep were then relocated to the Livestock Research Centre (Roseworthy Agricultural Campus) where they were randomly assigned to individual pens of approximately 1.5x1.2 metre dimensions. Once in the pens, acclimatisation to the ‘low’ quality pellet diet continued for one week, at the end of which (day 21) sheep were allowed ad libitum access to the feed. Sheep were introduced to the apparatus for measuring HR and VO$_2$ between days 14 and 34. Daily feed intake was measured throughout the entire trial. Refused feed was removed daily before feeding measured. Clean water was available at all times.

After two weeks of ad lib. access to the low quality diet (day 35), the HR and VO$_2$ of each individuals were measured simultaneously using an ADinstruments physiograph machine and gas analyser for a period of 3-5 minutes. This procedure was repeated one week later on day 42 for each individual. Following the second physiograph measurement, feed intake was restricted to 50% of ad lib. intake, or a minimum of 600 g/day over a four week period. On day 70, another measurement of VO$_2$ and HR was conducted for each sheep. The trial ended on day 77 when numerous sheep in the trial succumbed to copper toxicity as a result of chewing on copper water pipes in the pens.

Sheep were condition scored (Jeffries 1961) and weighed weekly prior to feeding. At the time of weighing each week, ultrasound scan measurements of loin eye muscle (EMD) and back-fat (BF) depth over the 12$^{th}$-13$^{th}$ rib, 100mm from the midline were taken using an Aquila Vet Ultrasound machine (ESAOTE, Genova, Italy) with a 6 MHz straight probe.
**Procedure for measuring VO$_2$ and HR**

An ADInstruments PowerLab 8/35 physiograph machine, in conjunction with Lab Chart Pro software interface was used to acquire and record the VO$_2$ and HR data. HR was measured utilising an ADInstruments single channel Bio Amp connected to the Power Lab machine. Three ECG leads (positive, negative and earth) connected to the BioAmp were attached to the sheep with the use of ‘Ambu Blue sensor L’ disposable ECG electrodes (Ambu, Copenhagen). The negative and earth electrodes were placed behind each front shoulder of the sheep, whilst the positive electrode was placed in front of the left shoulder at the junction of the neck and thorax. Sheep were clipped at the site of electrode attachment and skin cleaned with 98% ethanol to remove grease and dirt, ensuring optimal skin contact. VO$_2$ was measured with the use of a 1000mL Flow hood connected to an ADInstruments ML206 gas analyser. A face mask was constructed using a two litre soft drink bottle and silicon o-ring to ensure a secure fit over the muzzle of the sheep during measurement (Figure 3.2). Sheep were familiarised with having the face mask placed over their muzzle on a daily basis as soon as they were placed into
individual pens within the livestock centre. The familiarisation procedure involved gentle restraint of the sheep whilst the face mask was placed over the muzzle for 3-5 minutes, or until the sheep settled. This training procedure occurred daily for 13 days after sheep entered the livestock centre. On the measurement days, VO$_2$ and concurrent HR was measured for 3-5 minutes per sheep, or until a relatively steady HR was achieved for more than one minute. This ensured that VO$_2$ and HR were measured over a range of heart rates and that the resting heart rate and its concurrent VO$_2$ could be adequately quantified (Brosh 2007).

Figure 3.2: Face mask apparatus for measurement of respiratory parameters in conjunction with the ADInstruments PowerLab 8/35 Physiograph machine and ML206 gas analyser
Statistical Analyses

Factors contributing to variance in VO₂

All statistical analyses were conducted using GENSTAT (2012) 15TH Edition (VSN international). Data checking revealed a scale effect on the variance of the VO₂ data, which was duly normalised by log transformation of VO₂ values. HR values did not exhibit a scale effect. In order to determine which attributes were significantly contributing to variance in VO₂, a linear model was fitted using all measured terms and their interactions:

\[ VO₂ = Weight + Feed \text{ intake} + Back\text{-fat} + EMD + HR + HR.\text{Diet} + HR.\text{Measurement day} + Breed + Sheep + Diet.Breed + Diet.Sheep + Measurement day.Breed + Measurement day.Sheep + HR.Breed + HR.Sheep + HR.Diet.Breed + HR.Diet.Sheep + HR.\text{Measurement day.Breed} + HR.\text{Measurement day.Sheep} + \text{Residual} \]

Where Day (1, 2, 3), Breed (Merino or Border Leicester) or Sheep (1-20) were factors and the remaining terms were covariates. In order to determine the components contributing to variance in VO₂, the percentage of sums of squares for each trait was calculated, and the terms with minimal (<1%) effect were removed from the model. Because of the large data set, this approach was deemed more conservative than a P<0.01 significance level. The data for each dietary treatment (ad lib. and restricted) was separated and analysed separately to remove the diet effect. The reduced model used for each dietary treatment was as follows:
\[ VO_2 = \text{Weight} + \text{Feed intake} + \text{Backfat} + \text{HR} + \text{Breed} + \text{Sheep} + \text{Sheep Measurement day} + \text{HR Sheep} + \text{Residual} \]

The model for the restricted quantity, low quality diet did not include the sheep by measurement day interaction as there was only one measurement day for that dietary treatment.

**Relationship between measurement day, feed intake and respiratory parameters**

Following determination of the critical variance components, an average \( VO_2 \), \( O_2 \) pulse and HR value was calculated for each sheep to reduce the within-sheep variability. \( VO_2 \) and \( O_2 \) pulse (mL \( O_2 \)/ beat) values were analysed using a linear mixed model with fixed effects of measurement day (1, 2 or 3), HR (beats per minute, covariate), Weight (kg, covariate) and feed intake (kg, covariate). Heart rate data were analysed with a linear mixed model containing fixed effects of measurement day (1, 2 or 3), Weight (kg, covariate) and feed intake (kg, covariate). Individual sheep was fitted as a random effect in all models to account for repeated measures on the same sheep. Significance for all traits was defined as \( P<0.05 \).

### 3.4 Results

**3.4.1 Factors contributing to variance in \( VO_2 \)**

Summary statistics parameters recorded at the time of \( VO_2/HR \) measurement are presented in Table 3.1. The coefficient of variation values did not differ between measurement days and thus it is unlikely the effect of any given day will be dramatically skewing the results. Another finding of note from Table 3.1 is the reduction in mean HR as the trial progressed.
Table 3.3.1: Summary statistics for sheep measures undertaken at the time of simultaneous HR and VO2 measurement across three measurement days and two dietary regimes, namely ad libitum (AL) and restricted (RL).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day</th>
<th>Weight (kg)</th>
<th>BF (mm)</th>
<th>Feed Intake (Kg/day)</th>
<th>HR (beats per minute)</th>
<th>VO2 (mL/min)</th>
<th>O2 Pulse (mL/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>35</td>
<td>Max</td>
<td>87.2</td>
<td>10.6</td>
<td>2.8</td>
<td>202</td>
<td>840</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>56.8</td>
<td>4.5</td>
<td>1.0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>74.5</td>
<td>6.9</td>
<td>1.9</td>
<td>113</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>8.6</td>
<td>1.7</td>
<td>0.5</td>
<td>26</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV</td>
<td>11</td>
<td>24</td>
<td>25</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>Max</td>
<td>88.0</td>
<td>11.1</td>
<td>2.5</td>
<td>137</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>53.5</td>
<td>4.5</td>
<td>0.8</td>
<td>21</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>74.2</td>
<td>6.9</td>
<td>1.8</td>
<td>90</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>8.7</td>
<td>1.6</td>
<td>0.5</td>
<td>17</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV</td>
<td>11</td>
<td>23</td>
<td>26</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td>RL</td>
<td>70</td>
<td>Max</td>
<td>82.9</td>
<td>10.7</td>
<td>0.8</td>
<td>143</td>
<td>520</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min</td>
<td>55.7</td>
<td>1.0</td>
<td>0.1</td>
<td>55</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>70.6</td>
<td>6.3</td>
<td>0.5</td>
<td>84</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SD</td>
<td>7.8</td>
<td>2.1</td>
<td>0.2</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CV</td>
<td>11</td>
<td>32</td>
<td>44</td>
<td>19</td>
<td>37</td>
</tr>
</tbody>
</table>
The scatterplot of every HR and VO₂ value for all sheep from the three measurement days is presented in Figure 3.3. There was a moderate positive correlation between these two traits ($r^2=0.16$).
Figure 3.3: Scatterplot of heart rate against VO$_2$ for 18 sheep across 3 separate measurement days and ad lib and restricted dietary regimes ($r^2=0.6$).
Regression analyses of the VO₂ data for each dietary regime revealed that when sheep were fed *ad libitum*, a total of 68% of the variance in VO₂ could be explained by the parameters measured (Table 3.2, Figure 3.4). The greatest contributors to variance were sheep weight (17%) and individual sheep differences (30%). These individual sheep differences in VO₂ were still present after feed intake, BF, HR and breed had been taken into account. Feed intake, BF, and breed accounted for a small proportion of the variance (Figure 3.4). HR also had a small effect on VO₂ (3%), indicating that change in HR does not contribute to a large proportion of variation in VO₂. The sheep by measurement day interaction accounted for 9% of the variance in VO₂ consumption, indicating some re-ranking of sheep between measurement day. The HR by sheep interaction refers to the O₂ pulse (slope of VO₂ on HR), thus it would appear that there was some variation in the O₂ pulse between sheep (4%), even after individual sheep and measurement day characteristics have been taken into account (Figure 3.4).
Table 3.3.2: Analysis of variance for sheep and measurement day effects on VO2 measured on both Ad libitum (AL) and restricted levels (RL) of a 'low' quality diet.

<table>
<thead>
<tr>
<th></th>
<th>AL</th>
<th></th>
<th>RL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>&lt;.001</td>
<td>1</td>
<td>&lt;.001</td>
<td>1</td>
</tr>
<tr>
<td>Feed intake</td>
<td>&lt;.001</td>
<td>1</td>
<td>&lt;.001</td>
<td>1</td>
</tr>
<tr>
<td>Backfat</td>
<td>&lt;.001</td>
<td>1</td>
<td>0.092</td>
<td>1</td>
</tr>
<tr>
<td>HR</td>
<td>&lt;.001</td>
<td>1</td>
<td>&lt;.001</td>
<td>1</td>
</tr>
<tr>
<td>Breed</td>
<td>&lt;.001</td>
<td>1</td>
<td>&lt;.001</td>
<td>1</td>
</tr>
<tr>
<td>Sheep</td>
<td>&lt;.001</td>
<td>18</td>
<td>&lt;.001</td>
<td>15</td>
</tr>
<tr>
<td>Sheep.measurement day</td>
<td>&lt;.001</td>
<td>17</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>HR.sheep</td>
<td>&lt;.001</td>
<td>19</td>
<td>&lt;.001</td>
<td>19</td>
</tr>
<tr>
<td>Residual</td>
<td>n.a.</td>
<td>954</td>
<td>n.a.</td>
<td>265</td>
</tr>
</tbody>
</table>
Overall, 76% of the variance in VO$_2$ could be accounted for by the measured parameters (Figure 3.5). When compared to the *ad lib.* feeding level, weight contributed to 50% less variance in VO$_2$ (8%). The individual sheep effect remained one of the greatest contributors to variance (30%). Feed intake had a slightly greater effect (3%) whilst breed effects were diminished (2%). The effect of backfat was not significant (Table 3.2). The greatest change observed was in the effect of HR, which contributed to 29% of the total variance in VO$_2$. As there was no measurement day interaction fitted, no comment can be made regarding day to day variation in VO$_2$ of individual sheep. The contribution of O$_2$ pulse (HR by sheep interaction) was similar to that observed for the *ad lib.* data set, contributing to 5% of the variance (Figure 3.5).
3.4.2 Relationship between measurement day, feed intake and respiratory parameters

Regression analyses revealed that measurement day, HR, weight and feed intake contributed to a considerable proportion of the variance in VO$_2$. Analyses of HR and respiratory traits with the variables included in the model is presented in Table 3.3. Average VO$_2$ consumption was significantly affected by measurement day, heart rate and weight, but not feed intake. HR itself was affected by day of measurement and weight, but not feed intake. O$_2$ pulse was not significantly affected by HR or intake, but was significantly affected by measurement day, and approached significance for weight (Table 3.3).
Table 3.3.3: Tests of significance (f-probabilities) for heart rate and respiratory traits (n=60)

<table>
<thead>
<tr>
<th>Trait</th>
<th>VO&lt;sub&gt;2&lt;/sub&gt; (mL/minute)</th>
<th>Heart rate (Beats/minute)</th>
<th>O&lt;sub&gt;2&lt;/sub&gt; pulse (mL/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement Day</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Heart rate</td>
<td>&lt;0.001***</td>
<td>n.a.</td>
<td>0.102</td>
</tr>
<tr>
<td>Weight</td>
<td>0.043*</td>
<td>0.01**</td>
<td>0.055</td>
</tr>
<tr>
<td>Feed intake</td>
<td>0.08</td>
<td>0.521</td>
<td>0.332</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001, n.a. (not applicable)

Comparison of the mean values of VO<sub>2</sub> for each measurement day revealed that the days 1 (ab lib.) and 3 (restricted) were similar whilst the value for Day 2 (ad lib.) was approximately 50mL/minute greater (Table 3.4). Mean HR was highest on Day 1 (108 beats/minute), whilst the mean HR for days 2 and 3 was almost 20 beats/minute lower at 88 and 89 beats/minute respectively. Thus, differences in O<sub>2</sub> pulse appear to be driven by differences in VO<sub>2</sub>, with the values for day 1 and 3 being closer to one another (2.95 and 2.65 mL/beat respectively), and significantly lower than the 3.56 ml/beat value obtained for Day 2 (Table 3.4).
Table 3.3.4: Predicted means for measurement day effects on VO2, O2 pulse and Heart rate traits (n=60). Values presented are means ± s.e. Means within a row with different letters are significantly different.

<table>
<thead>
<tr>
<th>Trait</th>
<th>VO2 (mL/minute)</th>
<th>Heart rate (beats/minute)</th>
<th>O2 pulse (mL/beat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 (Ad lib)</td>
<td>266 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.95 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 2 (Ad lib)</td>
<td>320 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.56 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 3 (Restricted)</td>
<td>271 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.65 ± 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Checking the estimated mean slope effects in Table 3.5 reveals VO2 increased by 0.0015, 0.0024 and 0.0444 L/minute for every beat, kg bodyweight and kg intake increase respectively. For every kg increase in bodyweight and feed intake, HR was increased by 0.753 and 3.9 beats/minute. The slope of HR on O2 pulse revealed a slight negative relationship (-13ml decrease in O2 uptake/beat with every beat increase) whilst a kilogram increase in weight or feed intake resulted in a 29 and 290 ml/beat increase in O2 pulse (Table 3.5).

Table 3.3.5: Regression coefficients for heart rate and respiratory traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>Slope on HR</th>
<th>Slope on Weight</th>
<th>Slope on Feed intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2 (mL/minute)</td>
<td>1.5 ± 0.1</td>
<td>2.4 ± 1.6</td>
<td>44.4 ± 24.8</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>n.a.</td>
<td>0.753 ± 0.32</td>
<td>3.9 ± 6.0</td>
</tr>
<tr>
<td>O2 pulse (mL/beat)</td>
<td>-13.0 ± 6.0</td>
<td>29.0 ± 18.0</td>
<td>290.0 ± 29.7</td>
</tr>
</tbody>
</table>

n.a. (not applicable)
3.5 Discussion

Body weight contributed to 8 and 16% of the total variance in VO\textsubscript{2} for ad libitum and restricted fed sheep respectively. The differences in body weight observed between sheep are most likely due to differences in overall body frame size, body condition (particularly fatness) or gut fill. Larger-framed sheep will have greater tissue mass which incurs a greater oxygen demand (Suarez 1998). Larger frame size also facilitates larger lung size which allows greater O\textsubscript{2} uptake to occur (Tenney and Remmers 1963). The positive slope of VO\textsubscript{2} on weight observed illustrates this effect (Table 3.5).

As back fat was included in the model after weight, there is potential for differences in body fatness to be encapsulated in the weight difference and be contributing to variance in VO\textsubscript{2}. Energy expenditure of adipose is approximately one-third of than of muscle (Wang et al. 2010) it would be expected that sheep with proportionally greater adipose tissue at the same weight would have a lower VO\textsubscript{2}. Although the accuracy of the use of ultrasound-measured back fat to predict carcass fatness is variable (Jones et al. 1982; Fortin and Shrestha 1986; Silva et al. 2006; Leeds et al. 2008), the lack of access to other techniques and the inability to perform serial slaughter meant it was the only possible measure that could be undertaken. Given that only one ultrasound fatness measurement was taken at each measurement of VO\textsubscript{2}, it is likely that the measurement of fatness isn’t truly representative of body fat level, and that differences in body composition remain encapsulated within the weight effect.

Although feed intake was included in the model, there is potential for the effect of intake and gut fill level to also be within the weight effect. Dietary energy concentration and total MEI are known to affect the VO\textsubscript{2} of ruminants (Osuji 1974; Rometsch et al. 1997; Brosh 1998; Lohrke et al. 2013). Increased feed intake means that there is a larger substrate base to oxidise, which stimulates mitochondrial activity to facilitate a greater rate of oxidation (Baldwin 1995; Lohrke et al. 2003). Total daily feed intake was not significant for O\textsubscript{2} pulse or HR, but approached
significance for VO\textsubscript{2}, indicating that the effect of feed intake on variance in VO\textsubscript{2} was genuine (Table 3.2). However, the potential for variation in gut fill and stage of food substrate oxidation to vary is quite high whilst sheep were on the \textit{ad lib.} feeding regime, and thus differences in feeding pattern will determine the level of gut fill at the time of VO\textsubscript{2} measurement. As feeding pattern was not measured it is likely the effects of feed intake remain within the weight effect. The greater effect of weight on VO\textsubscript{2} for the \textit{ad lib.} compared to the restricted feeding level (17 vs 8\%) is likely due to feed intake induced increases in metabolism (Figures 3.3 and 3.4).

The dichotomy between the feeding levels in the effect of HR on VO\textsubscript{2} is an interesting occurrence that may be due to a number of factors. As outlined previously, increased and/or variable gut fill and the resultant change in oxygen consumption on the \textit{ad lib.} feed regime may have caused a dissociation between HR and VO\textsubscript{2}, leading to the reduced effect of HR on VO\textsubscript{2} (Table 3.3). The mean VO\textsubscript{2} value for day 2 was elevated, yet the HR was similar to that of day three, so it is likely that the elevated VO\textsubscript{2} values from day 2 are dissociating the relationship (Table 3.1). Alternatively, an insufficient or unrepresentative range of HR may have been measured and may also contribute to the lack of relationship with VO\textsubscript{2}. The decrease in mean HR as the trial progressed does indicate that the subjects were continuing to acclimatise to the measurement procedure (Table 3.1). As such, there may have been stress induced changes to HR and VO\textsubscript{2}. Although training had been undertaken to ensure familiarisation with the procedure, it appears this may not have been sufficient. Continued measurement over time would have further validated this, however the premature termination of the trial due to copper toxicity prevented this. Acute copper toxicity causes haemolysis (Gopinath and Howell 1975) and lipid peroxidation (Bremner 1998) which may have affected the respiratory measures under the restricted feeding regime.

Body weight had significant effects on HR, with the slope on weight indicating an increase in HR as bodyweight increased (Tables 3.2 and 3.3). The mid body weight ranges observed were
29 and 25kg for the *ad lib.* and restricted levels respectively. Using the slope on weight value this should correspond to an approximately 22 and 19 beat per minute range in heart weight between the smallest and largest sheep at each feeding level. The potential effects of level of intake on weight will have been greater on the *ad lib.* level and thus there is potential for the relationship between HR and VO\(_2\) to dissociate.

Individual ‘sheep’ effects accounted for almost one third of the variance in VO\(_2\) on both *ad lib* and restricted feeding regimes. This result indicates that once bodyweight, feed intake, HR and breed have been taken into account, a considerable degree of variation exists in ‘resting’ oxygen consumption, and thus, ‘resting metabolic rate’ between individuals. Up to 20% of the variance in resting energy expenditure of humans has been attributed to differences in body composition (Bader *et al.* 2005). As total body composition is believed to be inadequately measured in the trial, some of the differences between sheep may result from differences in deposition or mobilisation of fat and muscle and the resultant energy costs incurred (Rattray *et al.* 1974; Li *et al.* 2008). Variation in stress/excitement during the measurement procedure may also explain some of the sheep variance in VO\(_2\). However, human studies have shown that although psychological stress may increase respiration rate, actual VO\(_2\) and metabolic demand were unaffected (Masaoka *et al.* 2001; Traustadottir *et al.* 2005). Despite this, in studies of cattle, Brosh *et al.* (2007) did note that stressed/excited individuals exhibited a proportionately greater increase in HR than VO\(_2\), which will lead to underestimations of O\(_2\) pulse and EE. As no measure of stress response was taken during this experiment, the impact of stress on VO\(_2\) cannot be quantified.

Although stress and body composition effects may be included within the ‘sheep’ effect, the considerable contribution of ‘sheep’ to variance in VO\(_2\) indicates that there are still significant differences between sheep in resting oxygen consumption. If not all of the variation between sheep is in differing tolerance to stress, there are a number of physiological explanations as to
why sheep may differ in VO$_2$. Firstly, there are three different haemoglobin genotypes in sheep, which have been shown to have differing affinity for oxygen (Dawson and Evans 1966; Nienhuis and Anderson 1972). The interactions of haemoglobin genotype with blood potassium type (high or low) may also affect their function (Darcel and Avery 1960). Thus, difference in Haemoglobin genotype and resultant affinity for oxygen, may affect the rate at which oxygen is consumed and distributed to body tissues (Banchero and Grover 1972; Altaif and Dargie 1978). Additionally, variation in mitochondrial haplotype has been identified within and between breeds of sheep (Arora et al. 2013) and has been correlated with differing levels of production, which has the potential to lead to differences in oxygen consumption (Henry et al. 2011). Another explanation may be variation in lung size determining the physical capabilities for air flow through the alveoli and thus impact the amount of oxygen consumed (Mortola and Maskrey 2011). As no measures of these parameters were undertaken, these explanations remain speculative.

The sheep-by-measurement day interaction observed on the ad libitum feeding regime may be attributable to differing stress response to measurement procedure, variable level of gut fill and heat load at the time of measurement (Brosh et al. 1998) or variation in temperature conditions (Hofman and Riegle 1977). As no measure of gut fill or stress response was undertaken these factors cannot be quantified. As the sheep were housed in a temperature-controlled shed, the environmental variability was likely to be very low and should have had minimal effect.

The sheep-by-HR interaction (the O$_2$ pulse) contribution to variance in VO$_2$ was small, yet indicates that there is some variation between individuals in their oxygen pulse. Individual variation in level of feed intake has been reported to affect O$_2$ pulse in some studies (Barkai et al. 2002, Brosh et al. 2004), but not in others (Brosh et al. 1998, Arieli et al. 2002). The mean daily feed intakes measured ranged from 1.32-3.16 kg, which based on the estimated effects
should elicit a relatively small 0.53ml/beat range in O₂ pulse (Table 3.5). Feed intake differences may well be contributing to the individual variation in oxygen pulse.

Body weight effects also approached significance for O₂ pulse, which is not unexpected given that both HR and VO₂ as independent parameters were significantly affected by weight. An increase in oxygen uptake per heart beat as weight increases is logical, as larger animals will have a larger heart and thus stroke volume, and/or greater level of oxygen requirement due to a larger mass of body tissue (Bishop and Spivey 2013). The potential confounding effects of body composition and gut fill need to be kept in mind when considering the effect of weight on O₂ pulse.

The lack of a significant effect of HR on O₂ pulse is a desired result, as it means that O₂ pulse does not significantly change as HR changes, and thus, short-term measurements of O₂ pulse as a representative value for a given individual over a range of HR measured long term (Brosh et al. 1998). The similar mean O₂ pulse values on days 35 and 70, despite the significantly higher HR on day 35 support the constancy of O₂ pulse theory. Plotting the HR vs O₂ pulse for each separate day highlights the constancy of O₂ pulse across HR on days 35 and 70 (Figure 3.6). Overall, the slope of O₂ pulse on HR observed in this experiment was -0.013ml O₂/beat per beat increase in HR (Table 3.5) which although not zero, relates to a negligible 0.02 MJ/day increase in energy expenditure. The dissociation between HR and O₂ pulse on day 42 is most likely responsible for this.
Figure 3.6: Scatterplot of HR against Oxygen pulse of 20 sheep over three separate measurement days
The most likely cause of variation in VO$_2$ between measurement days is the environmental conditions. VO$_2$ is computed by the physiograph machine at a standard temperature and pressure (STP) (Maud and Foster 2006). Variation in temperature and pressure can be adjusted for to account for their effects on VO$_2$. However, STP was not adjusted for in this experiment and will have contributed to variability in measurement day. Although housed in an air conditioned building should reduce the variability between days, observation of the maximum temperatures for the three measurement days were 26, 34 and 29 degrees Celsius respectively (Bureau of Meteorology 2013). The effect of greater ambient temperature on calculations of VO$_2$ during day 42 is most likely responsible for the increased VO$_2$ values observed.

3.5 Conclusion

Investigation of the factors contributing to variance in the VO$_2$ of sheep has revealed that 68-76% of the total variance in VO$_2$ can be explained by bodyweight, feed intake, back fat depth, breed, individual sheep differences in VO$_2$ and differences in O$_2$ pulse between sheep. The results indicate that the O$_2$ pulse theory as proposed by Brosh et al. (1998) holds true, and that VO$_2$ remains constant across HR for a given individual. Reductions in mean HR as the trial progressed indicate that greater training is required to acclimatise animals to the measurement of VO$_2$. In addition, the daily variation in temperature and pressure may affect the measurement of VO$_2$ and should be considered in further trials. In order to reduce the effects of daily variation within and between sheep and further investigate its effect on O$_2$ pulse, more than three measurement periods are recommended, and potentially multiple measurement periods within a given day. Unfortunately the premature conclusion of the experiment due to copper toxicity issues prevented more than three measurements from being taken. If measurements of EE are to be taken in an outdoor situation, it is recommended that O$_2$ pulse measurements be undertaken whilst animals are outside, perhaps in addition to some initial measurements.
indoors. Comparison of outdoor vs. indoor measured $O_2$ pulse values should facilitate a greater understanding of the effect of variable intake, body composition, and environmental conditions. Proper quantification of bodyweight, composition and level of gut fill at time of measurement are all recommendations to ensure greater accuracy of HR and VO$_2$ predictions.
4. Live weight change is the best indicator of resilience in sheep

4.1 Abstract

‘Resilience’ is a term often used in the livestock industries to denote an animals’ ability to endure harsh conditions and remain in productive and healthy condition. Whether the best measure of resilience is live weight and/or body condition (fatness) is not well defined. This experiment measured the performance of 18 wethers from three breeds (Meatmaster, Merino and Merino cross Border Leicester) over two measurement periods (outdoors (P1) and indoors (P2)). When grazing a senescent pasture (P1), initially heavier individuals were fatter, and lost more subcutaneous fat, but not necessarily bodyweight itself during the period. When animals were housed indoors, and feed quality was restricted using a low quality ration (P2), the range in body weight change was considerably higher and included a subset of individuals which lost weight. As there was no measurable loss in BF depth this loss in weight was most likely due to mobilisation of muscle tissue, visceral organs and internal fat depots. Rumenoreticulum, kidney and liver weights were correlated with the rate of weight gain, highlighting that internal organ mass change comprises a component of total weight change.

These results highlight significant individual variation in response to nutritional restriction through quantity (P1) and quality (P2). The potential for different responses in weight, adipose tissue and organ mass change, overall average daily gain (ADG) is the best indicator of ‘resilience’ to nutritional stress.

4.2 Introduction

‘Resilience’ is a term used in the livestock industries to denote an animals’ ability to endure harsh conditions and remain in a productive and healthy state. Whether the best measure of
resilience is live weight and/or body condition (fatness) is not well defined. In this Chapter, the second experiment of this study (Experiment 2) is introduced. This experiment was designed to study energy retention, intake and expenditure of sheep under sub-optimal nutrition. The experiment evaluated the performance of the same subjects in both indoor and outdoor environments, as animal performance measured in controlled experimental conditions often differs to that in the field (Lawrence et al. 2012). Three different breeds (Merino, Border Leicester x Merino and Meatmaster) were used in this experiment, aiming to maximise variation in the traits of interest within a smaller sample of subjects.

Due to the breadth of measurements and large amount of data collected, different components of the experiment will be presented in Chapters 4 through 7, with chapter 8 summarising the correlations between the results of each chapter. This chapter will outline the general materials and methods for the whole experiment, with specifics clarified within each relevant chapter.

In addition to the outline of the experiment as a whole, this chapter reports on the weight and body composition measures to quantify the extent of individual variation observed. This will then provide information as to what measure/s are the best to define ‘resilience’ as a trait.

The potential measures of ‘resilience’ that are able to be measured easily in the live animal are bodyweight, subcutaneous fat depth and loin muscle depth. The change in these measures over time gives an indication of the ability for animals to retain energy and deposit it as body tissues. This experiment also provides insight into the mass of internal fat reserves and organs which will allow relationships to be drawn with measures in the live animal. Likewise, carcase weight and dressing percentage also provides information about energy retention within body tissue that will inform on the accuracy of live animal measures.
4.3 General materials and methods

Animals and their management

This experiment was partitioned into two consecutive experimental periods (Figure 4.1). Period 1 encompasses the entire time sheep were housed in the paddock and grazing available pasture, from days 0 to 84. Period 2 refers to the experimental period once sheep were removed from pasture and placed indoors, from days 84 to 125 (Figure 4.1).

Eighteen wethers of three genotypes (Medium wool Merino (MO), Border Leicester cross Merino (XB) and Meat Master composite (MM)), approximately 7-9 months of age were used in this trial. The MO and XB wethers were sourced from Martindale Holdings Pty Ltd, Roseworthy, South Australia. The MM wethers were sourced from Garryowen Pty Ltd, Wudinna, South Australia. Sheep were placed in a 4ha paddock at the University of Adelaide’s Roseworthy campus, South Australia on day 0 (Figure 4.1).

Available pasture consisted of annual ryegrass (Lolium multiflorum) and barley grass (Hordeum vulgare), with a small proportion of clover (Trifolium spp.). Sheep were permitted to graze ad lib. throughout the course of Period 1. Pasture availability was determined (as feed on offer in kg dry matter/ha) on a weekly basis. When the pasture availability dropped below 400kg DM/ha on day 75, sheep were introduced to a ‘low’ quality pelletised ration of approximately 7 MJ/ME kg and 7% crude protein (as per manufacturer’s analysis) (Figure 4.1). Feed was introduced gradually over a seven-day period via two Magnus Industries EZYFEED 18 bag sheep feeders (www.magnus.com.au). The end of this acclimatisation period denotes the end of Period 1.

Period 2 began when sheep were moved into the Livestock Research Centre, Roseworthy Campus, South Australia where they were randomly assigned to individual pens 1.5 metres wide and 1.2 metres deep. Individual daily feed intake was measured for the entire period.
Refusals were removed daily at the time of feed measurement and replaced with fresh feed. Clean water was available at all times.

After two weeks of individual intake measurements, each sheep underwent a one week period in a metabolism crate so that daily *ad lib.* feed intake, urine and faecal output could be measured. As only 9 metabolism crates were available, these measurements were undertaken in two ‘rounds’, with 9 sheep measured between days 98-105, with the remaining 8 measured from days 105-112 (Figure 4.1).

Once all metabolism crate measures were completed, all sheep were rested for 2 days. On day 114, each sheep was fitted with a jugular catheter and blood samples taken regularly 0, 2, 4, 6, 8, 12, 16, 20 and 24 hours. Specific details of the catheterisation procedure and measurements are addressed in Chapter 7.

*Measurement of HR and VO₂*

Throughout the trial each individual’s heart rate (HR) and VO₂ were measured simultaneously using an ADinstruments physiograph machine ADInstruments PowerLab 8/35 physiograph machine, using the methodology outlined in chapter 3. Measurements were taken for each sheep on days 10, 45, 52 and 58 during Period 1, and days 84 and 90 during Period 2.
Figure 4.1: Experimental timeline for experiment 2
**Weight and body composition measurement**

Sheep were conditioned scored (Jeffries 1961) and weighed weekly at 9 a.m. At the time of weighing each week, ultrasound scan measurements of loin eye muscle and back fat depth over the 12\textsuperscript{th}-13\textsuperscript{th} rib, 100mm from the midline were taken using an Aquila Vet Ultrasound machine (ESAOTE, Italy) with a 6 MHz straight probe. Scan traits were recorded as eye muscle depth (EMD, mm) and back fat depth (BF, mm).

**Slaughter and organ dissection**

Sheep were slaughtered by Menzel Meats Pty Ltd, Kapunda, South Australia as per industry standard procedure. Feed and water was removed 12 hours before slaughter when sheep were transported to the abattoir’s holding yards. Once slaughtered, and the skin removed, carcasses were individually tagged. All internal organs were individually bagged and labelled as removed for further dissection. Following removal of the internal organs, Hot Standard Carcass Weight (HSCW) was recorded for each individual. 48 hours post-slaughter, carcasses were weighed again to obtain the Cold Standard Carcass Weight (CSCW). At this time, the tail circumference, length and width was measured on the MM carcasses to quantify the distribution of fat in this area.

Bagged organs were taken back to Roseworthy Campus so that individual weights could be recorded. The heart, kidneys, lungs, liver and spleen were separated and the mental, heart and kidney fat were separated from their respective organs and the weight recorded. The rumenoreticulum, omasum and abomasum components of the digestive tract were dissected, the contents removed and a clean empty weight recorded. Before the rumen was emptied, a 3ml sample of rumen fluid was taken and frozen for further VFA analysis if required. Once the rumenoreticulum was cleaned and weighed, a 1cm square tissue sample was taken from the ventral and dorsal sac of the rumen, and separately placed
into tubes containing a 1% formalin solution. The large intestine, small intestine and caecum were cleaned and individually weighed.

Statistical Analyses

All statistical analyses were performed using Genstat 15th Edition (VSN international). General linear models were used to analyse all traits. Distribution and residual plots were checked for each trait before analysis was conducted. Significance of results for all traits was defined as P<0.05.

In order to predict average daily gain (ADG) values for bodyweight, BF, and EMD traits, a simple linear model was fitted to the data for both Period 1 and 2 separately. In order to simplify the analyses, each period was analysed separately as the last day of period 1 also serves as the first day of period 2. The model included fixed effects of sheep and the sheep-by-date interaction as per:

(bodyweight, BF, EMD) = Sheep + Sheep.Date + Residual

Predicted ADG (sheep.date slope predictions) values for each sheep were generated from this analysis and compiled into a separate dataset. A simple linear model with fixed effects of breed was used to analyse the variation between breeds and predict mean values. Sheep was fitted as a random factor to account for repeated measures on each individual.

4.4 Results

The results presented for all data from this experiment are for 17 sheep, as one of the XB wethers did not acclimatise to being housed individually indoors, and did not consume any of its ration. In accordance with the animal welfare guidelines the sheep was removed from the experiment.
4.4.1 Period 1 Body Weight and Composition Change

Initial bodyweight, BF and EMD measures were significantly different between sheep when commencing P1 (Table 4.2). There was a 12.5kg range in initial weight, although when expressed as metabolic bodyweight the range was 3.4kg\(^{0.75}\). Initial measures of BF and EMD covered a range of 3.6mm and 11.3mm respectively (Table 4.1). The ADG of bodyweight (sheep-by-date effect on weight) was significantly different between individuals with a range of 103g/day. No negative values for weight gain were recorded in this period. Individual differences in ΔBF were also significant, with a 0.027 mm/day range in values. Despite a greater range in gain values (0.107mm/day), the individual differences in ΔEMD were not significant (Table 4.2). It is worth noting that the minimum values for ΔBF and ΔEMD were negative, but positive for ADG, indicating that despite no negative bodyweight loss, changes in body composition were occurring (Table 4.1).

4.4.2 Period 2 Body Weight and Composition Change

At the start of P2, initial weights varied by 11.5kg. Expression as metabolic bodyweight reduced the range to 2.9kg\(^{0.75}\). There was a smaller range of BF (2.9mm) but greater EMD (14.4mm), than in P1 (Table 4.2). Individual differences in ADG approached significance (Table 4.1), despite a considerably greater range (600g/day) and negative weight gains for some individuals (Table 4.2). The 0.085mm/day variation in ΔBF significantly differed between sheep (Table 4.1). Interestingly, no negative value was observed for ΔBF (Table 4.1). Despite a loss of EMD for some individuals, and a greater range than observed in P1 (0.422mm/day vs. 0.107mm/day) differences in ΔEMD were not significant (Table 4.1).
<table>
<thead>
<tr>
<th>Period</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (kg)</td>
<td>57</td>
<td>51</td>
<td>63.5</td>
<td>3.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Initial metabolic weight (kg)</td>
<td>20.7</td>
<td>19.1</td>
<td>22.5</td>
<td>0.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Initial BF (mm)</td>
<td>3.3</td>
<td>1.9</td>
<td>5.5</td>
<td>0.9</td>
<td>25.8</td>
</tr>
<tr>
<td>Initial EMD (mm)</td>
<td>26.7</td>
<td>20.8</td>
<td>31.8</td>
<td>2.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Weight ADG (g/d)</td>
<td>80.1</td>
<td>21</td>
<td>124</td>
<td>20</td>
<td>47.7</td>
</tr>
<tr>
<td>ΔBF (mm/d)</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>53.4</td>
</tr>
<tr>
<td>ΔEMD (mm/d)</td>
<td>0.01</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>1358.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (kg)</td>
<td>63.7</td>
<td>58.5</td>
<td>70</td>
<td>3.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Initial metabolic weight (kg)</td>
<td>22.5</td>
<td>21.1</td>
<td>24.0</td>
<td>0.87</td>
<td>3.9</td>
</tr>
<tr>
<td>Initial BF (mm)</td>
<td>5</td>
<td>3.5</td>
<td>6.4</td>
<td>0.82</td>
<td>16.3</td>
</tr>
<tr>
<td>Initial EMD (mm)</td>
<td>27</td>
<td>21.2</td>
<td>35.6</td>
<td>2.98</td>
<td>11.0</td>
</tr>
<tr>
<td>Weight ADG (g/d)</td>
<td>-30.8</td>
<td>-300.0</td>
<td>300.0</td>
<td>140</td>
<td>136.1</td>
</tr>
<tr>
<td>ΔBF (mm/d)</td>
<td>0.03</td>
<td>0.01</td>
<td>0.09</td>
<td>0.02</td>
<td>74.5</td>
</tr>
<tr>
<td>ΔEMD (mm/d)</td>
<td>0.01</td>
<td>-0.11</td>
<td>0.32</td>
<td>0.1</td>
<td>422.5</td>
</tr>
</tbody>
</table>
Table 4.2: Tests of significance (F-probabilities) for bodyweight and ultrasound scan traits. From model: (bodyweight, BF, EMD) = Sheep + Sheep.Date + Residual

<table>
<thead>
<tr>
<th>Period</th>
<th>Trait</th>
<th>Sheep</th>
<th>Sheep.Date (ADG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weight</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BF</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>EMD</td>
<td>&lt;0.001</td>
<td>0.068</td>
</tr>
<tr>
<td>2</td>
<td>Weight</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>BF</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>EMD</td>
<td>&lt;0.001</td>
<td>0.075</td>
</tr>
</tbody>
</table>

4.4.3 Correlations between weight/scan traits within periods

In P1, initial weight was correlated with greater subcutaneous fat depth, but not loin muscle. There was no relationship between start weight and gain of weight or change in body condition traits. Greater initial fatness was however correlated with greater losses in BF (r= -0.8) (Table 4.3, Figure 4.3). Similar to the relationship observed with BF, sheep that had an initially greater EMD, tended to exhibit lower, or negative values for EMD change (r= -0.5) (Table 4.3, Figure 4.4).

Within P2, initial weight wasn’t strongly related to initial BF and EMD measures, nor the change values for those traits (Table 4.3). The greatest correlation with P2 initial weight was with weight gain (Table 4.3). Sheep which were heavier at the beginning of the period tended to have lower rates of gain.
### Table 4.3: Correlation coefficients for initial and gain values for weight and ultrasound scan traits

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>P2</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial Wt.</td>
<td>Initial BF</td>
<td>Initial EMD</td>
<td>ADG</td>
<td>Δ BF</td>
<td>Δ EMD</td>
<td>Initial Wt.</td>
<td>Initial BF</td>
<td>Initial EMD</td>
<td>ADG</td>
<td>Δ BF</td>
</tr>
<tr>
<td>Initial Wt.</td>
<td></td>
<td>0.5</td>
<td>0.1</td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BF</td>
<td></td>
<td>0.4</td>
<td>-0.3</td>
<td>-0.8</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial EMD</td>
<td></td>
<td>0.0</td>
<td>-0.3</td>
<td>-0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG</td>
<td></td>
<td>0.4</td>
<td>-0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ BF</td>
<td></td>
<td>-0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ EMD</td>
<td></td>
<td>-0.5</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Wt.</td>
<td></td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BF</td>
<td></td>
<td></td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial EMD</td>
<td></td>
<td>-0.1</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG</td>
<td></td>
<td></td>
<td>-0.3</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ BF</td>
<td></td>
<td></td>
<td>-0.1</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ EMD</td>
<td></td>
<td></td>
<td>-0.3</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.4 Correlations between weight and ultrasound scan traits and organ weights

Correlations between P1 traits and organ/carcass measures will not be reported given the 125 day time frame between measures.

Animals with greater ADG during P2 tended to have heavier kidneys, liver and rumenoreticulum (Table 4.4). As would be expected, greater ADG during this period was associated with greater HSCW (Figure 4.2).
Table 4.4: Correlation coefficients for initial and gain values of weight and ultrasound scan traits against dissected organ traits

<table>
<thead>
<tr>
<th></th>
<th>Initial Wt.</th>
<th>Initial BF</th>
<th>Initial EMD</th>
<th>ADG</th>
<th>ΔBF</th>
<th>ΔEMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Fat wt.</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Heart Fat wt.</td>
<td>0.2</td>
<td>0.0</td>
<td>-0.3</td>
<td>0.0</td>
<td>-0.1</td>
<td>-0.2</td>
</tr>
<tr>
<td>Kidney wt.</td>
<td>-0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Heart wt.</td>
<td>0.3</td>
<td>-0.1</td>
<td>0.1</td>
<td>-0.4</td>
<td>0.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>Lungs wt.</td>
<td>0.7</td>
<td>0.0</td>
<td>-0.2</td>
<td>-0.1</td>
<td>0.0</td>
<td>-0.3</td>
</tr>
<tr>
<td>Liver wt.</td>
<td>0.0</td>
<td>0.3</td>
<td>0.0</td>
<td>0.7</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Spleen wt.</td>
<td>0.3</td>
<td>-0.5</td>
<td>-0.2</td>
<td>-0.3</td>
<td>0.1</td>
<td>-0.4</td>
</tr>
<tr>
<td>Rumenoreticulum wt.</td>
<td>0.3</td>
<td>0.1</td>
<td>-0.2</td>
<td>0.5</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
<tr>
<td>Omasum wt.</td>
<td>0.1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Abomasum wt.</td>
<td>0.2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>S.I. wt.</td>
<td>-0.1</td>
<td>-0.4</td>
<td>-0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>-0.1</td>
</tr>
<tr>
<td>L.I. wt.</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Caecum wt.</td>
<td>0.3</td>
<td>0.0</td>
<td>0.1</td>
<td>0.3</td>
<td>-0.1</td>
<td>-0.1</td>
</tr>
</tbody>
</table>
4.5 Discussion

This chapter aimed to determine the optimal measure of resilience under sub-optimal nutrition. The candidate traits evaluated were weight, subcutaneous fat and loin muscle depth. When comparing the results from the two periods, the reader is urged to bear in mind that the nutritional regimes were quite different. The most appropriate measure of resilience is that with the greatest consistency of response and relationship to organ and carcass traits across environments.

During P1, initial weight was not related to weight gain in the period. However, these initially heavier animals tended to be fatter, and predisposed to losing BF during the measurement period. Initial measures of muscle were not significantly related to ADG performance. Conversely, when fed a ‘low’ quality pelleted ration *ad libitum* (P2), sheep of greater initial weight were prone to losing more weight, which was not necessarily exhibited as a loss in BF or EMD. Thus, the change in weight during this period was as a result of other, unmeasured fat...

Figure 4.2: Scatterplot of hot standard carcase weight (HSCW) vs. P2 weight ADG (P2=40 days)
and muscle depots, and/or changes in masses of the rumenoreticulum, liver and/or kidneys. Error in fat and muscle measurement may also contribute to the lack of relationship.

Animals that had greater weight gains had heavier empty rumenoreticulum mass. Rumen mass does not reliably change with energy intake level, but will more reliably increase with physical bulk of the feed (Rompala et al. 1988; Rompala et al. 1990; Sun et al. 1994a; Ortigues and Doreau 1995). Subsequent research by Sainz and Bentley (1997) found that the fore-stomachs of steers fed a fibrous diet exhibited hyperplasia with a resultant increase in organ mass that was greater than concentrate fed counterparts. Increased physical bulk of the feed results in greater levels of distention, and greater resistance to muscular contractions during rumination, hence the increase in cell number and organ mass (Rompala et al. 1988). Increased energy intake can also increase rumen weight, with Mcleod et al. (2007) reporting increased in rumen weight with MEI, and positive effects of ruminal infusions of starch hydrolysate. These increases in mass were as a result of rumen cell hyperplasia rather than hypertrophy (Mcleod et al. 2007). Although the animals in the experiment reported here were provided with similar quality feed in both periods, they did differ in physical form (pasture vs. pellets) and as such the potential for differences in intake level between periods do exist. A relatively greater MEI in P2 may have facilitated sufficient increase in energy level to allow ruminal hyperplasia. Whether the mass of the rumenoreticulum increased during P2 and thus contributed to a proportion of the weight, or were initially larger and facilitated the increased ADG via increased nutrient uptake is unclear.

The greater liver weight of sheep with higher weight gains in P2 is not unexpected. The liver is an active metabolic organ that responds to nutrient content of the diet, in particular the energy and amino acid content (Ortigues and Doreau 1995; Sainz and Bentley 1997). Dietary protein increases the total protein content of the liver, thereby increasing its mass. Research by Sainz and Bentley (1997) determined that increases in energy intake facilitated liver cell hypertrophy,
resulting in an increase in organ mass. Restrict feeding will result in a decrease in liver mass concurrent with the energy intake, with Drouillard (1991a and 1991b) suggesting that liver weight will remain depressed long after re-feeding. Conversely, Wester et al. (1995) and Sainz and Bentley (1997) reported rapid recovery of the liver following re-feeding. As a rapidly dynamic and responsive organ, the relationship observed between liver weight and ADG represents the variation in the level of energy intake between individuals and would thus appear to concur with the previously reported response of this organ.

The relationship between kidney weight and P2 ADG was much the same as observed for liver weight. Another metabolically active organ, kidney mass has been shown to respond positively to increased dietary energy and protein content (Wester et al. 1995). Mcleod et al. (2007) reported greater kidney mass of high-intake steers compared to their low-intake counterparts. The protein effects on kidney mass were highlighted by Fluharty and McClure (1997), who reported greater kidney weights in lambs fed ad libitum energy and 125% of their daily protein requirements. Although the energy and protein content of the diet fed in this experiment was low, differences in MEI and/or microbial protein production may facilitate greater kidney growth. The positive relationship between kidney weight and ADG illustrates this relationship, and will be further investigated in chapter 5.

4.6 Conclusion

During both periods, individual variation in bodyweight, fat and muscle change were observed, successfully indicating that individual variation does exist in response to the nutritional stress imposed on these sheep. The different responses between period likely reflect differences in diet composition, and the stage of animal maturity. When grazing a senescent pasture (P1), initially heavier individuals were fatter, and lost more subcutaneous fat, but not necessarily bodyweight itself. The lack of weight loss is most likely due to changes in skeletal growth as
the sheep were young and still developing. When energy was restricted using a low quality ration (P2), the range in body weight change was considerably higher and included a subset of individuals which lost weight. As there was no measurable loss in BF depth this loss in weight was due to mobilisation of muscle tissue, visceral organs and internal fat depots. Rumenoreticulum, kidney and liver weights were correlated with the rate of weight gain, highlighting that internal organ mass change comprises a component of total weight change.

The presence of variation in body weight and composition change supports the anecdotal claims that differences do occur in ‘resilience’. Bodyweight and the change in bodyweight over time appears to be the best indicator of ‘resilience’ of sheep under nutritional stress as it is correlated with muscling, subcutaneous and internal fatness, visceral organ masses and carcass weight. Given that internal fat, organ and carcass weights are not easily measured in the live animal, bodyweight change remains the easiest and most practical gauge of performance in a commercial setting. Throughout the following chapters discussing experiment 2, weight fat and muscle measures will continue to be discussed, but when referring to ‘resilience’ the author here on will be referring to bodyweight change.
5. Resilient sheep had greater digestible energy intake

5.1 Abstract

Bodyweight change (ADG) is able to encapsulate changes in fat, muscle and organ mass under different conditions, and is thus the best measure of resilience to sub-optimal nutrition. Having defined a measure of resilience, this chapter investigates the variation in feed intake parameters observed during P2 of experiment 2. A 1.44 kg range in daily feed intake (DFI) was measured coupled with the 18 and 25% range in dry matter and energy digestibility respectively. This resulted in a 5MJ/day range in digestible energy intake (DEI) between the sheep in this experiment. The strongest trait correlations were observed between DFI and DEI with ADG (r=0.5 and 0.6 respectively). The strength of these correlations relative to those with ultrasound fat and muscle measurements further strengthens the use of ADG as a measure of resilience. Accounting for animal weight and ADG to calculate an ‘adjusted feed intake’ (AFI) value revealed that there was a 0.53 kg/day range in feed intake required to maintain the same bodyweight and ADG (or level of ‘resilience’). This suggests that when feed quantity and quality are reduced, the energy expenditure of some individuals within a population is lower, requiring a lower gross intake of feed. Thus, under ad libitum feeding conditions, the gross level of intake is the primary driver of resilience, but the existence of variation in AFI indicates the potential for physiological variation in efficiency of gain and/or energy expenditure.
5.2 Introduction

Chapter 4 outlined the extent of variation in body weight and condition and determined that weight change is the most appropriate measure of ‘resilience’. This chapter reports on the relationships between these parameters and feed intake and digestibility traits. The aim of this chapter is to investigate the second mechanism proposed in chapter one:

2. Resilient sheep have greater energy intake when under sub optimal nutrition

Feed intake has a direct effect on sheep performance on low-quality feedstuffs as it is a major determinant of how much energy a given individual can access. In addition, the extent of energy extraction (digestibility) of an individual also determines how much energy they can obtain from a given weight of feed (Wilkes et al. 2012). The general relationship between intake and digestibility in ruminant species is negative, although the exact relationship will vary with physical form of the feed and rate of intake (Kelly et al. 1993; Lourenco et al. 2010). Thus, it is possible for animals to employ different digestive strategies to obtain sufficient energy from the feed (increase throughput at the cost of digestibility or retain feed for longer and extract more from it) depending on the physical form of the feed (McDonald et al. 2002), gut morphology (Purser and Moir 1966; Munn et al. 2008) or rumen microorganisms (Hegarty et al. 2004).

The extent of variation in energy intake between individual sheep, and the relationships with the measurements of energy retention are examined in this chapter.
5.3 Methods

*Animals and their management*

Individual feed intake was measured from the time sheep were moved to the livestock research centre on day 84 until they were transported to slaughter on day 125 (Figure 4.1). After being given 14 days to acclimatise to their new environment and feed, sheep in pens numbered 1-9 were placed into individual metabolism crates (Figure 5.1) on day 98 (Figure 4.1) for a one week period. Sheep in pens numbered 10-18 were then placed into the metabolism crates on day 105 for one week also. Daily *ad lib.* feed intake, urine and faecal output were measured for each sheep and a 10% volume/weight sample taken and immediately frozen for further analysis. Once all sheep were returned to their individual pens, they were allowed to settle for two days.

![Individual metabolism crate (Magnus Industries Pty Ltd.)](image)

*Figure 5.1: Individual metabolism crate (Magnus Industries Pty Ltd.)*
Measurement of sheep energy balance

Faecal samples from the metabolism study were pooled for each sheep on a proportional basis. A 10% weight sample was then taken, weighed and dried in an oven for ~24 hours at 80°C. Dried samples were weighed and the dry matter content of the faeces determined. Samples were then ground to pass a 0.2mm screen using a commercial feed mill grinder and then formed into pellets weighing approximately one gram. The gross energy content of pelleted samples was determined using a Parr 1281 Bomb Calorimeter (Parr Instrument Company, Illinois USA) at the SARDI Pig and Poultry Production Institute Nutrition Research Laboratory, Roseworthy, South Australia. Feed samples were also milled and the energy content determined by bomb calorimetry. The total energy retained was estimated from total energy intake minus total faecal energy output. Apparent energy digestibility was calculated for each individual by calculating the energy retained as a proportion of the energy intake. No account was made for urinary or methane energy.

Statistical Analyses

All statistical analyses were performed using Genstat 15th Edition. General linear models were used to analyse all traits. Distribution and residual plots were checked for each trait before analysis was conducted. Significance of results for all traits was defined as P<0.05.
Feed intake and Digestibility data

To determine factors affecting feed intake, the following model was fitted:

**MODEL 5.1**

\[
\text{Daily feed intake} = \text{Pen type (Crate vs. Pen)} + \text{Crate time (1or2)} + \text{Period 2 Mid-weight} + \\
\text{Period2ADG} + \text{Period2Mid-BF} + \text{Period2ΔBF} + \text{Period2Mid-EMD} + \text{Period2ΔEMD} + \\
\text{Energy digestibility}
\]

The random effects of sheep and day were fitted to account for repeated measures on the same individuals over multiple days (i.e. each sample on each day was analysed).

In order to predict mean daily feed intake values for each sheep, a simple linear model was fitted with fixed effects of initial weight and sheep as per:

**MODEL 5.2**

\[
\text{Daily feed intake} = \text{Initial weight} + \text{Sheep} + \text{Residual}
\]

Adjusted Feed intake (AFI) was calculated in a two-step process. Firstly, a linear model was used to analyse feed intake as proposed by Koch *et al.* (1963), and provide a residual intake value for each sheep. The model contained fixed effects of Period 2 metabolic mid-weight, Period 2 average daily gain and sheep as per:
MODEL 5.3:

\[ \text{Daily Feed intake} = \mu + \beta_1(P2 \text{ Mid Weight}^{0.75}) + \beta_2(P2 \text{ ADG}) + \text{Sheep} + \text{Residual} \]

The mid weight and ADG values used were those obtained from the previous analysis of weight data. Pen and metabolism crate data were analysed separately to generate individual pen and crate AFI values. Day was fitted as a random effect to account for repeated measures. Once the residual value was determined for each individual, an AFI value was calculated based on the deviation of the residual value for each individual from the population mean intake:

\[ \text{Adjusted Feed Intake (AFI)} = \mu + \text{Residual Feed Intake} \]

Energy digestibility data were analysed using a linear model with fixed effects of crate time (1 or 2) and the crate time-by-sheep interaction.

In summary, each sheep had individual intake measured for a period of 45 days, including 7 days in a metabolism crate. The 7 days spent in the metabolism crate created 7 daily measures of faecal and urine output in addition to feed intake.

5.4 Results

Feed intake was significantly affected by housing type (Table 5.1) with a mean intake 0.26kg/day higher when sheep were in floor pens as opposed to the metabolism crate (Table 5.1). Crate measurement time (1 or 2) did not affect feed intake (Table 5.1).
Table 5.0.1: Tests of significance (F-probabilities) for housing type parameters on daily feed intake.

<table>
<thead>
<tr>
<th>Feed intake type (kg)</th>
<th>F Pr.</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen</td>
<td>&lt;0.001</td>
<td>1.83</td>
<td>1.18</td>
<td>2.62</td>
<td>0.36</td>
<td>23</td>
</tr>
<tr>
<td>Crate</td>
<td></td>
<td>1.56</td>
<td>0.84</td>
<td>3.07</td>
<td>0.59</td>
<td>42</td>
</tr>
</tbody>
</table>

The association between metabolic-mid-weight and feed intake was significant, with the slope being 0.23kg increase in daily intake for every 1kg increase in metabolic mid-weight (Table 5.2). Feed intake was also significantly associated with ADG, with a slope of 4.13kg in feed intake for every 1kg increase in ADG (Table 5.2).

When mid-values for BF and EMD, and their rates of gain were fitted as independent variables to model 5.1, they had no significant effect on feed intake (Table 5.2). The effect of dry matter digestibility (DMD) on intake was significant, with a 0.3kg decrease in DFI associated with every 1% increase in DMD (Table 5.2). Energy digestibility was not significantly related to intake (Table 5.2). The relationship between DMD and energy digestibility was positive as would be expected (r=0.54). Although DMD did not vary as greatly as energy digestibility, there was still within breed individual variation (Figure 5.2).
Table 5.0.2: Variance and best linear unbiased estimates for housing and sheep parameters regressed on mean daily feed intake.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F Pr</th>
<th>Slope</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic Mid weight (kg^{0.75})</td>
<td>0.039</td>
<td>0.23</td>
<td>0.09</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0.002</td>
<td>4.13</td>
<td>1.21</td>
</tr>
<tr>
<td>Mid BF (mm)</td>
<td>0.721</td>
<td>-0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>BF Gain (mm/d)</td>
<td>0.364</td>
<td>-3.43</td>
<td>8.39</td>
</tr>
<tr>
<td>Mid EMD (mm)</td>
<td>0.482</td>
<td>-0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>EMD Gain (mm/d)</td>
<td>0.994</td>
<td>0.57</td>
<td>1.22</td>
</tr>
<tr>
<td>Dry matter digestibility (%)</td>
<td>0.013</td>
<td>-0.03</td>
<td>1.65</td>
</tr>
<tr>
<td>Energy digestibility (%)</td>
<td>0.155</td>
<td>-0.013</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 5.2: Scatterplot of energy digestibility vs. dry matter digestibility
Calculation of individual mean values for each sheep revealed a 120% (1.44kg/day) difference between the highest and lowest daily feed intake, which when considered with metabolic mid weight and ADG, gave a 32% (0.5kg/day) difference between the highest and lowest Adjusted feed intake (AFI) (Table 5.3). Adjusting intake for weight and gain halved the CV, with AFI having the lowest variance of all intake related traits. As the energy content of the ration fed was 7MJ/kg, the difference in AFI represents a 3.71 MJ/day range in daily gross energy intake. A 19.8 and 24.9% differential in dry matter and energy digestibility was present which resulted in a 5 MJ/day range in daily DEI (Table 5.3). Both DM and E digestibility were far less variable (CV=11% and 8% respectively) than DEI (18%), indicating that the variance in DEI is largely driven by variance in mass of intake rather than digestibility (Table 5.3).

Due to the large variation between sheep, analysis of individual predicted mean values for DFI, AFI, DMD and E digestibility revealed that there were no significant breed differences for any of these traits (Table 5.3).
Table 5.0.3: Summary statistics, tests of significance (F-probabilities) and best linear unbiased estimates for feed intake and digestibility traits.

<table>
<thead>
<tr>
<th>Breed Mean</th>
<th>Mean</th>
<th>Max</th>
<th>Min</th>
<th>SD</th>
<th>CV</th>
<th>Fpr.</th>
<th>MM</th>
<th>MO</th>
<th>XB</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI (kg/d)</td>
<td>1.8</td>
<td>2.62</td>
<td>1.18</td>
<td>0.36</td>
<td>20</td>
<td>0.21</td>
<td>1.98</td>
<td>1.68</td>
<td>1.62</td>
<td>0.14</td>
</tr>
<tr>
<td>Dry matter digestibility (%)</td>
<td>50.1</td>
<td>58.59</td>
<td>40.0</td>
<td>0.04</td>
<td>8</td>
<td>0.34</td>
<td>48.22</td>
<td>50.23</td>
<td>52.15</td>
<td>1.78</td>
</tr>
<tr>
<td>Energy digestibility (%)</td>
<td>60.4</td>
<td>73.08</td>
<td>48.2</td>
<td>7.13</td>
<td>11</td>
<td>0.58</td>
<td>57.97</td>
<td>61.29</td>
<td>62.4</td>
<td>3.08</td>
</tr>
<tr>
<td>DEI (MJ/day)</td>
<td>7.4</td>
<td>9.54</td>
<td>4.51</td>
<td>1.37</td>
<td>18</td>
<td>0.57</td>
<td>7.89</td>
<td>7.23</td>
<td>7.04</td>
<td>0.59</td>
</tr>
<tr>
<td>AFI (kg/d)</td>
<td>1.8</td>
<td>2.15</td>
<td>1.62</td>
<td>0.17</td>
<td>9</td>
<td>0.72</td>
<td>1.91</td>
<td>1.85</td>
<td>1.77</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Sheep with greater initial BF depth tended to have greater DEI during P2 (r=0.2, Table 5.4). A strong positive correlation was observed between DFI and resultant ADG during P2 (Table 5.4). Once the energy digestibility of each individual was taken into account to calculate DEI the correlation with ADG was strengthened (Table 5.4, Figure 5.3). The significantly lower ADG of one particular individual reduces the strength of this correlation considerably, with the removal of value increasing the correlations to 0.85 and 0.70 for DFI and DEI respectively.
Figure 5.3: Scatterplot of P2 Body weight gain vs. average daily digestible energy intake Line of best fit for complete dataset (black), equation: $y=0.0614x-0.3483$, $r^2=0.332$. Line of best fit with outlier removed (Red), equation: $y=0.0574x-0.2944$, $r^2=0.61$.

P2 initial weight was positively associated with DMD, but not any other intake-related trait. Initial EMD was not strongly related intake or digestibility (Table 5.4). The rates of BF and EMD were slightly positively correlated with DEI ($r=0.2$ and 0.3 respectively), which as for weight gain were slightly higher than those observed for the correlation with DFI (Table 5.4).
Table 5.0.4: Correlations between initial and gain values for weight and body composition traits with feed intake parameters from Period 2.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Initial BF</th>
<th>Initial Weight</th>
<th>Initial BF</th>
<th>Initial EMD</th>
<th>ADG</th>
<th>Δ BF</th>
<th>Δ EMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI (kg)</td>
<td>0.2</td>
<td>0.0</td>
<td>0.2</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Dry matter digestibility (%)</td>
<td>0.0</td>
<td>0.3</td>
<td>0.0</td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>E Digestibility (%)</td>
<td>-0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>-0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>DEI (MJ/day)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>0.6</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>AFI (kg/day)</td>
<td>-0.2</td>
<td>-0.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

5.5 Discussion

The 1.44 kg range in DFI measured coupled with the 18 and 25% range in dry matter and energy digestibility resulted in a 5MJ/day range (4.5-9.5 MJ/day) in DEI across the sheep in this experiment. This indicates that there was large individual variation in voluntary intake, digestive performance and resultant energy intake. The 0.53kg/day range in AFI highlights the presence of variance in efficiency of gain and/or basal metabolic rate (Nkrumah et al. 2004b).

5.5.1 Feed intake and Digestibility

Gross weight of feed consumed and the resultant level of nutrient extraction are intimately linked. Feed intake was negatively related to digestibility as expected (Kelly et al. 1993; Lourenco et al. 2010; Wilkes et al. 2012). Furthermore, the precise relationship between intake
and digestibility is affected by many factors, including the physical size and capacity of the rumen (Munn et al. 2008), physical form and fibre content of the feed (Pralomkarn et al. 1995), level of mastication and particle breakdown pre-digestion (Doreau et al. 2003), rumen microbial population (Hegarty et al. 2004) and previous dietary regime, particularly during development of the rumen (Abou Ward 2008). Evidence suggests that inherent genetic differences may exist in the ability to cope with poor quality diets (Barriere et al. 1992; Lee et al. 2002). It is likely that one or more of these factors are causing differences in digestibility observed in these results.

Despite their respective intake and digestibility differences, the average daily DEI for each breed did not differ significantly (Table 5.4). The relatively large standard error (±0.59 MJ/day) does indicate a degree of individual variability, which will contribute to the lack of significant breed effect. A greater number of animals from each breed, sourced from numerous different localities would be required to test the true breed differences.

5.5.2 Feed intake and resultant growth

ADG was affected by the feed intake and metabolisable energy intake of the sheep in this experiment (Table 5.2). The relationship between intake and growth is well reported, with strong correlations (r=0.8) reported between ADG and intake in cattle (Nkrumah et al. 2004b). Vieira et al. (2013) used data from 13 studies of Sante Ines ram lambs, reporting a significant, moderate correlation (r=0.6) between ADG and intake across a range of different diet ingredients and compositions. Most previous research has focused on the use of diets that are of high quality and designed to optimise growth. This work differs in that animals were offered a low quality ration ad libitum and allowed to exhibit their intake and resultant growth capability. As a result, the growth rates obtained may be low, but the positive relationship with intake remained. The low ADG exhibited by one sheep reduced the strength of the relationships
considerably. However, no signs of internal parasitism or caecal inflammation were observed on post-mortem to suggest this individual was physiologically impaired, and as such the values obtained may actually be representative of that individual’s ability to perform on a low quality diet.

The inverse of the slope of ADG on intake was 4.13 kg intake for every kg of gain, and can be defined as a value of efficiency of gain for the sheep in this experiment. Values in the literature range from 3-3.5:1 for Sante Ines lambs (Cirne et al. 2013), to 5.5:1 for Pelibuey lambs (Salinas-Chavira et al. 2013) and 6.5:1 for Dorper and Rambouillet lambs (Yeaman et al. 2013). Using values of mean DFI from Table 5.3 and ADG from Table 4.3, a total feed conversion ratio of 17.7 kg feed/kg gain is achieved. Given the low quality nature of the diet consumed this value is not unreasonable.

5.5.3 Adjusted Feed Intake

Accounting for body size and rate of gain provides a residual feed intake (RFI) value, which is expressed as deviations from the population mean. Adding each individual's RFI value to the population mean intake creates the trait referred to herein as adjusted feed intake (AFI). AFI was far less variable than DFI (CV=9% vs. 20% respectively, Table 5.3). Despite breed differences in RFI being reported in cattle (Retallick et al. 2013) and lambs (Kirschten et al. 2013), no significant difference in AFI was observed. Once again it must be remembered that most tests of RFI are undertaken on diets that optimise growth, and that the low quality diet fed here, stimulated variable performance and has exacerbated the variation in the residual component of intake, and thus AFI. Nonetheless, the presence of individual variation in AFI may provide clues as to differences in overall efficiency and the ability to maintain condition on a low quality diet.
5.6 Conclusion

The aim of this chapter was to quantify the extent of individual variation in energy intake of sheep when fed a low-quality diet *ad libitum*. A significant amount of individual variation in feed intake and digestibility traits were expressed. The significant relationship between DMD and intake was not unexpected, and is of primary importance when explaining the variation in intake.

In the context of this experiment, resilience, as defined by ADG of sheep under poor quality nutrition was most significantly influenced by the gross level of energy intake. This effect on ADG was largely driven by gross feed intake (r=0.5), with the inclusion of energy digestibility raising the strength of the correlation to 0.6.
6. Resilient sheep had greater energy expenditure

6.1 Abstract
Oxygen uptake per heartbeat, known as the oxygen pulse, is constant for a given individual sheep across a range of HR (Chapter 3). The primary aim of the current work was to further validate the O₂ pulse technique, and then use individual oxygen pulse values with longer term measures of HR to calculate energy expenditure. Overall, 92% of the total variance in VO₂ could be accounted for. The most significant finding from this is the small proportion of variance in VO₂ as a result of differences in O₂ pulse, and the apparent constancy of pulse between environments and across measurement days. Garmin HR and GPS monitors were attached to sheep during P1 to measure activity level and HR whilst sheep were grazing. Despite considerable efforts to maintain contact and signal conductivity, the use of externally-mounted HR electrodes in sheep proved unsuccessful and no consistent measures of long-term HR could be recorded. As a result, only short term measures of HR and VO₂ could be used to predict energy expenditure (EE). Calculated EE values were positively correlated with ADG (resilience), contradicting the hypothesis that lower EE would be associated with greater resilience. Development of internally-planted logging devices to measure diurnal changes in HR should allow total daily EE to be calculated with greater accuracy and validate the true association with resilience.

6.2 Introduction
At a given level of energy intake, lower energy expenditure means greater energy availability for maintenance and growth of body tissues. Thus, when energy supply is limiting, lower energy expenditures increase the threshold at which body tissues will start to be mobilised to provide
energy to survive. Differences in energy expenditure may occur via differences in basal energy expenditure (metabolic rate), or differences in activity level and thus total energy expenditure (Koong et al. 1985; Canas et al. 2003; Lachica and Aguilera 2005).

This chapter characterises the individual variation in energy expenditure, as determined by the use of heart rate and oxygen consumption measures. Further validation of this technique was also undertaken as per chapter 3. The use of GPS to monitor activity in grazing sheep will also be discussed. It is hypothesised that lower measures of energy expenditure will result in greater energy availability to the animal, which will be expressed via greater growth or maintenance of body weight.

6.3 Methods

Procedure for establishing the VO$_2$ and HR relationship

Throughout the trial each individual’s heart rate (HR) and oxygen consumption (VO$_2$) were measured simultaneously using an ADInstruments physiograph machine and gas analyser (Section 3.2). Measurements were taken for each sheep on days 10, 45, 52 and 58 during Period 1, and days 84 and 90 during Period 2.

From day 0-10, sheep underwent familiarisation with the respiratory flow mask twice-daily. This involved the sheep being held with the mask over their muzzle until they would calmly stand and breathe into the mask.

On the measurement days, VO$_2$ and concurrent HR was measured prior to feeding for approximately 5 minutes per sheep, or until a relatively steady heart rate was achieved for more than a minute. This ensured that VO$_2$ and HR were measured over a range of heart rates and that the basal rate and its concurrent VO$_2$ could be adequately quantified.
Field measurement of heart rate was undertaken using Garmin Forerunner 910 XT heart rate/GPS monitors (Figure 6.1). The Garmin units consist of an elastic strap with built in contact electrodes, which remotely transmit to a watch capable of logging data for approximately 20 hours. 6 Garmin units were used in this study, and thus on a given day the HR of only six sheep were measured. The units were rotated around the 18 sheep on a 3 day rotation, where sheep were randomly allocated to a measurement day, ensuring that within a 3 day measurement block all sheep were measured.

In order to fit the measurement devices, sheep were clipped around their girth behind the shoulders to remove wool and ensure firm contact of the electrodes with the skin. Before the strap was attached to the sheep, the skin was cleaned with 80% ethanol solution to remove grease and dirt. The strap was then placed around the girth and tightened firmly, ensuring the electrode pads maintained firm contact with the skin, as per (Figure 6.2). Elastic cohesive bandage was wrapped around the girth of the sheep over the HR strap to provide additional pressure, and minimise movement of the strap. Once secured, the watch was turned on and the recording sequence commenced. As each sheep was fitted with the device, it was released into a small holding yard until all sheep were fitted and they could be released back into the paddock as a mob. Sheep were mustered the following morning so that the devices could be removed and data downloaded. The units were charged and then fitted onto the next six sheep. In order to enable consecutive days measurement, whilst encompassing the vital time for grazing during the day (early morning and late evening), devices were fitted by 2pm, and then removed around 10am the following morning.
Considerable difficulties were encountered using the Garmin units to measure HR for extended periods of time. High ambient temperatures, dirt and grease (lanolin) prevented conductivity and thus constant HR measurement. Numerous different electrode, strapping and padding methods were employed to overcome this with no success. The measurement of HR using these monitors was ultimately abandoned. Some GPS data were collected during the trial whilst efforts were made to obtain consistent HR measures.

Figure 6.1: Garmin Forerunner 910XT heart rate and GPS monitor chest strap and recording watch
Statistical analysis

**VO$_2$, HR, O$_2$ pulse and Energy Expenditure Data**

In order to determine the magnitude of the components contributing to variance in heart rate, a linear model was fitted with fixed effects of day and environment, and random effects of sheep-by-day, sheep-by-environment and sheep-by-day-by-environment interaction.

In order to predict a singular mean HR value for each sheep, a linear model was fitted with date of measurement as a fixed effect, and sheep as a random effect to account for repeated measures on the same individual.

Upon inspection, the data for VO$_2$ and O$_2$ pulse revealed a skewed distribution. Log, square root and cube root transformations were applied to the data and the distributions re-assessed. The cube root transformation normalised the data appropriately and was used for analysis. Heart
rate data for each sheep on each day was calculated as a standardised residual, called New (standardised) HR for the analysis of VO₂ and O₂ pulse, by the following:

\[
New \,(\text{standardised})\,HR = \frac{(Old\,HR - Mean\,HR)}{SD}
\]

In order to determine the significant components contributing to variance in VO₂ the following model was fitted:

\[ VO₂ = Environment\, (indoors\,or\,outdoors) + new\,HR. \]

The random effects fitted in the model were as follows:


Temperature Humidity Index was calculated by the following equation:

\[
THI = (1.8 \times T + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times T - 26))
\]

Where:

T= temperature (°C)

RH= Relative Humidity (%)
**O₂ pulse predictions**

O₂ pulse values were predicted using the following model:

\[(O₂ \text{ pulse}) = \text{Environment (indoors or outdoors)} + \text{Date} + \text{Environment-by-Date}\]

Initial modelling included random effects of:

\[\text{Sheep} + \text{Sheep-by-Environment} + \text{Sheep-by-Date} + \text{Sheep-by-Environment-by-Date}\]

This was then simplified to a model containing a random effect of sheep alone to account for repeated measures on each individual.

**Estimation of daily energy expenditure**

The technical issues encountered whilst trying to gather long term HR data mean that no long term HR data could be used in conjunction with estimated O₂ pulse values to determine a daily energy expenditure value. To gain some appreciation for daily energy expenditure, predicted mean HR values for each sheep (obtained from the 6 measurement days) were used in conjunction with predicted O₂ pulse values to calculate a nominal energy expenditure as per the following:

\[\text{Energy Expenditure (MJ per day)} = (\text{Predicted mean HR (bpm)} \times 60 \times 24) \times (\text{Oxygen pulse} \times 0.02047)\]

(Brosh et al. 2002)
The daily energy expenditure values were then analysed using a linear model with fixed effects of breed and sheep.

*GPS data*

Due to the technical difficulties associated with measuring HR using the Garmin Forerunner units, a limited number of complete measurements of GPS logged activity were available. Two complete, 17-19 hour measurement periods were collated for each individual. HR data were too incomplete to contemplate analysis so only locomotion data were extracted for analysis. From these measurements, summary data was calculated for each of the following traits:

- Average distance travelled
- Average speed
- Average moving speed

In order to determine individual animal and day effects for each trait a linear mixed model with fixed effects of sheep and measurement date. Individual sheep predictions were calculated for each trait to be used for correlating with other traits in Chapter 8.

Breed effects were tested using a linear mixed model with fixed effect of breed, and random factors sheep and measurement day.
6.4 Results

6.4.1 Factors contributing to variance in VO₂ and Heart Rate

VO₂ measurements

Of all the VO₂ data collected, 92% of the variance in individual oxygen consumption could be explained by the parameters measured (Figure 6.3). Individual sheep differences only accounted for 6% of the total variance as compared to the 30% reported in Chapter 3. The greatest contributor to variance in VO₂ was the day effect which amounted to 48%. The sheep-by-environment effect alone did not comprise a quantifiable proportion of the variance. Fitting the temperature humidity index did not account for any of the variance. The sheep-by-day-by-environment interaction contributed to almost one third, indicating that the VO₂ of sheep varied between the days in each environment. O₂ pulse (sheep-by-New HR) didn’t have a significant effect, indicating that O₂ pulse values didn’t differ between sheep. Sheep-by-environment-by-New HR contributed to very little variance, indicating minimal variation in O₂ pulse between indoor and outdoor conditions. There was however, an 8% contribution of Sheep-by-day-by-environment-by-New HR, which indicates the potential for variation in pulse between days within environmental housing treatment. The unexplained residual variance in VO₂ comprised a mere 8% (Figure 6.3).
Figure 6.3: Percentage of sums of squares for components contributing to variance in VO2.

Heart rate measurements

Calculation of the accuracy of HR values recorded revealed that measurement of 60, 10s intervals (10 minutes) of HR, the accuracy of the HR values is over 98.5%, and continues to increase thereafter (Figure 6.4). Thus, while 5 minutes gave a good indication, a cumulative measurement period of at least 10-15 minutes is recommended to ensure an accurate representation of a given individuals HR profile.
Figure 6.4: Accuracy of HR value measurement vs. duration of measurement period defined as true correlation with heart rate.

Analysis of all measurements of HR revealed that 94% of the variance in this trait could be accounted for by taking into account sheep, day and environment factors (Figure 6.5). 50% of the variance in HR was attributed to different sheep response to the environment in which the HR was measured (indoor vs. outdoors). The individual sheep effect of 16% illustrates that differences in HR exist between different individuals, whilst the lack of a sheep by day interaction shows that the HR measured for an individual on any given day is representative of its HR. The presence of a sheep-by-day-by-environment interaction indicates some variation between days within an environment, yet its effect may be included in the sheep-by-environment interaction if day is confounded with environment.
6.4.2 Environment, breed and individual effects on metabolism traits

There was no significant environmental effect on VO$_2$ measured in this experiment (Table 6.1). Breed and sheep effects on VO$_2$ were also not significant (Table 6.1).

Analysis of the predicted mean HR value for each sheep within each environment revealed that there was a significant effect of environment, but no breed or sheep difference (Table 6.1). Analysis of HR data for each environment separately revealed a mean HR of 75.8 beats/minute for indoors and 93.2 beats/minute for outdoors, with the values measured indoors showing a higher CV than outdoors (Table 6.2). Although the breed effect was not significant, the MM sheep had the highest mean HR, followed by the XB and then MO in both environments (Table 6.2).

Predicted O$_2$ pulse values did not differ between environments (Table 6.1). It must be noted that although the means were not significantly different the standard errors of both VO$_2$ and O$_2$ pulse were significantly larger for the outdoor measurements than those taken indoors (Table 6.1).
Initial modelling to extract individual predictions of O$_2$ pulse revealed that the environment by date interaction was not significant, and thus was removed from the model. The sheep-by-environment-by-day and sheep-by-day terms had very low variance components, and were also removed from the random effects. The low sheep-by-day variance, in conjunction with the short term of the trial, and relatively small changes in body weight was deemed sufficient reason to not need to calculate the slope of pulse change over time. As a result, only sheep was fitted as a random effect in the final model and a singular prediction of O$_2$ pulse for each sheep obtained. There was no significant difference between breed in O$_2$ pulse, nor was there any sheep effect within breed (Table 6.1).

From calculations based on a predicted daily HR and O$_2$ pulse value, daily energy expenditure for indoors and outdoors was calculated. There was a significant environmental effect on EE, with calculations for outdoors returning larger mean values of expenditure (Tables 6.1 and 6.2). Significant breed differences were observed in mean daily EE, with the MM sheep having the highest value whilst indoors. The MO had the lowest indoor expenditure whilst XB sheep were intermediate between the two (Table 6.3). Whilst outdoors, the MM sheep had the highest expenditure, and the XB had only a slightly lower value. The MO sheep had the lowest mean expenditure outdoors with a value 0.57 MJ/day less than the XB. Within breeds there were no significant sheep differences in daily EE observed (Table 6.2).
Table 6.0.1: Tests of significance (F-probabilities) for O\textsubscript{2} pulse, HR and EE traits

<table>
<thead>
<tr>
<th></th>
<th>Environment</th>
<th>Breed</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO\textsubscript{2} (L/min)</td>
<td>0.142</td>
<td>0.102</td>
<td>0.465</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>&lt;0.001</td>
<td>0.157</td>
<td>0.574</td>
</tr>
<tr>
<td>O\textsubscript{2} pulse (ml/beat)</td>
<td>0.167</td>
<td>0.107</td>
<td>0.473</td>
</tr>
<tr>
<td>EE (MJ/d)</td>
<td>0.003</td>
<td>0.023</td>
<td>0.761</td>
</tr>
</tbody>
</table>

Table 6.0.2: Best linear unbiased estimates of breed effects on O\textsubscript{2} pulse, HR and EE traits

<table>
<thead>
<tr>
<th></th>
<th>Overall Sheep Means</th>
<th>Breed Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
</tr>
<tr>
<td>Outdoor (P1) HR (beats/min)</td>
<td>93.2</td>
<td>78.4</td>
</tr>
<tr>
<td>Indoor (P2) HR (beats/min)</td>
<td>75.8</td>
<td>61.1</td>
</tr>
<tr>
<td>O\textsubscript{2} Pulse (ml/beat)</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Outdoor (P1) EE (MJ/d)</td>
<td>6.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Indoor (P2) EE (MJ/d)</td>
<td>5.3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Different postscript letters indicate significant difference
6.4.3 GPS-derived outdoor grazing measurements

The average distance travelled (expressed as distance within an hour) was significantly affected by both sheep and measurement date factors (Table 6.3). The overall mean distance covered within an hour was 280m, with a 190m range in measured values (Table 6.3). Both total average speed and average moving speed were differed between sheep and measurement day (Table 6.3). Total average speed was 0.27kph (0.075ms$^{-1}$) with a 0.16kph (0.044ms$^{-1}$) range. Mean moving speed of all sheep was 4.5kph, or 1.24ms$^{-1}$ across a range of 2.7kph (0.76ms$^{-1}$).

Average distance travelled within an hour did not differ between breeds, with predicted mean values ranging from 0.28km for the MO to 0.30 km for the MM (Table 6.3). Average total speed overall was similar for all three breeds, but average moving speed differed significantly with MO sheep exhibiting the fastest speed at 4.6kph (1.3ms$^{-1}$). The mean speed of the XB sheep was 4% lower at 1.2ms$^{-1}$, whilst that of the MM was some 16% lower than the MO at 1.1ms$^{-1}$ (Table 6.3).

All correlations between activity measures and HR, O2 pulse and calculated EE were low (Table 6.4).
Table 6.0.3: Summary statistics and tests of significance (F probabilities) and best linear unbiased estimates for breed, sheep and date effects on Garmin Forerunner 910XT measured HR and GPS traits.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Max</th>
<th>Min</th>
<th>SD</th>
<th>CV</th>
<th>Breed</th>
<th>Sheep</th>
<th>Date</th>
<th>MM</th>
<th>MO</th>
<th>XB</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Distance travelled/ hr (km)</td>
<td>0.40</td>
<td>0.23</td>
<td>0.06</td>
<td>21.4</td>
<td>0.34</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>0.28</td>
<td>0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>Average Speed (kph)</td>
<td>0.30</td>
<td>0.24</td>
<td>0.07</td>
<td>25.9</td>
<td>0.11</td>
<td>0.03</td>
<td>0.002</td>
<td>0.31</td>
<td>0.29</td>
<td>0.27</td>
<td>0.02</td>
</tr>
<tr>
<td>Average moving speed (kph)</td>
<td>6.11</td>
<td>3.42</td>
<td>0.67</td>
<td>14.9</td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>3.97</td>
<td>4.61</td>
<td>4.41</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Table 6.0.4: Correlations between activity traits and HR, oxygen consumption and calculated energy expenditure values

<table>
<thead>
<tr>
<th>Distance/hour</th>
<th>Average Speed (kph)</th>
<th>Average moving speed (kph)</th>
<th>Outdoor HR</th>
<th>Indoor HR</th>
<th>O\textsubscript{2} Pulse</th>
<th>OUTDOOR EE</th>
<th>INDOOR EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance/hour</td>
<td>1.0</td>
<td>1.0</td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Average Speed (kph)</td>
<td>1.0</td>
<td>1.0</td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Average moving speed (kph)</td>
<td>1.0</td>
<td></td>
<td>-0.1</td>
<td>-0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Outdoor HR</td>
<td>1.0</td>
<td>1.0</td>
<td>0.2</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor HR</td>
<td>1.0</td>
<td></td>
<td>0.2</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O\textsubscript{2} Pulse</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OUTDOOR EE</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>INDOOR EE</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
6.4.4 Correlations between metabolic parameters and body weight/composition change traits

Oxygen pulse was positively associated with initial weight EMD and BF in both periods (Table 6.5). Gain of weight, EMD and BF in P2 were all positively related to $O_2$ pulse. HR exhibited no strong correlation with any trait other than P2 EMD gain. Calculation of EE revealed that weight, fat and muscle gain were all associated with energy expenditure. As a result of their relationship with gain of weight, EMD and BF in P2 were all positively related to $O_2$ pulse. HR exhibited no strong correlation with any trait other than P2 EMD gain.
Table 6.0.5: Correlations between metabolic and growth performance traits of 17 wethers of three breeds across two experimental periods

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th></th>
<th>P2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial Weight</td>
<td>Initial EMD</td>
<td>Initial BF</td>
<td>ADG</td>
</tr>
<tr>
<td>O2 Pulse</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>OUTDOOR (P1) HR</td>
<td>-0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>INDOOR (P2) HR</td>
<td>-0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>OUTDOOR (P1) EE</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>-0.1</td>
</tr>
<tr>
<td>INDOOR (P2) EE</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>-0.1</td>
</tr>
</tbody>
</table>
6.5 Discussion

The difficulties surrounding long term measurement of heart rate of sheep using contact ECG electrodes was a major limiting factor in this experiment. Although work in this field has previously been conducted with sheep, there is insufficient detail reported regarding the application of HR monitors, or the level of success. Arieli et al. (2002) and Aharoni et al. (2003) used ‘Dansoft’ manufactured data logger ‘fastened to a harness attached to the thorax behind the legs’, whilst Landau et al. (2006) used ‘external electrodes and data loggers’ made by the same company, but neither authors gave specific details on location and attachment of electrodes. Research presented by Animut et al. (2005) reports the use of Polar S610 HR monitors with no specific detail of the attachment procedure whilst Beker et al. (2010) also used Polar HR monitors, placing one electrode behind the shoulder and the other at the base of the neck on the opposite side, though no further specific details are given (Beker et al. 2009; Beker 2010). Barkai et al. (2002) provided more detail in their methodology, stating that the flank was shorn before the attachment of a flexible chest band, to which Dansoft HR loggers were attached. The authors do mention the use of nickel coins under the chest band to serve as electrodes and ensure conductivity. Although they do not elude to difficulties in measuring HR, the number of ‘satisfactory’ HR data files obtained varied for the four treatments imposed, and would tend to indicate that sometimes difficulties obtaining complete HR data were encountered. Interestingly, since the completion of this study, personal communications with Dr Dean Revell, (formerly CSIRO Western Australia) and Arieh Brosh (Israel) have revealed that they had similar difficulties in obtaining good electrode contact with sheep. Given the lack of detail in the literature, and the significant difficulties encountered in this experiment, further investigation into methods of improving HR measurement in sheep need to be undertaken, and reported to the scientific community.
Despite difficulties obtaining long term measures of HR, the short term measures undertaken in conjunction with those of VO\textsubscript{2} proved successful. The accuracy of such short term measures for the estimation of long term energy expenditure at first seems questionable but the accuracy calculations have shown that ten minutes of HR elicited 98.5% accurate values of HR for a given individual. The correlation between indoor and outdoor measures of HR indicate its consistency long term between environments. In addition, neither HR nor VO\textsubscript{2} contributed significantly to the variance in one-another indicating that they were steady during measurement and did not increase disproportionately of one another. Initial studies of the technique by Brosh \textit{et al.} (1998) involved measurement for 15-20 minutes, with most other studies being conducted for 10-20 minutes (Arieli \textit{et al.} 2002; Barkai \textit{et al.} 2002; Aharoni \textit{et al.} 2003; Landau \textit{et al.} 2006). Brosh (2007) concluded that 5-15 minutes would be sufficient to enable stable measurement of both HR and oxygen consumption parameters. The results of this trial concur with those existing in the literature, and thus, even well-trained subjects require at least 10 minutes acclimatise to the measurement procedure.

Large environmentally-induced differences in HR (Table 6.2) are not unexpected and concur with previous research highlighting similar differences between confined and free ranging animals in HR (Barkai \textit{et al.} 2002; Landau \textit{et al.} (2006). There are a number of potential causes for the increase in measured HR observed in sheep in the outdoor situation. Heat load has been shown to increase HR in sheep (Barkai \textit{et al.} 2002), and thus may have contributed to the increased HR observed in sheep outdoors. Ambient temperature and solar radiation both contribute to heat load and thus sheep outdoors were exposed to these elements (Sleiman and Saab 1995; Barkai \textit{et al.} 2002). Total energy intake, and pattern of feeding has a direct influence on metabolic heat production and may also have increased HR outdoors (Baldock \textit{et al.} 1988; Brosh \textit{et al.} 1998). Landau \textit{et al.} (2006) found that grazing sheep had a bi-modal pattern to HR associated with grazing behaviours (morning and evening) whilst when confined HR was
mono-modal and more associated with feeding behaviour. As sheep indoors had access to feed at all times it is likely that their feeding pattern was more constant and thus increases in HR associated with feeding may not have been as great as those outdoors (Mohr and Krzywanek 1993; Arieli et al. 2002). As no successful measures of long term HR were achieved this cannot be investigated.

The 16% contribution of individual sheep differences to variance in HR may be due to inherent differences in HR between individuals (Syme and Elphick 1982; Baldock et al. 1988) or differences in response to handling during the measurement (Hargreaves and Hutson 1990). The lack of sheep by day interaction however, suggests that differences in handling and a given sheep’s response to handling may not be playing a role and the effects observed may be due to genuine differences between individuals. The presence of a minor sheep by day by environment effect may be due to handling effects, but is more likely due to environment specific characteristics such as heat load. Despite the contribution to the variance component, analysis of the data revealed that there was no significant effect of ‘sheep’ on HR, nor was there a breed effect. Thus it would appear that individual differences at the time of HR and VO2 measurement is not a significant source of variation that may contribute to variation in energy expenditure.

A large proportion (92%) of the total variance in VO2 could be explained by sheep, day and environment effects, the interaction between them as well as the HR-environment interaction (Figure 6.4). This is considerably higher than the 68% variance explained as presented in Chapter 3. The large contribution of day variance (48%) indicates that perhaps this is due to the greater number of measurement days and total data volume. Differences between the environmental conditions encountered may be thought to contribute to this day variance yet there was no significant contribution of environment nor the sheep-by-environment interaction. The lack of effect of temperature humidity index effect indicates that temperature-induced changes in VO2 are unlikely to be the cause of this variation. The 30% contribution of sheep-
by-day-by-environment does however indicate the fact that sheep could respond differently between days within an environment. Due to the lack of temperature effect, the daily variance observed is thus most likely due to differences in response to handling and pre-measurement heat load as a result of exercise or feed intake (Brosh 2007). As no pre-measurement quantification of activity or intake is possible during P1 this remains unknown.

The apparent lack of environmental effects on VO$_2$ at the time of measurement draws attention to the presence and role of inherent sheep differences. However, individual sheep only contributed to 6% of the variation in VO$_2$, which is considerably lower than the 30% measured in experiment 1. The reduction in sheep variance in this experiment may be attributed to a more thorough training program, as well as greater number of measurement days, which would reduce the incidence of stress and handling effects altering respiration parameters. The lower sheep variance in these results is an interesting finding in comparison to those obtained previously that may indicate differences in acclimatisation to the measurement procedure.

Although understanding environmental and individual sheep components of VO$_2$ variance are important, the significance of oxygen pulse (sheep by HR interaction) and its various interactions is of far greater importance when considering implications for measuring energy metabolism. The lack of sheep and sheep-by-HR interaction indicates that there is little variation between sheep in their oxygen uptake as heart rate changes. This is not dissimilar to the minor 4% contribution from experiment 1, as presented in Chapter 3. Grouping individuals in genotype similarly revealed no significant ‘breed’ effects on O$_2$ pulse. However, the predicted mean pulse values for the XB were some 13% higher than that for the MO and MM, which were the same, although greater samples sizes would be required to validate this.

Despite no effect of environment on O$_2$ pulse (Sheep-by-HR-by-environment interaction), inclusion of day within the interactions elicited an 8% contribution to total variance. Thus there
is the potential for differences in O$_2$ pulse difference between measurement days. Observation of the slopes of environment by HR for VO$_2$ reveals slopes that approach zero, though that for the indoor measurements is slightly negative, indicating that as HR increases O$_2$ pulse will decrease. In addition, the variability of outdoor measurements was considerably greater than that for indoors. Interestingly, work by Barkai et al. (2002) found no effect of measurement day on O$_2$ pulse, indicating the potential for an unaccounted for variable in these results. Despite the presence of some daily variation, the lack of environmental differences means that for the purposes of these results, a universal measure of O$_2$ pulse can be applied for each individual based on measurements in both environments.

There are a number of potential causes for day to day variation in O$_2$ pulse. Heat load as a result of MEI has been shown to confound measurements (Barkai et al. 2002; Brosh et al. 2004) although contradictory reports of no effect exist (Arieli et al. 2002; Aharoni et al. 2003). Dietary composition and time of measurement post feeding seems to have a greater effect, with energy dense diets shown to increase pulse values (Brosh et al. 1998). Thus, differences in feeding pattern and intake composition would have had a greater effect on the sheep when outdoors, and thus may explain the greater degree of variability in these traits. When indoors, feed was available at all times and as a result the influence of the heat increment of feeding are likely to be less. Repeated measures of O$_2$ pulse over the experimental period are likely to ameliorate the effects of intake reduced variation and lead to greater accuracy of results.

Despite no significant individual differences in EE, there was a significant effect of environment and breed type on the calculated values. EE values calculated outdoors were higher than those for indoors, presumably driven by the higher HR values observed in this environment. Interestingly, MM sheep had the highest EE indoors, followed by the XB and then MO. Once again this is driven by the higher HR of the MM. Outdoor EE means for the MM and XB were similar, although the MM remained highest. It is interesting to note the higher
pulse value of the XB did not infer a greater EE value in either environment, despite quite similar mean HR values. Thus it appears that the EE differences calculated are largely as a result of differences in HR rather than O$_2$ pulse.

The increased association between oxygen pulse and P1 initial weight observed is because more oxygen is required to fuel greater tissue mass of those larger individuals. Species comparisons show strong linear increases in heart weight, haemoglobin weight and total blood volume as body weight increases. As a result, stroke volume will increase and a proportional increase in oxygen pulse will be observed (Astrand et al. 2003). The correlation between P2 initial weight and O$_2$ pulse was lower, though still positive (r=0.3). As the sheep entered the experiment at a young age and on a good plane of nutrition, the relationship between the initial weight at the commencement of the trial and O$_2$ pulse can be expected to be stronger than that observed following variable weight changes on a sub-optimal diet (P2).

The positive relationship between O$_2$ pulse and Back fat measures in both periods is possibly driven by alterations in stroke volume. Human studies have shown that stroke volume increases with adiposity (Alexander et al. 1962; Pflieger et al. 1994). However, the levels of subcutaneous adiposity measured in this experiment were not extremely high, and the lack of internal fat measures makes the potential effects of adiposity on stroke volume impossible to quantify.

The relationship between P1 BF and O$_2$ pulse may also be driven by the positive correlations observed between initial weight and BF. Interestingly, P2 initial weight was not strongly correlated with O$_2$ pulse, yet the initial measure of BF in P2 was (Table 4.3). Weak correlations between P2 initial weight and P1 measures of weight and BF, along with the moderate positive correlation between P1 and P2 BF measures (r=0.4) indicate that BF had a greater effect on the O$_2$ pulse measured during this experiment than body weight itself. As no total adiposity measure was taken it is somewhat difficult to separate the bodyweight vs. adiposity effects on O$_2$ pulse.
In contrast to the relationship with BF, P2 measures of EMD correlated poorly with bodyweight in either period, thus the positive correlation between EMD and O$_2$ pulse are independent of the proposed bodyweight effects. Studies of humans have reported positive correlations between fat-free body mass and heart volume, with resultant correlations in tissue oxygen uptake (Patrick and Cotes 1978; Cotes et al. 1980). The relationship between fat-free mass and cardiac output is stronger than with adipose mass, and is unrelated to arterial pressure, diabetic state, body habits and age (Collis et al. 2001). No work has been conducted in sheep to determine individual variation in musculature and its relationship with cardiac parameters. Based on human findings, it is not unreasonable to postulate differences in muscle mass and potentially heart function (particularly stroke volume). This difference in muscle mass may arise due to genetic predisposition or differing ‘fitness’ as a result of different environmental background. As no measure of stroke volume, whole-body musculature nor ‘fitness’ capacity was made herein this is purely speculative.

The positive correlations between gain values for BF and EMD in P2 with O$_2$ pulse would indicate that those individuals with a greater oxygen uptake were those that exhibited greater gains in fat and muscle. It must be remembered that these sheep were not on an optimum diet and growing to their full potential, and thus ‘greater gains’ of the highest performing individuals are only slightly positive, whilst the lower performing individuals had negative values for these traits. As a result gain values may be hard to interpret clearly as individual response to the dietary regime was variable.

Based on the independent effect of bodyweight on EMD, increased gain of fat and muscle of initially musclier animals and their associated higher values of O$_2$ pulse, it appears that sheep with greater muscling were able to maintain condition or grow under the nutritional conditions imposed. It is not clear whether the increased O$_2$ pulse of these individuals is causative of the increased muscularity and growth potential, and symbolises an animal’s ‘fitness’. In contrast,
the increased muscling and growth potential may be due to other metabolic or digestive efficiency parameters, and as a result of their greater growth performance, an increase in O$_2$ pulse is caused. As the whole body musculature, ‘fitness’ level and developmental history were not recorded it is difficult to determine the correct explanation.

Given the large data pool behind the predictions of O$_2$ pulse and investigations into its variance, it is likely that the values obtained are representative of the true values for each individual. As the HR used to calculate EE was over short periods of time, they are not representative of the daily HR patterns and as such are limiting these results. The causes for increased EE in grazing animals pertain to the energy costs associated with locomotion and grazing activities (Osuji 1974; Lachica and Aguilera 2003).

Compared to confined measures, increases in field EE are reported to range from 25-92% for small ruminants (Coop and Hill 1962; Animut et al. 2005). Terrain (Lachica et al. 1997; Animut et al. 2005), forage availability (Davies and Southey 2001; Animut et al. 2005) and forage quality (Lachica and Aguilera 2003; Aharoni et al. 2013) all affect grazing EE by altering the expenditure to find and harvest, then masticate and digest feed. As these variables were not captured, one may only speculate on their potential differences and whether or not the differences in EE measured short-term are representative of inherent basal differences in EE.

The use of summary statistics from GPS recordings of two, 17-19 hour periods for each sheep has provided some insight into locomotion differences between the sheep.

The average distance travelled was significantly affected by individual sheep and day of measurement, with the mean hourly distance being 280m, with a 190m range in measured values. There was no breed effect on average distance travelled. Extrapolating these figures to 24 hour estimates of distance travelled gives a range of approximately 2-11km/day, with a mean of 6.7 km. For comparison, sheep in a rangeland environment travelled an average of 7.9
km/day (Munn et al. 2013) whilst Beker et al. (2010) reported a range of 2.3-3.4 km/day for sheep grazing grass/forb pastures in confinement. Thus, the extrapolated mean lies within an acceptable range for sheep under moderate confinement with a low feed availability. The large degree of individual variation may be due to differences in tolerance to temperature and its resultant reduction in grazing behaviour (Thomas et al. 2008) or energy requirement and thus drive to source adequate energy (Lin et al. 2011; Falzon et al. 2013). As no measure of lying/standing activity, or grazing activity was measured with equipment such as IceTag™ units (Beker et al. 2010) or neckband bite counters (Kawamura et al. 2006) quantifying the exact behaviours during this time is somewhat difficult. Nonetheless it is interesting to note the potential for individual variation in distance travelled and thus be aware of the potential implications on total energy expenditure of sheep.

The lack of relationship between predicted EE and activity measures is not surprising, as measurements of HR and VO$_2$ used to predict EE when confined. Thus, the value calculated is that of confined EE, with the additional influence by the heat increment of feeding at the time of measurement. Successful measurement of diurnal changes in HR would have reflected the variation in activity level and allowed estimation of total EE.

Average speed and in particular average moving speed may provide insight into grazing behaviour and rate of sheep. Both average speed and moving speed were significantly affected by sheep and day, though only average moving speed was affected by breed. MO sheep moved at the fastest rate, followed by XB and MM. Differences in speed of movement could be attributed to stride length differences. Aharoni et al. (2013) studied cattle throughout the year, finding that Baladi cows spent a greater time foraging than Beefmaster x Simford cows in all seasons, taking longer steps despite their smaller frame. Animal experience, pasture composition and the animals favour for certain plants are likely to affect grazing behaviour to a greater extent and thus will ultimately affect the speed at which animals move. (Ahmadi and
Peiravi 2010). The presence of this breed effect may highlight differences in diet selection/preference, but as no measure of exact grazing behaviour was taken this cannot be quantified.

6.6 Conclusion

This experiment involved the further application of the measurement of HR and VO$_2$ to measure EE of sheep when under sub-optimal nutrition. Calculation of the repeatability and accuracy of HR determined that 10 minutes of measurement is sufficient to ensure stable, accurate measures of HR, which concurs with the published literature. This is of course, provided an adequate training regime is undertaken beforehand to ensure familiarisation with the handling and measurement procedure.

More than 90% of the variance in both HR and VO$_2$ were accounted for by individual sheep, day and environment effects. The most significant finding from this is the small proportion of variance in VO$_2$ as a result of differences in O$_2$ pulse, and the apparent constancy of pulse between environments and across measurement days. Whilst body size is positively correlated with O$_2$ pulse, it appears that muscularity has a greater influence, presumably through effects of heart size and stroke volume. The apparent consistency of O$_2$ pulse for a given individual concurs with the results from experiment 1 (Chapter 3) and further validates the previously reported theory that an individual’s O$_2$ pulse is relatively constant and can thus be used to infer their EE based on measurements of HR alone.

The significant difficulties encountered with measuring HR of sheep whilst free ranging was a major limiting factor to the results of this experiment. The lack of clear instruction and lack of reports of accuracy within the literature makes it hard to clarify the extent of such difficulties in other research and why they are occurring. Despite considerable effort and different
application techniques, the use of external contact electrodes to measure HR accurately and continuously in sheep appears almost impossible. It is thus recommended that future studies draw upon the use of surgically implanted logging devices to improve the quality of data obtained (MacArthur et al. 1979; Wild et al. 1998; Signer et al. 2010).

Due to the constancy of $O_2$ pulse, EE calculations are driven by differences in HR and thus in order to draw conclusions regarding EE, long term measures of HR over numerous days are required. Understanding individual patterns of HR whilst grazing in the field are crucial to determining variation in EE and its resultant effects on the energy balance of sheep.

There were no significant ‘sheep’ effects on HR, $O_2$ pulse or calculated daily EE, which indicates that individual differences in these metabolic parameters may not be responsible for differences in weight and body condition maintenance during ‘tough’ conditions. Despite the lack of significant individual variation, the positive correlations between $O_2$ pulse, EE and the gain of body weight, BF and EMD would indicate that higher performing or ‘resilient’ animals tended to have a greater measured EE. This contradicts the proposed hypotheses that lower EE would facilitate greater energy retention.
7. Resilient sheep did not differ in endocrine profile

7.1 Abstract
Differences in endocrine response of sheep may provide insight into underlying variation in physiology under nutritional stress. Leptin and ghrelin have both been identified in the role of feed intake regulation, as well as regulation of energy balance and growth hormone secretion respectively. The control of metabolic rate and resultant heat production are largely underpinned by thyroid gland activity, and therefore, concentrations of tetra-iodothyronine/thyroxine (T₄) and 3-3′-tri-iodothyronine (T₃) (Todini 2007). Therefore, measurement of these four endocrine parameters could provide information on potential variation in energy input and expenditure in sheep. Eighteen wethers from three breeds were individually penned and fed a low quality diet for a 41 day period. On day 39, jugular catheters were placed in each sheep and blood samples taken at time points 0, 2, 4, 6, 8, 12, 16, 20 and 24 hours for analysis of plasma leptin, ghrelin, insulin and total T₄ and T₃ concentrations. A three-fold range in fasted ghrelin was identified which was moderately associated with feed intake and average daily weight gain. As expected, leptin levels were well associated with adiposity and served as a useful measure of differences in energy retention. Thyroid hormone levels were not as strongly correlated with as growth as expected, but the associations with digestibility measures indicate its role in gut motility and intake.

7.2 Introduction
The endocrine control of metabolism is a complex process involving a myriad of hormonal and neuronal factors (Sartin et al. 2010). Differences between sheep in their endocrine control of metabolism may provide valuable information regarding variation in the response to nutritional
stress. This chapter reports on the endocrine measures taken in experiment 2 of this study with specific reference to their role in energy balance.

Leptin and ghrelin are two hormones that have been identified in the role of feed intake regulation and as a result have received significant interest in recent times. Produced primarily by adipose tissue, leptin plays an important role in assessing body energy balance and feed intake regulation (Houseknecht et al. 1998; Sartin et al. 2010). As leptin is produced by adipose cells, it is also a useful measure of body fat reserves (Blache et al. 2000; Wylie 2011). Ghrelin is produced in the abomasum and is involved in stimulation of feed intake, although the exact mechanism in ruminants is unclear (Udum and Tanriverdi 2013). Comparison of basal ghrelin levels, and their response to feeding events may allow further understanding of the control of this hormone as well as potentially explain differences between individuals in their voluntary intake (Wertz-Lutz et al. 2010).

In addition to intake regulation, the endocrine regulation of metabolic rate itself can provide information regarding variation between individuals. The control of metabolic rate, and the on-flow effects of feed intake and resultant heat production are largely underpinned by thyroid gland activity (Todini 2007). Thyroid hormones exist in two main forms, tetra-iodothyronine/thyroxine (T₄) and 3-3’-tri-iodothyronine (T₃). T₃ is the biologically active form, which is produced when T₄ is deiodinated in the peripheral tissues. These hormones act on a multitude of tissues, increasing metabolic rate by stimulating cardiac function, respiration rate, lipid metabolism and the availability of glucose (Capen and Martin 1989; Seijan et al. 2012; Segar et al. 2013). The broad effect of these hormones has made them popular subjects for research, particularly in studies of environmental and nutritionally induced stress (Salem et al. 1991; Lourenco et al. 2010; Abdollahi et al. 2013; Johnson et al. 2013; Seijan et al. 2013).
Individual variation in hormone levels between sheep fed a low quality diet were quantified to test the following two hypotheses:

a) That higher plasma leptin and ghrelin levels will be associated with greater level of feed intake, and;

b) That plasma levels of both $T_4$ and $T_3$ thyroid hormone will be associated with greater energy expenditure, and will indicate a reduction in energy retention.

7.3 Methods

As outlined in the experimental protocol in Chapter 4.2, sheep were fitted with jugular catheters on day 114 of the experiment. To enable regular blood sampling, 1mm internal diameter polyethylene catheters were installed as per the University of Adelaide School of Animal and Veterinary Science approved protocol. Sheep were fasted from the morning before catheters were inserted. All sheep were catheterised in the morning within the space of 4 hours. Once the sheep were catheterised, they were allowed to settle for 2 hours. An initial blood sample was taken (time 0), followed by one at 2, 4, 6, 8, 10, 12, 16, 20, 22 and 24 hours post initial bleed. After the last blood sample, catheters were removed and sheep were left to settle.

20ml of blood was taken per sample, half of which was placed into a heparinised vacutainer, whilst the other half was placed into an EDTA vacutainer. Samples were centrifuged at 3000rpm for 15 minutes and plasma pipetted into eppendorf tubes and frozen.
Blood hormone analysis

All hormonal analysis was conducted by Dr Margaret Blackberry from the University of Western Australia. Plasma leptin, ghrelin, insulin, T3 and T4 was measured for each sheep at time points 0, 2, 4, 6, 8, 12, 16, 20 and 24 hours.

Plasma leptin was measured in duplicate using a double-antibody radioimmunoassay (Blache et al. 2000; Zhang et al. 2004). Each sample was processed in a single assay with a limit of detection of 0.05ng/mL. The assay included six replicates of three control samples (0.45, 1.06 and 1.86ng/mL) that allowed calculation of the intra-assay CV of 3.9, 2.1 and 7.1% respectively.

Plasma insulin was assayed in duplicate using a double-antibody radioimmunoassay (Tindal et al. 1978; Zhang et al. 2004). Each sample was processed in a single assay and the limit of detection was 6.33µU/mL. Six replicates of three control samples (2.32, 4.87 and 10.29µU/mL) were included in the assay to determine intra-assay CV values of 6.2, 3.2 and 2.8% respectively.

Plasma ghrelin was measured in duplicate using a modified double-antibody radioimmunoassay as outlined by Miller et al. (2009). All samples were processed in a single assay, measured in duplicate. The limit of detection was 49 pg/mL. The assay included three control samples (76.4, 708.6 and 1362.1pg/mL) measured in duplicate to allow determination of intra-assay CV values of 8.3, 4.6 and 4.2% respectively.

Plasma concentrations of T3 and T4 were measured using a double-antibody radioimmunoassay (Dawson et al. 1996; Zhang et al. 2004). T3 samples were assayed as duplicate 20µl aliquots with a limit of detection of 0.02ng/mL, whilst T4 samples were assayed as duplicate 20µl (One in 40 dilution) aliquots with a limit of detection of 0.12 nM/L. Four replicates of two control samples containing 0.42 and 0.92ng/mL were used in the T3 assay to obtain intra-assay CV
values of 3.3 and 8.6% respectively. Control samples used in the T4 assay comprised two samples of 1.5 and 4.22 nM/L replicated four times to obtain intra-assay CV values of 5.1 and 3.0% respectively.

**Statistical Analysis**

In order to test differences in hormone levels across the entire time profiles measured a linear mixed model was fitted with fixed effects of time (0, 2, 4, 6, 8, 12, 16, 20 and 24 hours), breed (MM, XB, MO), sheep (-17) and the sheep by time interaction.

Fasted and peak levels were tested using a linear mixed model with fixed effect of Breed (MM, XB and MO) and sheep (1-17).

**7.4 Results**

7.4.1 Variation in plasma insulin, ghrelin and leptin levels

Plasma insulin level changed over the blood sampling period (Table 7.1, with a marked increase in plasma insulin at 4 hours post-feeding after which levels decreased (Figure 7.1). There was no overall difference between sheep in plasm insulin level nor between sheep at any given time point (time by sheep interaction) (Table 7.1). There was no significant breed difference in insulin level (Tables 7.1 and 7.2). Fasted and peak insulin level did not differ significantly between breed and sheep.
Both fasted and peak levels of insulin were not strongly correlated with any of the initial or gain values for weight and body composition in P2 (Table 7.3). Fasted insulin was positively correlated with both mean daily feed and digestible energy intake. Total insulin concentration (AUC) was negatively related to DMD, but not feed intake. AUC was also negatively associated with $O_2$ pulse ($r=-0.4$) and EE ($r=-0.4$) (Table 7.3).

Plasma ghrelin concentration did not change significantly over the measurement period, yet individual sheep exhibited an almost three fold difference between the minimum and maximum fasted levels (Table 7.1). Despite the differences in fasting level, there was no interaction between sheep and the time of measurement (Table 7.1). Breed differences were not observed in either fasted or peak levels of ghrelin.

No notable correlation was observed between ghrelin and body weight and composition values during P2 (Table 7.2). Fasted ghrelin and total ghrelin concentration (AUC) were not well

Figure 7.1: Mean plasma Insulin profile post fasted re-feeding from 17 wethers. Values presented are mean ± SEM.
correlated with intake measures, but exhibited mild negative correlation with both measures of digestibility (Table 7.2).

Plasma leptin level varied significantly over the measurement period, and between sheep, though no interaction between sheep at individual measurement times (Table 7.1). Given the lack of sheep–by-time interaction, the average leptin profile for all sheep was used to observe the changes in this hormone throughout the measurement period (Figure 7.2). Post-feeding, leptin levels decreased slightly, then gradually increased to a maximum level at 20 hours, after which they began to decline again (Figure 7.2).

Figure 7.2: Plasma Leptin profile post fasted re-feeding from 17 wethers. Values presented are mean ±SEM.
There were no breed differences in both fasted and peak leptin, nor any significant individual variation within breed (Table 7.2). Total Leptin concentration (Area under the curve) and mean leptin were not affected by sheep or breed effects (Table 7.2).

The correlation between BF and fasted leptin was positive, albeit minor (Table 7.2). The strongest trait correlations were observed between ADG and all leptin measures, with higher leptin levels associated with higher ADG. Mean DFI was similarly correlated with leptin measures which is the likely cause of the relationship with ADG (Table 7.3).

7.4.2 Variation in plasma thyroid hormone levels

Plasma concentration of T₄ did not vary significantly over the measurement period (Table 7.1). However, there was a significant effect of breed and individual sheep on T₄. MO sheep had the highest average T₄ levels at 226.3 nM/mL, whilst the MM and XB exhibited similar levels to one another at 147.8 and 151.7 nM/mL respectively (Figure 7.3). The change in T₄ level over the course of the measurement period did not differ significantly between sheep (Table 7.1). Fasted T₄ levels were not significantly affected by breed or sheep effects (Table 7.2).
P2 initial EMD was the most strongly correlated trait with both initial and average plasma T4, with P2 initial BF and ADG of fat and muscle exhibiting weaker, yet still positive correlations.

In contrast to T4, plasma levels of T3 varied significantly over the course of the measurement period, as well as being significantly affected by breed and sheep (Table 7.1). Breed differences in T3 were as for T4 level, with highest levels for the MO sheep (1.05 ng/ml), whilst the levels measured for the XB and MM were considerably lower at 0.67 and 0.57 ng/ml respectively (Figure 7.4, Table 7.2). Fasting levels of T3 were not significantly different between breeds, although significantly differed between individual sheep (Table 7.2). As for all other traits there remained no interaction between measurement time and individual sheep (Table 7.1).
Figure 7.4: Best linear unbiased estimates of average plasma T3 levels for wethers of three breeds.

Values presented are means ± SEM.

Initial weights in both periods were negatively correlated with measures of T3, with the plasma levels of this hormone decreasing as sheep body weight increased (Table 7.2). The initial values for EMD and BF exhibited slight positive correlations with initial T3 (Table 7.2). Gain values for both BF and EMD were stronger, with higher plasma T3 levels associated with greater rates of loin muscle and fat (Figure 7.5). The relationship between EMD gain and plasma T3 appears to be weaker than that of BF gain, and is under the leverage of the high gain value for a particular sheep with high EMD gain (Figure 7.5).
Figure 7.5: Scatterplot of P2 BF and EMD loin tissue depth gain vs. initial plasma T3 levels for 17 wethers.

Table 7.0.1: Tests of significance (F-probabilities) for time, breed and sheep effects on feed intake and metabolism related hormones

<table>
<thead>
<tr>
<th>Trait</th>
<th>Time</th>
<th>Breed</th>
<th>Sheep</th>
<th>Time.Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µU/ml)</td>
<td>0.01</td>
<td>0.64</td>
<td>0.34</td>
<td>0.87</td>
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<tr>
<td>Ghrelin (pg/ml)</td>
<td>0.61</td>
<td>0.09</td>
<td>0.05</td>
<td>0.98</td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>0.03</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.62</td>
</tr>
<tr>
<td>T4 (nM/l)</td>
<td>0.69</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.99</td>
</tr>
<tr>
<td>T3</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 7.0.2: Tests of significance (F-probabilities) for breed effects on feed intake and metabolism related hormonal parameters.

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>CV</th>
<th>Breed</th>
<th>Sheep</th>
<th>MM</th>
<th>MO</th>
<th>XB</th>
<th>Pooled SEM</th>
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</thead>
<tbody>
<tr>
<td>Fasted Insulin (µU/ml)</td>
<td>4.22</td>
<td>12.90</td>
<td>2.61</td>
<td>33</td>
<td>0.92</td>
<td>0.58</td>
<td>7.63</td>
<td>8.08</td>
<td>8.29</td>
<td>1.17</td>
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<tr>
<td>Peak Insulin Level (µU/ml)</td>
<td>8.76</td>
<td>20.58</td>
<td>2.84</td>
<td>21</td>
<td>0.94</td>
<td>0.91</td>
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<td>13.17</td>
<td>13.77</td>
<td>1.27</td>
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<tr>
<td>Fasted Ghrelin (pg/ml)</td>
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<td>682.74</td>
<td>102.10</td>
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<td>1.90</td>
<td>2.02</td>
<td>0.17</td>
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<tr>
<td>AUC Leptin (Ng/ml)</td>
<td>15.75</td>
<td>32.29</td>
<td>4.5</td>
<td>18</td>
<td>0.364</td>
<td>0.249</td>
<td>30.58</td>
<td>18.11</td>
<td>23.97</td>
<td>1.09</td>
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<td>3.62</td>
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<td>0.69</td>
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<td>2.43</td>
<td>2.43</td>
<td>0.21</td>
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<tr>
<td>Initial T4 nM/L</td>
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<td>217.4</td>
<td>32.25</td>
<td>19</td>
<td>0.32</td>
<td>0.02</td>
<td>0.82</td>
<td>0.62</td>
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<td>261.3</td>
<td>36.3</td>
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<td>0.177</td>
<td>147.8</td>
<td>226.3</td>
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<tr>
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<td>0.23</td>
<td>0.17</td>
<td>176.50</td>
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<tr>
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<td>1.05</td>
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Table 7.0.3: Correlation coefficients (r-values) for endocrine and growth performance traits. Coloured cells denote correlations of greatest strength.

<table>
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<th>Trait</th>
<th>DFI</th>
<th>DEI</th>
<th>T digestibility</th>
<th>Dry matter digestibility</th>
<th>OJP</th>
<th>P2 EE</th>
<th>P2 Initial Weight</th>
<th>P2 Initial EMD</th>
<th>P2 Initial BF</th>
<th>P2 ADG</th>
<th>P2 ΔBF</th>
<th>P2 ΔEMD</th>
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</thead>
<tbody>
<tr>
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<td>-0.1</td>
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<td>0.0</td>
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<td>-0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>-0.1</td>
</tr>
<tr>
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<td>0.1</td>
<td>-0.2</td>
<td>-0.3</td>
<td>-0.2</td>
<td>0.0</td>
<td>-0.1</td>
<td>-0.2</td>
<td>-0.1</td>
<td>-0.1</td>
<td>-0.3</td>
</tr>
<tr>
<td>AUC Insulin (µU)</td>
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<td>0</td>
<td>-0.3</td>
<td>-0.4</td>
<td>-0.4</td>
<td>-0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-0.1</td>
<td>-0.3</td>
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<tr>
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<td>0.1</td>
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<tr>
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<td>0.2</td>
<td>0.5</td>
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<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
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<tr>
<td>AUC Ghrelin (pg)</td>
<td>0</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Fasted Leptin (Ng/ml)</td>
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<td>0.2</td>
<td>-0.2</td>
<td>-0.1</td>
<td>-0.1</td>
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<td>0.2</td>
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<tr>
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<td>0.3</td>
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</tr>
<tr>
<td>AUC Leptin (Ng)</td>
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<td>0.3</td>
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<td>0.3</td>
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<tr>
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<td>Initial T3 Ng/ml</td>
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<td>-0.1</td>
<td>-0.2</td>
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<td>0.2</td>
<td>0.1</td>
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<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
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</tbody>
</table>
7.5 Discussion

7.5.1 Variation in plasma insulin, ghrelin and leptin levels

The known response to feed intake by insulin explains the significant effect (Table 7.1) of measurement time (Ouellet et al. 2001). Upon consumption of feed, direct absorption of glucose stimulates insulin production from the β-cells of the islets of Langerhans within the pancreas (Pineda and Dooley 2003; Catunda et al. 2013). Insulin then acts on the hypothalamus to regulate body energy balance via orexigenic or anorexigenic pathways (Miller et al. 2011; Daniel et al. 2013; Catunda et al. 2013). The lack of differences in total level and magnitude of peak concentration indicate there was little overall variation in total insulin response. Previous research has shown that feeding sheep at different levels of bulk volume (Tsiplakou et al. 2012) or energy (Zhang et al. 2005a; Caldeira et al. 2007) has been shown to alter insulin response. The flow on effects of altered insulin feedback are changes in hypothalamic gene concentration of orixegenic (Nueropeptide-Y and Agouti-related protein) and anorexigenic (pro-opiomelanocortin and cocaine/ampethamine-regulated transcripts) factors which leads to an increase or suppression of feed intake (Miller et al. 2007; Miller et al. 2009).

Given the known effects of differing feeding/energy level it is evident that the differences in energy intake observed in this experiment were not sufficient to elicit a significant response in basal level or total concentration of insulin. This is most likely due to the low quality of the feed used in the experiment.

The similar insulin response between individuals is the most likely reason for the lack of relationship with weight and condition traits. Given the variable level of intake and resultant body weight and composition change, the relationship between intake level,
insulin response and growth cannot be expected to be straight-forward. Leon et al. (2004) reported that in restrict-fed heifers there was no change in insulin concentration as body condition decreased although when fed sufficient energy to gain condition, there was an increased insulin response and relationship with weight gain. As the nutritional regime imposed in this experiment did not result in excessive energy availability, the lack of relationship between insulin and weight/composition change is not unexpected.

Although fasting and peak level did not differ between sheep, analysis of all ghrelin measurements highlighted the presence of individual differences. Variation in ghrelin concentration has been reported between selection lines of sheep and have correlations with growth and carcass performance (Ingham et al. 2011; Bahrami et al. 2012), but the direct implications of this variation in ghrelin on intake has not been quantified. As the diet fed in this experiment did not differ in physical or chemical composition, any dietary induced variation thus come as a result of differences in level of intake, frequency of feeding, or overall sensitivity to ghrelin concentration.

Fasted ghrelin concentration was positively associated with feed intake and negatively related to digestibility traits, indicating it may well play a role in intake regulation. Currently the secretion of ghrelin in ruminants is not fully understood, with some reports claiming an increase before feeding events, decrease post-feeding and then rise again to pre-feeding levels (Hayashida et al. 2001; Sugino et al. 2002; Miura et al. 2004; Wertz-Lutz et al. 2006; Wertz lutz et al. 2010; Ozfiliz et al. 2011). The results reported here suggest no change in ghrelin concentration over time post-feeding. However, these studies involve set levels of feeding at programmed times, unlike the *ad libitum* supply in this experiment which may ameliorate the concentration of the
hormone. Udum and Tanriverdi (2013) also found no difference in the fasting ghrelin levels in both ad libitum and twice daily fed lambs. It is likely that programmed feeding of a set amount stimulates a rhythmic pattern of neuronal induced ghrelin concentration which were not detected here (Grouselle et al. 2008). Other evidence suggests that the volume of feed consumed is not the primary control mechanism, but rather the chemical composition (Volatile fatty acids produced in the rumen) (Tschop et al. 2000; Fukomori et al. 2011), and energy density of the feed (Ozfiliz et al. 2011; Sajedianfard et al. 2012) influences ghrelin secretion. Although the feed composition was constant during this experiment, differences in energy and dry matter digestibility may cause changes in ghrelin concentration, as evident by the positive relationship observed between total concentration and energy digestibility.

Whilst positive correlations existed with P2 initial weight, they were small and as a result no clear effect of body weight on ghrelin secretion can be concluded. It is interesting to note that the correlations between BF, EMD, ADG and BF gain were all positive, albeit small. Given the positive effect of ghrelin on growth hormone production it is not unreasonable to expect these positive relationships. The nutritional stress placed upon these sheep and the variable response in weight and condition change is likely to be the cause of these weak correlations. In humans, ghrelin level is negatively correlated with body-mass-index (Purnell et al. 2003; Greenman et al. 2004; Kellokoski et al. 2005) and fat cell volume (Purnell et al. 2003). The evidence for such relationships in ruminant species is limited at this time and warrants further exploration.

Leptin is another hormone reported to have a regulatory effect on ghrelin in ruminants and may be responsible for the differences observed in this experiment. The exact relationship between leptin and ghrelin in ruminants is unclear, with some reports
indicating an inhibitory effect (Tschop *et al.* 2001; Kirsch *et al.* 2012) whilst Udum *et al.* (2012) reported that leptin levels responded to feeding events in the same manner as ghrelin. Given that statistical analysis revealed variation between time points and individual sheep in leptin level, either scenario seems possible. In order to determine the relationship between circulating leptin and ghrelin, regression of total concentration for each reveals a very weak correlation (Figure 7.6). Thus, the results of this experiment do not clearly indicate a strong relationship between these two hormones.

**Figure 7.6:** Scatterplot of total plasma ghrelin vs. total plasma leptin concentration over 24 hours from 17 wethers of three different breeds.

The significant change in leptin over the measurement period is not unexpected as the main source of variation in leptin concentration in ruminants throughout the day is feeding events (Blache *et al.* 2000; Marie *et al.* 2001). Leptin levels have been shown to increase 2-8 hours post-prandially in sheep, yet due to the *ad lib.* nature of feeding
regime utilised in this experiment the exact timing of feeding events is difficult to quantify. Average plasma concentration oscillated for the first 8-10 hours post feeding, indicating multiple feeding events. After this time presumably sheep were satiated and intake decreased as the leptin concentration rises to a peak at 20 hours post feeding. It is interesting to note that fasting leptin level wasn’t affected by breed and sheep effects, reiterating the need for repeated measures of fed sheep to determine variation in leptin level (Wylie 2011).

Aside from its potential role in the regulation of feed intake, plasma leptin levels are a useful tool for reflecting adipose tissue content of sheep. In order to ensure an accurate measure of plasma leptin is taken, without the effects of intake or secretory pattern difference, animals need to be in a fed, ‘energy-neutral’ state (Houseknecht et al. 1998; Wylie 2011). As the animals in this trial were never in such a state, the average leptin concentration from the entire measurement period and/or the total leptin concentration (AUC) should give the most accurate representation of differences in leptin secretion and thus adiposity. The correlation between subcutaneous BF and plasma leptin was negligible. Previously reported correlations between subcutaneous BF and leptin range from 0.3-0.83 (Blache et al. 2000; Delavaud et al. 2000). ADG however was positively, albeit weakly, to leptin, which is likely a reflection of the intake:leptin relationship. It must be noted that the previously reported relationships were undertaken in adult sheep, and those used in this experiment were under 12 months of age at the start of the experiment, and thus still maturing during this period. Studies of growing Holstein steers by Vega et al. (2002) reported low circulating leptin remained low during the initial growing phase, after which it begins to increase with adipose tissue deposition. Thus there is potential for the relationship between subcutaneous BF and measured leptin level of the wethers in this trial to be weaker than expected.
Despite the weak correlation with initial values of BF, there was a tendency for lower mean plasma leptin sheep to have greater levels of BF gain during P2. These individuals may be leaner due to age, physiological maturity (Vega et al. 2002) or differing plane of nutrition prior to the experiment (Faulconnier et al. 2007). It is not unreasonable to expect these leaner individuals to exhibit a greater ‘drive’ for increased body condition, as the inhibiting effect of higher plasma leptin levels would not be present (Sartin et al. 2010; Sami et al. 2013). Quantification of the rate of total body fat gain may well increase the strength of this correlation but cannot be quantified with the data collected in this experiment.

In order to test the relationship between plasma leptin level and body adiposity further, the dissected adipose tissue weights post-slaughter can be compared to the measured levels of leptin. In order to eliminate individual variation in distribution, the weights of heart, kidney and omental fat were summed to provide a ‘total internal adipose tissue’ measure. Mean plasma leptin level and total internal adipose tissue (Figure 7.7) were strongly correlated ($r=0.77$). This correlation highlights that the measures of subcutaneous BF taken in this trial inadequate measures of the total body fat content due to the presence of internal fat depots and tail fat in the MM sheep.
Figure 7.7: Scatterplot of total internal adipose tissue weight (cumulative weight of the heart, kidney and omental fat weight) vs. Mean plasma leptin level of 17 wethers from three breeds.

Thus it would appear that as per the literature, leptin is a good indicator of total adipose tissue content. If trials are to be conducted with young animals on sub-optimal nutrition, subcutaneous BF may not be an accurate indicator of adiposity, and that whole-body scanning (DEXA or CT) and/or dissection of the carcass needs to be undertaken to allow accurate measurement of adiposity.

7.5.2 Variation in plasma thyroid hormone levels

The lack of variation over time in plasma T₄ level indicates that the production of T₄ by the thyroid gland was fairly constant over the measurement period (Chopra et al. 1975). As the ‘storage’ form of the hormone, it can be expected that T₄ would be relatively stable in its production within the course of a day. Conversely plasma T₃ level (as the ‘active’ hormone) changed significantly over time (Chopra et al. 1975). Plasma T₃ levels depend on the de-iodination of T₄ in the peripheral tissues, and is thus more
susceptible to change over a short course of time (Chopra et al. 1975; Ward et al. 2008; Vonhamme et al. 2013). Observation of the mean plasma level for all sheep in the trial reveals that both T₃ and T₄ levels followed a similar pattern, exhibiting the highest levels at the 8 hour time point (Figure 7.8). The difference in plasma T₄ level between the highest and lowest measures was 12%, whereas the difference between the highest and lowest T₃ measure was 34%, confirming the greater fluctuation of T₃. Both thyroid hormones undergo circadian rhythms of concentration, although at which the peak level occurs does vary between experiments (Nazifi et al. 2008). Weeke and Gunderson 1978, Souza et al. (2002) and Nazifi et al. (2008) all reported peak levels of both T₃ and T₄ in the late afternoon to early evening. The measurement time point of 8 hours occurred at approximately 17:00-18:00 hours and thus the peak level of both T₃ and T₄ levels correlates with the results observed in previous studies.

Figure 7.8: Average plasma T₃ (Ng/mL) and T₄ (nM/L, expressed /100) concentration over a 24 hour period.
Thyroid hormones are involved in muscle cell differentiation and development and thus the increase in thyroid hormones as muscle mass increased (based on initial values) is not unreasonable (Hocquette 2010). Initial measures of plasma T3 were positively correlated with rates of BF and EMD gain in P2, yet the average T3 concentration showed either zero (BF) or low positive (EMD) correlations. The measures of initial T3 had a considerably greater CV than the average T3 measure and both measures of T4 which is the most likely cause of the increased correlation between BF and EMD gain and this trait. Fasting does reduce plasma thyroid hormone levels, irrespective of nutritional status (Wronska-Fortuna et al. 1993). As T3 fluctuates throughout the day, an average plasma level from repeated measures will provide a greater indication of circulating levels than a singular fasted measurement. Thus the average T3 level would be expected to provide the greatest insight into endocrine: growth relationships and as a result it can be concluded that no consistent relationship existed between this hormone and growth performance in P2.

Thyroid hormones also influence gut motility, and thus the resultant flow rate of digesta (Barnett et al. 2012). The negative relationship between DMD and both measures of T4 and average T3 suggests such an occurrence (Table 7.2). The positive associations of both gross feed intake and DEI with average T3 and T4 concentration is consistent with the increased throughput and lower DMD. Ekpe et al. (2000) found that restrict feeding cattle reduced T3 whilst leaving T4 level unaffected. Yambayamba et al. (1996) found that restrict feeding reduced both T3 and T4 levels, and these levels remained low for 10 days following re-feeding. The lower thyroid levels suggest that energy expenditure is lower during the initial phase of re-feeding, leading to the increased ‘compensatory’ growth often observed during these initial periods (Yambayamba et al. 1996; Hornick et al. 2000). Although the sheep in this trial were restricted by nutritional quality,
differences in intake capacity and digestive efficiency, along with inherent differences in metabolism and appetite will have affected the degree of restriction imposed, and as such, the thyroid hormone response may not be as clear.

The significant breed differences in average levels of both thyroid hormones elude to potential differences in energy expenditure (Kim 2008). The MO sheep in this trial had plasma T₄ levels of 49 and 54% greater than the XB and MM sheep respectively. The differential in plasma T₃ between breeds was even greater, with the MO levels 57 and 85% higher than the XB and MM. Conversely, differences between breeds in cold tolerance have also lead to increases in circulating T₄ levels, with Ile de France sheep maintaining lower hormone levels than their Churra-da-Terra-Quente counterparts (Lourenco et al. 2010). As all animals were housed in a temperature controlled shed at 22 degrees Celsius, the likelihood of temperature extremes affecting thyroid status is unlikely. Variation in feed intake and resultant Metabolic heat load can also increase thyroid hormone levels (Valtorta et al. 1982; Lourenco et al. 2010; Seijan et al. 2013), however the low energy nature of the diet used coupled with the lack of breed differences in DFI and DEI observed (Table 5.4) render this effect unlikely. Thus, it is probable that the results obtained are indicative of true differences in metabolic activity and/or energy expenditure between these breeds.

Previous examples of breed differences in thyroid hormone levels can provide insight into the implications of the results. Eryavuz et al. (2007) reported 40% lower T₃ levels in fat-tailed Akkaraman sheep compared to Anatolian Merinos which was indicative of a lower basal metabolic rate. Higher T₄ levels have been associated with larger body size and growth potential (Williams et al. 2004), growth rates (Al-Damegh 2012), and wool growth rate (Abecia et al. 2005). As the sheep used in this trail were wethers of a
similar age and weight, fed a diet of poor quality (thus impairing energy intake, but not heat production), the effects of age and growth potential are likely to be minimal contributors to any differences. Conversely, breed differences in wool growth potential may be contributing to the differences in plasma thyroid hormone levels observed (Ryder 1979). Differences in circulating thyroid hormone have been identified between fleece-weight selection lines, although the results vary between experiments (Sun et al. 1994). Higher thyroid levels are associated with bulb cell proliferation, greater fibre elongation and thus fleece weight (Hynd 1994). As MM sheep are a ‘shedding’ breed with a clear moulting cycle and the XB and MO have more-or-less continual hair growth cycles, it is not unreasonable to expect that the MO sheep may have higher circulating thyroid hormones to meet their fibre growth demand (Todini 2007). The XB sheep are likely to have been producing a coarser, less dense fleece, and thus have a lower thyroid hormone requirement than the MO. Thus, the similar levels of T₃ and T₄ observed between the MM and XB does challenge this theory. However, as fleece growth and quality was not measured, further quantification of breed differences is not possible.

Having accounted for the potential effects of environmental conditions, residual differences in circulating thyroid hormones are most likely a reflection of differences in energy expenditure between the MO and both the XB and MM breeds. However, this variation does not correlate clearly with differences in growth performance. Further investigation into the cause and effect of these differences may allow a greater understanding of variable performance under sub-optimal conditions.
7.6 Conclusions

Within a small subset of animals, individual variation was identified in insulin, ghrelin, leptin and both thyroid hormones. The role of ghrelin in ruminants and its association with intake dynamics require further investigation before its role as a predictor of intake or growth potential can be concluded. Leptin levels were well associated with adiposity and served as a useful measure of energy retention differences. Thyroid hormone levels were not as strongly correlated with growth as expected, but the associations with digestibility measures may indicate its role in gut motility. Inherent breed differences were identified in thyroid hormones, but further investigations are required to highlight the change in metabolism through selection for wool vs. meat.

No endocrine measure of intake, energy retention or energy expenditure was strongly associated with resilience as defined by ADG. Thus, measurement of these parameters in the fasted animal may not be used as a predictor of performance under sub optimal nutritional conditions.
8. General Discussion

The analysis of body weight and composition change during the second experiment (Chapter 4) lead to the conclusion that body weight change is the best definition of resilience as it encompasses changes in fat, muscle and organs which cannot be easily measured in the live animal. This chapter draws together the results presented in Chapters 4-7, and discusses the validity of each of the three mechanisms proposed in Chapter 1.

Before this final interpretive analysis was conducted, the data for sheep #7 were removed. This was the sheep which exhibited extremely high weight loss during the trial (~300g/day). There is no evidence to suggest measurement error occurred, and the author is confident that the measurement reflects the sheep’s true response to the dietary treatment. However, as it is so divergent, for the purposes of partitioning variation it has been removed. In a commercial setting, removal of such poor performing individuals would be standard practice. Understanding what drives variation in resilience within populations with a tighter range of performance will increase the accuracy of selection tools and inform management and breeding decisions in the future.

To partition the various components that contributed to variation in ADG, a complex linear model was fitted will all initial, growth, carcase, organ and endocrine traits as fixed effects. All traits with a significance level ≥0.05 were removed, leaving the following model:
The variation in ADG was quantified as the standard deviation calculated as the square root of the total mean squares. The 336 g/d range in actual ADG measured was then divided by the SD of 104 g/d, revealing that the sheep measured lay within a range of 3.23 SD’s. The above model was then re-fitted with each trait added sequentially, and the residual mean square recorded as each trait was added to the model. The square root of each residual mean square was then calculated. The difference between the residual mean square values was calculated to represent the proportion of the SD attributed to the trait. This value was then multiplied by 3.23 to allow the proportion of variance attributed to each component to be presented in terms of the relative contribution to the 601 gram range in ADG measured, as presented in Figure 8.1. Overall, 60.2% of the variance in P2 ADG could be accounted for with the model.

Figure 8.1: Proportion of variation for components contributing to the actual total range in average daily gain measured over a 40 day period.
8.1 Do resilient sheep start off in better condition?

This mechanism is based on the principle that if animals can capitalise on periods of plentiful nutrition and lay down sufficient body reserves, then they will be able to draw-upon those reserves during times of nutritional deprivation. By this principle, an animal may lose a large proportion of its body weight during this time, but remain in a productive and healthy condition. However, under such a scenario, a high weight loss sheep would actually be deemed less resilient when using ADG as the primary trait to define resilience. In production systems without pronounced seasonal variations in feed availability, or when periods of extended feed restriction are encountered, these high weight loss animals may not be able to sustain themselves long term.

Both experimental periods imposed poor-quality nutritional conditions, and elicited considerable variation between individuals in ADG/resilience. Initial weight contributed to <1% of the variance in ADG, but fat and muscle had no significant effect (Figure 8.1). Thus, there was no major advantage conferred by greater initial weight or condition.

8.2 Do resilient sheep have a higher energy intake?

Overall, 48.3% of the total variance in ADG was attributed to digestible energy intake making it the most significant contributor to resilience (Figure 8.1). This follows the positive correlations between DEI and gain of weight (0.7), Δ BF (0.2) and Δ EMD (0.3) (Chapter 5). Over-and-above the effect of energy intake, final liver weight contributed to 7.6% of variance in ADG (Figure 8.1), which may be a reflection of the relative proportions of volatile fatty acids and the extent of protein digestion. No other
final organ weight significantly contributed to the variance in ADG, indicating that any changes in these traits were dependent on DEI and stage of maturity.

The mean plasma ghrelin concentration contributed to a statistically significant, albeit small proportion of ADG (0.3%). As reported in Chapter 7, measures of ghrelin were poorly correlated with DEI. Given that the effect shown in Figure 8.1 is after adjusting for weight and energy intake, the association between ghrelin and ADG indicates the presence of differences in growth hormone level or sensitivity, or other effects of ghrelin.

It is worth noting that individual variation in intake was still observed when animal weight and gain were adjusted for to provide AFI. The 0.5kg/d range in AFI measured indicates an economically viable range in feed intake to sustain a given level of ‘resilience’. The variance in residual measures of intake (whether expressed as RFI or AFI) are generally much lower on restricted quality or quantity diets (Bordas et al. 1995; Veerkamp et al. 1995; Silverstein 2006; Roberts et al. 2007; Wilkes et al. 2012; Lines et al. 2014 and Mauch et al. 2014). As the performance of these sheep was not quantified on a high-quality diet, they have not been ranked on AFI under these conditions. Nonetheless, should the quantity of the diet have been restricted, then the lower AFI animals should maintain their ‘resilience’, and the differences in ADG observed may increase.

8.3 Do resilient sheep have lower energy expenditure?

No measure of oxygen intake, heart rate or calculated energy expenditure contributed to the measured variance in ADG (Figure 8.1). Mean plasma T3 level did however contribute to 3% of the variance in ADG (Figure 8.1). The correlation between ADG
and T3 reported in Table 7.3 are slightly positive, indicating that greater thyroid activity is associated with greater cellular activity and growth, rather than energetic efficiency.

A primary aim of this study was to evaluate the use of heart rate and oxygen pulse in measuring the energy expenditure of sheep in the field. Analysis of VO$_2$ variability from both experiment 1 and 2 showed that within normal ‘non-stressed’ physiological ranges, VO$_2$ was constant as HR changed. This concurs with the published data on other ruminant species and provides further evidence to suggest that O$_2$ pulse with long term measures of HR can be used to calculate the energy expenditure of sheep. It must be noted that despite the apparent consistency of this trait, multiple short term measurements of O$_2$ pulse are recommended to increase the accuracy of predictions for each individual.

Whilst the relative ease of O$_2$ pulse measurement bode well for the measurement of EE, numerous difficulties were encountered measuring the heart rate of sheep whilst on range. Initial testing of HR systems over short periods of time were promising but the application of contact electrode HR logging devices to sheep whilst grazing resulted in highly variable, often inaccurate data collection. Despite concerted efforts to clip and clean the site of electrode contact, the ease of subsequent dust and grease accumulation and ability for sheep to rub against trees and fences lead to loss of electrode contact. The lack of papers published on the use of this method in sheep may reflect poor reporting or negative results. Further investigation into the conductivity of sheep skin and its effect on contact electrode function would be of interest. Alternatively, internally located sensors (subcutaneous, intra-abdominal) and logging devices could be sourced to measure HR, thereby circumnavigating any skin conductivity issues.
8.4 Conclusion

Under a low quality, *ad libitum* feeding regime, feed intake had the greatest effect on resilience as defined by weight change (ADG). The presence of such varied response to poor quality nutrition between individual’s sheep highlights the potential for genuine differences in resilience between sheep within a flock. The presence of difference in intake required to sustain a given level of weight and gain (AFI) may also exacerbate the variation in resilience observed when feed quantity and quality are restricted.

In a commercial setting, overall pasture availability and/or the extent of supplementary feed available can be recorded at a flock level, but the measurement of individual intake cannot be easily quantified. With regular weighing intervals, the weight change of a given cohort of sheep can be tracked and the more resilient individuals easily identified. When making such selections in the breeding flock, the productive status (Pregnant, lactating, dry) and the level of production (number/weight of lambs produced) should be considered when classifying an individual’s resilience. With good records of weight change, productive state and total level of production, adjustments can be made to determine the extent of weight change over and above that induced by these additional metabolic demands.

Future work recommended includes characterisation of body weight change and hormonal profiles of larger flocks of reproductively active sheep, coupled with select intake testing of high and low body weight loss animals. Advances in remote-sensing technology will hopefully make the measurement of HR in free-ranging sheep achievable, and allow the EE to be measured accurately in extensive grazing systems.
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## Appendix

Table Appendix.1: Ingredients and nutrient composition of low quality pellet ration used in experiment

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of ration</th>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>Barley</td>
<td>4.91</td>
<td>ME (MJ/kg)</td>
<td>7</td>
</tr>
<tr>
<td>Mill Mix</td>
<td>20.74</td>
<td>CP %</td>
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</tr>
<tr>
<td>Oat Offal</td>
<td>25</td>
<td>Fat %</td>
<td>3</td>
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<tr>
<td>Barley Offal</td>
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<td>Fibre %</td>
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<td>Wood Flakes</td>
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<td>ADF %</td>
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<td>Choline (mg/kg)</td>
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