Consequences of selection for residual feed intake in beef cattle

David Lines

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The University of Adelaide

Faculty of Sciences

School of Animal and Veterinary Sciences

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Declaration

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David Lines

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Abstract

Abstract

In all livestock systems, feed accounts for the greatest cost of production. Therefore, improvements in the production efficiency by reduced feed inputs would be a significant economic benefit to Australian ruminant production systems, particularly for beef cattle. Residual feed intake (RFI) is the difference between an animal's actual feed intake and that which would be expected based on production. Selection for residual feed intake enables a reduction in inputs (feed) with no or minimal change in outputs (mature weight and growth rate). However, the biological processes underpinning variation in residual feed intake are unclear.

Many authors have hypothesised that part of the variation in RFI may be due to differences in energetic efficiency through changes in heat production, these being in part due to differences in protein metabolism. Following three generations of divergent selection for RFI, eight High and eight Low-RFI heifers were fed at both 105 and 180% of predicted maintenance feed requirements. Between-RFI line and feeding-level differences were assessed for energy intake, protein metabolism, heat production, body composition, energy and nitrogen balance and digestibility. The RFI lines did not differ in protein metabolism or heat production. The High-RFI heifers deposited 51 and 56% more subcutaneous fat at the P8 rump and 12/13th rib sites, respectively, with no difference in eye muscle area gain or average daily weight gain. The greater fat deposition of High-RFI heifers was due to a larger ad libitum feed consumption compared with the Low-RFI heifers. Energy and nitrogen balance did not differ between the RFI lines. The energy transactions indicated no difference in the efficiency

of energy use on 105% maintenance, although when fed 180% of maintenance the differences in feed intake suggest variation in appetite as the mechanism contributing to RFI. All of the extra energy consumed by High-RFI heifers above maintenance and deposition of protein was associated with additional energy retained as fat.

Despite the variation in residual feed intake being accounted for by variation fat deposition high and low RFI animals still differ significantly in actual feed intake. A potential explanation of this difference could be variation in the energy status and appetite between high and low RFI animals. Eight High and eight Low-RFI heifers were fed at either 105 or 180% of predicted maintenance feed requirements. Plasma were analysed for glucose, insulin, non-esterified fatty acids (NEFA) and ghrelin from blood samples taken before during and after feeding. There was no difference between the circulating ghrelin of low and high RFI heifers, however, have a reduced feed intake compared to high RFI heifers. It could be hypothesised that the low RFI heifers had a reduced sensitivity to circulating ghrelin whilst the high RFI heifers appear to have weaker negative feedback mechanisms from fatness to reduce feed intake. Additionally, low RFI heifers may be more stressed and certainly appear to be mobilising adipose tissue to produce NEFA as an energy source.

The performance of low RFI-EBV Angus steers in a large commercial feedlot by reduced feed consumed with no adverse effects on final turnoff weight. Low RFI-EBV steers consumed on average 270kg less feed than medium RFI-EBV and high RFI-EBV steers, resulting in a saving of $53 (at $200/tonne) of feed per animal. Low RFI-EBV steers finished with less subcutaneous fat measured at the 7/8th rib, which may impact on market specifications. Dressing percentage and seam fat were higher in the low RFI-

EBV steers. Together, this would be expected to result in a greater yield of retail beef with no reduction in visual meat quality or marbling grade. Breeding to reduce RFI, may change distribution of carcass fat but the consequences may not be as severe as previously thought as not all fat depots appear to be equally affected. Meat tenderness may be slightly reduced, but with longer ageing periods, this is unlikely to be a problem.

Cows genetically differing in fatness appear to behave similarly to animals differing in RFI. Low fat genotype cows consume considerably less feed and energy than expected based on their weight, weight gain, growth of the calf and the growth of the gravid uterus. Thus, low fat genotype cows had a lower RFI during both periods of measurement than the high fat genotype cows. Low fat genotype cows had higher mature weights (as these genotypes appear to have a later maturity pattern) with no differences in the weight gains of cows and calves or the weaning weights of calves from these cows, similar to low RFI cows. High fat genotype cows had a greater appetite and ate more, as do high RFI cows. Both of these types of cows are possibly fatter as they have greater appetites and eat more (Chapter 8). Whilst not conclusive, high fat genotype cows and high RFI cows tend to both have higher calving rates, weaning rates and weaning weights per cow exposed. These differences between high and low fat genotypes cows are exactly as expected from cows divergent in RFI. The conclusion is that given the high phenotypic and genotypic correlations between fatness and RFI, selection for feed efficiency may be most easily and cheaply achieved by selecting for fatness.

Direct selection for feed efficiency in beef cattle (FCR) in the past has indicated some potential drawbacks. One issue is that FCR is highly correlated with average daily gain; therefore selection for high growth alone is much more cost-effective than measuring individual feed intake. Another problem is that this measure of feed efficiency would tend to select for animals with greater muscle mass and less fat deposition. Additionally, selection for increased FCR results in increased mature size and increasing the size and energy requirements of cows would not be a goal of most commercial operations.

Due to these issues with selecting for feed conversion ratio (FCR), it was anticipated that RFI may be an alternative to genetic selection for FCR(Koch *et al.*, 1963). It was thought that RFI could be used for genetic selection with much more confidence in beef production systems as it was supposed to be independent of average daily gain, body weight and mature size. However, all the evidence from the experiments conducted herein show that the only biological mechanisms that appear to be affected through selection for RFI is appetite and activity at constant weight and daily gain. The 2 main implications are not trivial: 1) animals that have a greater appetite and consume more energy at constant weight and daily gain, deposit more energy as fat, and 2) animals that deposit more energy as fat do this due to a greater appetite.

Evidence from this thesis concludes that reducing maintenance requirements through selection for RFI may not be possible and may be detrimental to animal fitness. However, if RFI is to be used as a tool for improving feed utilisation, then adjustment for body composition would need to be considered. Given that improving feed utilisation is only reasonable in the growing animal, then feed conversion would be much easier to implement given the high generic and phenotypic correlations between

FCR and growth rate. Currently, producers do not have good measures for the variation in feed utilisation for maintenance to target in selection programs. In the absence of such measures, producers should be encouraged to focus on measurable output traits in their selection programs.

Chapter 1

Review of Literature

CHAPTER 1: Literature Review

1.1 Introduction

In all livestock systems, feed accounts for the greatest cost of production. This can be harder to quantify in grazing systems than in the intensively managed grain based systems of the pig and poultry industries, however, it is still considered a significant expense (Archer *et al.*, 1999). Ruminant grazing systems have the additional burden of very low reproductive outputs compared to the pig and poultry industries and therefore, the proportion of total feed requirements for the breeding female is very large. Feed consumption of a cow can represent up to 75% of the total annual feed requirements in breeding operations (Gregory, 1972, Klosterman, 1976, Archer *et al.*, 1999). Of this, maintenance requirements of the cow may represent 60-75%, or about 50% of the total energy requirements of the breeding operation (Ferrell and Jenkins, 1984, Archer *et al.*, 1999). Therefore, improvement in the production efficiency by reduced feed inputs would be a significant economic benefit to Australian ruminant production systems, particularly for beef cattle.

1.2 Measures of feed efficiency

1.2.1 Gross efficiency and feed conversion ratio

Gross efficiency (GE) is defined as the ratio of production outputs versus feed inputs (Archer *et al.*, 1999). Feed conversion ratio (FCR) is the inverse of gross efficiency and is defined as the ratio of feed inputs versus production outputs. In meat production systems, the feed conversion ratio is the feed requirements (kg) per kilogram of meat produced over a growth period. Therefore,

$$GE\ (kg/kg) = \frac{ADG}{DFI}$$

$$FCR\ (kg/kg) = \frac{DFI}{ADG}$$

where *ADG* is average daily gain and *DFI* is daily feed intake.

However, it has been well documented that both gross efficiency and feed conversion efficiency are highly correlated, phenotypically and genetically, with their component traits (Cameron, 1998). Therefore, selection for these production traits is similar to selection for the input traits and provides little reason for measuring feed intake (Cameron, 1998, Archer *et al.*, 1999).

Selection experiments for feed conversion ratio have also demonstrated correlated changes in their component traits (Bishop *et al.*, 1991, Arthur *et al.*, 2001c, Arthur *et al.*, 2001b). For instance, the study by Bishop et al (1991) in beef cattle showed moderate phenotypic (r_p=-0.33) and genetic (r_g=-0.66) correlations between average daily gain (ADG) and FCR over a 140 day feed test. The studies of Arthur *et al.* 2001a and 2001b indicate stronger phenotypic and genetic correlations between ADG and FCR. These studies support the theory that selection for feed conversion ratio through increased growth rate will have similar effect to direct selection on feed conversion ratio. Koch *et al.* (1963) showed that this, i.e. selection for growth, equated to an 81% of the reduction in feed conversion ratio compared to direct selection for feed conversion ratio.

Salmon *et al.* (1990) in mouse selection experiments for growth rate showed that selection for high growth over 42 days (i.e. high average daily gain) resulted in a

significant reduction in the food conversion ratio of these mice compared to the low growth line. Salmon *et al.* (1990) concluded that when adjusted for stage of maturity, the relationship between growth rate and feed conversion ratio was greatly reduced. That is, that the relationship between growth rate and feed conversion ratio "may be accounted for by genetic differences in a factor associated with the elevation of their target mature body weight".

Genotypes with high growth rates (and hence, low feed conversion ratios) have higher mature weights and as such, higher feed requirements for the breeding female (Archer *et al.*, 1999). However, the increases in mature size of the breeding female have little effect on the production system efficiency. Therefore, whilst improvement in FCR may be of benefit for the finisher progeny, the benefits for the breeding herd are effectively nil (Archer *et al.*, 1999).

1.2.2 Maintenance

Maintenance requirements are defined as the energy requirement for body weight and energy stasis (Ferrell and Jenkins, 1985). Therefore, maintenance is defined as the ratio of body weight maintained to daily energy intake (Jenkins and Ferrell, 2002).

$$Maintenance\ (kg/MJ) = \frac{Weight\ maintained}{Metabolisable\ energy\ intake}$$

Maintenance may be an important measurement for the breeding herd. However, it is not practical to measure in growing animals due to the limitation that weight stasis must be achieved (Archer *et al.*, 1999).

1.2.3 Partial efficiency of growth

The partial efficiency of growth (PEG) is defined as the ratio between ADG and feed intake once the expected maintenance requirements have been subtracted (Kellner and Goodwin, 1909, Nkrumah *et al.*, 2004). Maintenance requirements can be estimated from body weight, feed intake and growth during the measurement period from feeding tables (e.g. ARC 1980, NRC 2000, SCA 1990).

$$PEG\ (kg/kg) = \frac{ADG}{DFI} - estimated\ daily\ maintenance\ requirements$$

The estimation of maintenance requirements from feeding tables assumes that no variation in maintenance requirements exists (Archer *et al.*, 1999) even though variation exists in important components of maintenance requirements such as body composition, liver and visceral weights, etc. Therefore, the partial efficiency of growth cannot be used as a selection criterion for reducing the maintenance requirements of the breeding portion of the herd as the maintenance requirement itself is not static.

1.2.4 Conclusion: Measures of feed efficiency

Selection for these measures of feed efficiency are unable to change the maintenance requirements of cattle. Selection for GE or FCR is phenotypically and genetically correlated with their component traits. Therefore, selection based on the component trait, i.e. growth rate, is currently more cost effective than measuring feed intake. Additionally, selection for GE or FCR will result in increasing the size of the animals and hence, will increase the maintenance requirements of breeding animals. Selection for maintenance may change maintenance requirements, but it is impractical to measure until the 'animal' reaches maturity, and therefore, expensive. Selection for partial efficiency of growth is unable to change maintenance requirements as it assumes that maintenance is static.

1.3 Residual Feed Intake

Residual feed intake (RFI) was first used by Koch *et al.* (1963), and is defined as the difference between the actual feed intake of an animal and its feed intake that would be expected based on its weight and growth rate over a period of time (Arthur *et al.*, 2004a). Residual feed intake can be predicted from the multiple linear regression below

$$DFI\ (kg/day) = \beta_0 + \beta_1 (MidWt)^{0.75} + \beta_2(x) + r$$

where:

DFI is the daily feed intake in dry matter averaged over the test period,

β_0 is the regression intercept,

$\beta_0 (MidWt)^{0.75}$ is the partial regression of DFI on the average metabolic weight $(MidWt)^{0.75}$ during the test period,

$\beta_0(x)$ is the partial regression of DFI on the production parameter x (which in the Australian beef industry is the average daily gain during the test period), and

r is the residual error in the model.

In this case, r is equal to the residual feed intake of the animal during the test period.

Residual feed intake has been used in production animals as a measure of the efficiency of feed use and included in selection indices in some livestock species. Depending on the production system, the partial regression of x ($\beta_0(x)$) on DFI changes to reflect the adjustment for the production parameter of importance in that system. For example, in laying hens, regressions have additionally included egg mass and body weight gain during the laying period (Luiting and Urff, 1991a). In pigs, other measures of production have been included, such as start test weight, lean carcass percentage, backfat thickness, lean tissue gain and fat tissue gain (De Haer *et al.*, 1993). In dairy

cattle, adjustments have been made for live weight change and milk production (Veerkamp *et al.*, 1995).

Residual feed intake is heritable. However, as Kennedy *et al.* (1993) highlighted, the heritability of residual feed intake can be variable depending on the genetic and phenotypic parameters in its component traits. In beef cattle, the heritability estimates range from 0.2 to 0.4 (Koch *et al.*, 1963, Herd and Bishop, 2000, Arthur *et al.*, 2001b). In other species, estimates have ranged from 0-0.8 for laying hens (Luiting, 1990), 0.22 in growing dairy heifers (Korver *et al.*, 1991), 0.19 in lactating dairy cows (Van Arendonk *et al.*, 1991), and 0.3 in finishing pigs (Foster *et al.*, 1983).

As residual feed intake includes adjustment of metabolic mid-weight and live weight gain, it has been reported to be genetically independent of these traits (Herd *et al.*, 2004). However, residual feed intake is genetically correlated with some beef production traits. For instance, residual feed intake has a positive genetic correlation with FCR. These genetic correlations have been reported as 0.66 (Arthur *et al.*, 2001b), 0.73 (Van Arendonk *et al.*, 1991) and 0.82 (Korver *et al.*, 1991). Robinson and Oddy (2004) reported high genetic correlations between RFI and subcutaneous fatness at rib and rump (P8) sites of r = 0.58 and r = 0.79, respectively. However, Arthur *el al.* (2001b) initially estimated very low genetic correlations of r = 0.17 and r = 0.06 for rib fat depth and P8 fat depth, respectively, although later, with additional data, these genetic correlations were amended to r = 0.68 and r = 0.71 for rib fat depth and P8 fat depth, respectively (Arthur *et al.*, 2004b). Live weight gain is not correlated with residual feed intake, although the direct effect of 200 day weight and 400 day weight have genetic correlations of -0.45 and -0.26, respectively (Arthur *et al.*, 2001c).

1.4 RFI and heat production

Studies in Angus cattle indicate that much of the variation in RFI may be accounted for by the heat production from various metabolic processors (Herd and Arthur, 2009). From these studies, differences in heat production alone accounted for 58% of the variation in RFI with the remaining variation was accounted for by physical activity (10%), body composition (5%) and “other” unaccounted processes (27%) (Figure 1.1) (Richardson and Herd, 2004). The proportions of variation in heat production associated with RFI were estimated as protein turnover, tissue metabolism and stress accounting for 37%, digestibility accounting for 10%, the heat increment of feeding and fermentation accounting for 9% and feeding patterns accounting for 2% (Figure 1.1) (Richardson and Herd, 2004, Herd and Arthur, 2009).

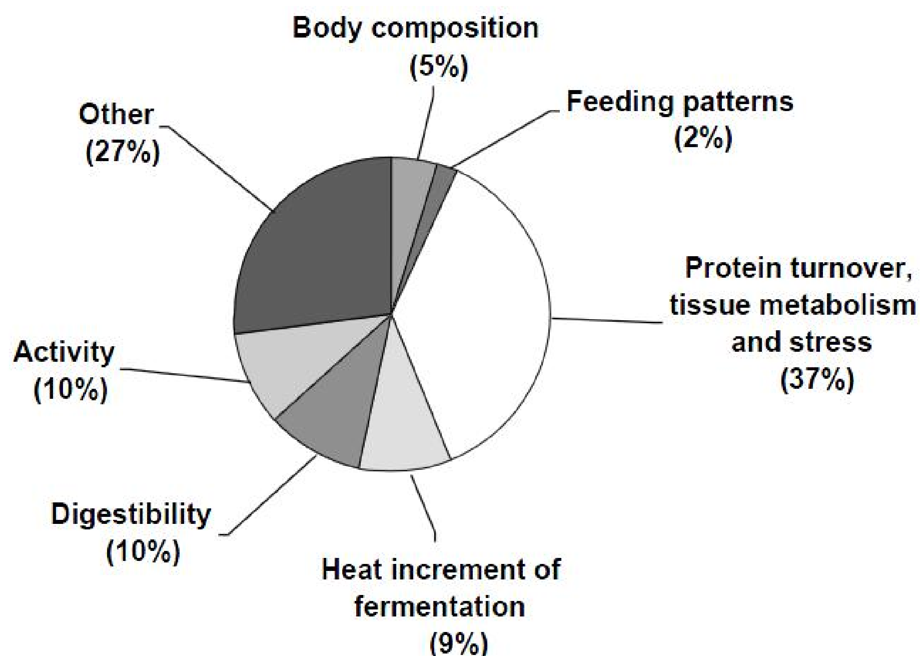

Figure 1.1: Contributions of biological mechanisms to variation in residual feed intake as determined from experiments on divergently selected cattle (Richardson and Herd, 2004).

1.5 RFI and protein turnover

Following divergent selection for residual feed intake in Angus beef cattle, Richardson and Herd (2004) estimated that protein turnover, tissue metabolism and stress accounted for 37% of the variation in RFI. These authors observed three trends regarding protein degradation and protein accretion in the whole body and the correlation with residual feed intake. Firstly, more efficient steers had a lower rate of protein degradation or more efficient mechanisms for protein degradation. Additionally, there was a negative correlation between residual feed intake and protein gain. Secondly, there was a positive association between the sire EBV for residual feed intake and plasma urea concentration at the start of the residual feed intake feedlot test. The third trend from the data was a positive correlation between indicators of liver protein catabolism and residual feed intake at both weaning and the start of the residual feed intake feedlot test.

1.5.1 Protein turnover

Protein turnover is the renewal or replacement of protein in the body by the continual synthesis and degradation thereof (Buttery, 1981). Protein accretion occurs where synthesis exceeds protein degradation. A significant proportion of the difference between the energy cost of protein deposition and protein accretion is a consequence of energy consumption associated with protein turnover. The turnover of protein in the body has a significant energy cost. The formation of one peptide bond during protein synthesis requires at least five ATP molecules and degradation requires at least one ATP (Oddy *et al.*, 1998). Waterlow (2006) equated the cost of synthesizing one gram of protein at a minimum of 4.5kJ g^{-1} protein. Whole body protein synthesis has been estimated as having a theoretical cost between 18-26% of heat production (Hawkins, 1991).

Intracellular protein degradation is an important process, not only for the turnover of proteins, but also for the regulation of individual proteins and for the growth and atrophy of tissues (Croall and DeMartino, 1991). Proteins are degraded within the cell by proteases, enzymes which cleave peptide bonds (Thompson and Palmer, 1998). There are three main intracellular proteolytic systems involving proteases: 1) calcium dependent proteases, 2) the ATP-ubiquitin dependent pathway, and 3) the lysosomal proteases. Two, if not three, of the proteolytic systems are required to completely release all the amino acids, which represents the end point of protein degradation (Goll *et al.*, 1992).

1.5.2 Protease systems

1.5.2.1 Calcium dependent proteases

Calcium dependent or activated proteases are members of the calpain system (Croall and DeMartino, 1991). The calpain system involves two proteases that are Ca^{2+} dependent, μ-calpain and m-calpain, and these seem to be associated primarily, though not exclusively, with the components found in skeletal muscle (Goll *et al.*, 2003). Therefore, it is not surprising that there is evidence to suggest that protein degradation in muscle is regulated by calcium ionophores (Zeman *et al.*, 1985). It has been suggested that the disassembly of myofibrils into filaments is the first step of skeletal muscle protein degradation and that the calpain system is responsible for this process with the end products degraded, in turn, by other protease systems (Goll *et al.*, 1992). Calpains have been localised to the cytoskeleton of non-muscle cells in addition to vesicles and the plasma membrane.

Calpains initiate the degradation primarily of skeletal muscle, principally those of myofibrillar origin (Goll *et al.*, 2003). They cleave the titin, desmin and nebulin proteins near the Z-disk of sarcomeres, separating their attachment to Z-disk proteins. The cleavage of desmin additionally releases α-actinin, initiating the disappearance of the Z-disk, and thereby, leaving a space in the myofibril. This enables the myosin and actin filaments to be released from the myofibril. These released filaments can either be reassembled or additional degradation of the troponin, tropomyosin and C-proteins can occur, which enables the complete disassociation of the myofibril (Goll *et al.*, 2003). The protein fragments of titin, desmin, nebulin, troponin, tropomyosin and C-protein, as well as the actin and myosin filaments, are degraded to amino acids through ubiquination by the proteasome or the peptidases of the lysosomal protease system.

Calpastatin is involved in the inhibition of myofibrillar protein disassembly that precedes protein degradation by the lysosomal and ATP-ubiquitin dependent proteases. Calpastatin inhibits calpains by binding to two of the calpain domains, domain IV and VI. This binding is Ca^{2+} dependent (Goll *et al.*, 2003). The Ca^{2+} originates from the calpain and fewer concentration of Ca^{2+} is required for calpain/calpastatin binding than is required for proteolysis. Calpastatin is able to inhibit 4 units of calpain for every unit of calpastatin (Dransfield, 1999).

1.5.2.2 ATP-ubiquitin dependent pathway

The ATP-ubiquitin dependent proteolysis pathway, as its name suggests, requires the activity of the 76-residue protein ubiquitin and an energy source (ATP) for its function (Hershko and Ciechanover, 1992). The primary determinant of protein targeting for degradation in this system is via the ubiquination of the target proteins (Hochstrasser,

1995). Ubiquination of the target protein is a complex system involving at least three enzymes (E1, E2 and E3) in addition to the short ubiquitin protein.

Ubiquitin proteins are activated by the ubiquitin activating enzyme E1. This process involves ATP as the energy source (Ciechanover, 1998). As the ubiquitin protein is activated, it binds to the E1 enzyme in the presence of ATP, releasing AMP and two phosphate ions. The E2 enzyme, which is a ubiquitin-conjugating enzyme or ubiquitin-carrier protein, transfers the activated ubiquitin protein to a cysteine-residue of the E2 enzyme complex (Ciechanover, 1998). Having completed the activation, the E1 enzyme is released from the ubiquitin protein and usually continues to activate ubiquitin proteins for ubiquination or is itself degraded. The E2-ubiqitin protein complex is acylated with the catalytic removal of the activated ubiquitin molecule by the E3 enzyme to a targeted protein substrate for degradation (Weissman, 1997). The E3 ubiquitin-protein ligase enzyme is ultimately responsible for target protein specificity (Weissman, 1997). The E3 enzyme continues to add ubiquitin to the protein substrate forming a poly-ubiquitin chain, which marks the protein substrate for degradation by the proteasome.

Before proteolysis of the ubiquitinated target protein substrate can occur, the 26S proteasome must be assembled. In its basic active state, the 26S proteasome is comprised of a 20S catalytic complex core associated by two 19S regulatory complexes (PA700), flanking the 20S complex on either side (Attaix *et al.*, 1998, Ciechanover, 1998). The 20S catalytic complex can degrade proteins completely in the absence of ATP if they are denatured. If the protein is stably folded, the 20s catalytic complex is unable to unfold the protein for degradation (Hochstrasser, 1996). The 700 kDa

proteasome activator (PA700) contains 6 subunits that are members of the ATPase family (Attaix *et al.*, 1998). These subunits are believed to provide energy for the assembly of the 26S proteasome. The remaining 19 subunits are believed to be involved in the recognition of the poly-ubiquitin chains of the targeted protein substrate. The other crucial function of the PA700 19S subunit is to unfold the protein substrate complex (Hochstrasser, 1996). This enables the 26S proteasome complex to degrade even the most complex protein substrates.

After the poly-ubiquitin chain attached to the substrate protein is bound by the PA700 complex, the protein is unfolded by the PA700 complex as it is enters the core of the 20S proteasome (Hochstrasser, 1995). This is an energetically dependent step using ATP. As the substrate protein chain enters the 20S proteasome core, it is cleaved into small peptides (Hochstrasser, 1995, Attaix *et al.*, 1998). These small peptides are released from the 26S proteasome complex by 'gills' located in the 20S proteasome wall. During this process, the poly-ubiquitin chain serves to anchor the protein substrate in place as it is being degraded (Hochstrasser, 1995). Disassembly of the poly-ubiquitin chain facilitates the release of the protein substrate as it travels through the 20S complex core. The disassembly of the poly-ubiquitin chain occurs via deubiquination in which the small ubiquitin chains are cleaved apart (Ciechanover, 1998).

There is an additional form of the proteasome that utilises the central 20S proteasome core, with either a 26S proteasome or a PA28 proteasome. The PA28 proteasome is very similar to the 26S proteasome, but the PA700 is substituted with a 28 kDa proteasome activator (PA28) (Hochstrasser, 1996). In this form, the PA28 stimulates the 20S proteasome peptidase activity with small protein substrates. However, the PA28

reduces the 20S proteasome's ability to break down full-sized proteins as presumably it cannot unfold them (Hochstrasser, 1996; Kuehn and Dahlmann, 1997).

1.5.2.3 Lysosomal proteases

The myofibrillar proteins released from the myofibrils by calpains and the proteasome are degraded by lysosomal proteases (Goll *et al.*, 2003). Lysosomal proteases, however, are not restricted to the degradation of only skeletal muscle proteins to amino acids but can degrade proteins of any origin. The lysosomal protease system is complex and involves lysosomes, autophagic mechanisms and proteases. The lysosome is described as membrane-bound vesicle found within the cytoplasm and contains many acid hydrolases (Bechet *et al.*, 2005). The lysosomal protease system creates an environment sealed by the lysosome membrane that enables the optimum performance of the hydrolase enzymes within the lysosome (Pillay *et al.*, 2002). Lysosomes have an acid, fluid filled cavity or lumen of pH 4-5. Unlike the other members of the protease systems (e.g. the calpains and the ubiquitin dependent proteasome), the lysosomal proteases are physically isolated from the cytoplasmic elements, and hence, rely upon the autophagic and other mechanisms to transport proteins into the lysosome for degradation by the lysosomal hydrolases (Bechet *et al.*, 2005). The mechanisms of transport into the lysosomes include macroautophagy, microautophagy, crinophagy and chaperone mediated transport (Blommaart *et al.*, 1997).

1.5.3 Contribution of protein turnover to the efficiency of feed use

Following divergent selection for residual feed intake in beef cattle, Richardson and Herd (2004) found that protein turnover, tissue metabolism and stress accounted for 37% of the variation in RFI. These authors made 3 observations regarding protein

degradation and protein accretion in the whole body and the correlation with residual feed intake. Firstly, they suggested more efficient steers had a lower rate of protein degradation or more efficient mechanisms for protein degradation. This conclusion was drawn based on the observation that there a negative correlation for residual feed intake and the proportion of chemical protein in the whole body as a percentage of live weight at slaughter (Richardson *et al.*, 2001). Additionally, there was a negative correlation between residual feed intake and protein gain. Secondly, there was a positive association between the sire EBV for residual feed intake and plasma urea concentration at the start of the residual feed intake feedlot test. The progeny of these high residual feed intake sires had lowered concentrations of blood urea compared to the progeny of low efficiency sires at the start of the feedlot test (Richardson *et al.*, 2004). Plasma urea was also positively correlated to average daily gain and average daily feed intake (Richardson *et al.*, 2004). This was evident at weaning as well. As urea is an end product of protein degradation, this implied that less efficient steers had a greater rate of protein degradation. However, urea is additionally donibated by the removal of NH_3 derived from the rumen. The third observation from the data was a positive correlation between blood aspartate amino transferase concentration and residual feed intake at both weaning and the start of the residual feed intake feedlot test. Elevated aspartate amino transferase concentrations indicated a higher degree of liver protein catabolism in the progeny from high residual feed intake sires (Richardson *et al.*, 2004).

In these experiments, indirect measures of protein degradation were also used by quantifying the urinary output of 3-methyl-histidine (3MH), creatinine and the 3MH:creatinine ratio in an animal house and over the whole experiment (feedlot + animal house) (Table 5.1). Notably, these indirect measures were not significant for

residual feed intake. In the animal house, creatinine was positively correlated (P<0.10) to the feed conversion ratio (FCR), but was negatively correlated, though not significantly, over the whole experiment (Table 5.2). The 3MH:creatinine ratio was negatively correlated (P<0.05) in the animal house, but over the whole experiment was positively correlated (P<0.01). These discrepancies between experimental locations may be due to extra stress in the animal house.

Protein turnover is energetically expensive (Oddy *et al.*, 1998). It is believed that protein synthesis requires much more energy than protein degradation in that the synthesis of a peptide bond requires at least five ATP molecules and the breaking the same bond requires one molecule of ATP. Therefore, hypothetically, if animals have constant growth rates regardless of the feed intake, those animals with more muscle protein turnover would require a higher residual feed intake to maintain the same growth as animals with low protein turnover.

Table 1.1: Correlation coefficients for measures of protein degradation

Trait	Creatinine (g/day)	3MH (mg/day)	3MH:creatinine ratio
Animal house			
Average daily feed intake (kg/day)	0.40*	0.31	-0.21
Average daily gain (kg/day)	0.01	0.27	0.36*
Feed conversion ratio(kg feed/kg gain)	0.25†	0.20	-0.57*
Residual feed intake (kg/day)	0.18	0.26	0.04
Final live weight	0.43*	0.40*	-0.44*
Feedlot and animal house			
Average daily feed intake (kg/day)	0.45**	0.30*	-0.40*
Average daily gain (kg/day)	0.31*	0.15	-0.33*
Feed conversion ratio(kg feed/kg gain)	-0.23	-0.001	0.48**
Residual feed intake (kg/day)	0.12	0.05	-0.22

†P<0.10; *P<0.05; **P<0.01; ***P<0.001
3MH = 3-methyl-histidine
(adapted from (Richardson *et al.*, 2004)

Indeed, protein turnover has been implicated as a major source of inefficiencies involved with residual feed intake. Protein turnover has been estimated to account for

15-20% of the resting metabolic rate (Waterlow, 1984) and contribute up to 50% of heat production (Webster, 1978). Others have stated that protein turnover contributes a maximum of 8% towards heat production (Tomas *et al.*, 1988). 3-Methyl-histidine and hydroxyproline, blood or urine release markers of skeletal muscle protein and collagen protein degradation respectively, have been shown to be positively correlated with heat production (Murdoch *et al.*, 2003). An increase in heat production has been linked with high feed intake and greater visceral organ weights (Ferrell and Jenkins, 1998). Visceral organ weights have been associated with variation in maintenance requirements and efficiency of gain due to differences in metabolic rate (Baldwin *et al.*, 1985, Ferrell and Jenkins, 1998).

Murdoch *et al.* (2003) found that under restricted feeding, gene expression of μ-calpain and m-calpain of the calpain proteolytic system and ubiquitin from the ATP-ubiquitin system were down-regulated. The calpains and ubiquitin also showed a positive correlation with average heat production (Murdoch *et al.*, 2003). Under feed restriction, reduction in protein turnover is another mechanism involved in conserving energy.

McBride and Kelly (1990) found that components of protein turnover were a significant energy sink in ruminants (Table 5.1). They suggested that heat production in the gastrointestinal tract is far greater for protein synthesis and degradation compared to whole body heat production. They also estimated that heat production for protein synthesis is five times greater than that of protein degradation (McBride and Kelly, 1990).

Table 1.2: Metabolic heat production

	Percentage of gastrointestinal heat production	Percentage of whole body heat production
Na^+, K^+-ATPase	28.5 - 62	5.7 - 12.4
Protein Synthesis	20.2 - 23.1	4.0 - 4.6
Protein Degradation	4.3	0.9
Total	53.0 - 90.4	10.6 - 17.9

(adapted from McBride and Kelly, 1990).

Tomas *et al.* (1991) pointed out that whole body protein turnover accounts for 15-25% of total heat production in mammals and suggested that this contribution may be as high as 20-30% in chickens. There was a clear relationship between feed conversion ratio and muscle protein breakdown rates, which was especially obvious in young chicks. Chicks selected for improved feed conversion ratio were also inadvertently selected for lower rates of muscle protein degradation. Selection for high food consumption also co-selected for high rates of muscle protein breakdown (Tomas *et al.*, 1991). The authors concluded that the rate of protein turnover is an important contributor of overall energetic efficiency and that the two traits are genetically associated. Protein synthesis appeared to be unchanged by genetic selection in chickens (Tomas *et al.*, 1991). The authors concluded that protein synthesis rates were more responsive to nutritional and environmental factors than genetic factors. This was confirmed in their study as plasma IGF-I concentration was highly correlated to protein synthesis but not correlated to feed conversion ratio. The results suggest that the contribution of protein turnover to FCR is primarily through protein degradation. Protein degradation was suspected of being regulated by genetic (intrinsic) mechanisms.

Another study involved the determination of liver protein turnover rates in rainbow trout on different diets, either low protein/high fat diet (LP/HF), a non-carbohydrate/high fat diet (NCH/HF) or a control diet (Peragén *et al.*, 2000). Both of the high fat diets decreased feed conversion indices, feed efficiency and the protein

conversion ratio. Both of the high fat diets also increased the rate of liver protein synthesis and degradation over the control diet. The NCH/HF diet decreased feed efficiency by 16%, increased protein synthesis by 211% and increased protein degradation 1243% over the control diet. The LP/HF diet had similar effects, resulting in a 58% reduction in feed efficiency, an increase in protein synthesis by 167%, and an increase in protein degradation by 881% over the controls. Overall, protein retention efficiency was reduced in the LP/HF and NCH/HF diets by 64% and 73%, respectively, over the control diet.

Myofibrillar fragmentation index was found to be higher in low efficiency cattle than high efficiency cattle ($P<0.05$) divergently selected for low and high residual feed intake (McDonagh *et al.*, 2001). Myofibrillar fragmentation index is a measure of the degradation of muscle proteins under post-mortem conditions (Hopkins *et al.*, 2000). The calpain/calpastatin system is believed to be the main cause of post-mortem myofibrillar fragmentation (Watanabe *et al.*, 1996). There was no significant difference between the low and high efficiency steers in m-calpain and μ-calpain activity. However, the calpastatin concentration was 13% higher ($P<0.05$) in animals selected for high efficiency than in low efficiency animals. The same steers selected for high efficiency also had a 7% lower level of myofibre breakdown.

Calpains initiate the breakdown of skeletal muscle in the live animal with calpastatin as the inhibitor of calpain activity (Goll *et al.*, 2003). Therefore, a few points can be postulated from the calpain and calpastatin concentrations in the muscle of animals selected for high and low residual feed intake. Firstly, selection for low residual feed intake co-selects for decreased muscle protein degradation. These animals are able to

better inhibit the calpain proteases that initiate muscle protein degradation. This is because they have a higher concentration of calpastatin and no difference in calpain concentration. Secondly, selection for low residual feed intake will hypothetically result in less tender meat, if the mechanism for reducing residual feed intake is by reducing protein degradation rate. Post-mortem protein degradation is highly correlated with tenderness such that higher rates of protein degradation are associated with higher tenderness scores (Pringle *et al.*, 1997).

Results from the divergent selection for residual feed intake in cattle indicate that feed efficiency can be related to myofibrillar fragmentation in that low efficiency animals had a 7% increase in myofibrillar fragmentation compared to high efficiency animals at day 1 post-slaughter (McDonagh *et al.*, 2001). However, there was no corresponding increase meat toughness as measured by shear force and compression. In fact, there was no significant difference in shear force, compression, m-calpain and μ-calpain between the two residual feed intake lines. Whilst there was no difference between calpain concentrations, the low efficiency line had 13% less calpastatin activity (McDonagh *et al.*, 2001). The increase in myofibrillar fragmentation is most probably due to decrease in calpastatin concentration, and hence, decreased proteolytic activity in the low efficiency line. Myofibrillar fragmentation was additionally found to be negatively correlated with the meat tenderness measures of shear force and compression, although there was no statistical relationships between ageing related changes in shear force and compression on calpain system activity (McDonagh *et al.*, 2001). The increase in myofibrillar fragmentation and decrease in calpastatin concentration did not result in an increase in meat tenderness within the residual feed intake lines. This is inconsistent with other findings and with current knowledge on the factors affecting tenderness.

However, the results were from animals after a single generation of divergent selection for residual feed intake. Given the differences in calpastatin and myofibril fragmentation. These results suggested that on-going selection for high residual feed intake may ultimately negatively affect meat tenderness (McDonagh *et al.*, 2001).

1.6 Conclusion

Protein turnover, tissue metabolism and stress account for 37% of the variation seen in residual feed intake in beef cattle. Of this, protein turnover has been estimated to contribute 15-20% of the resting basal metabolic rate. As it is so energetically expensive, if the rate of protein turnover can be reduced so that its contributions to basal metabolic rate is minimal, then this may be one method of improving the efficiency of feed use by the beef industry. There are gaps in the knowledge of protein turnover associations with residual feed intake, and there is no concrete evidence as to the association between residual feed intake and protein turnover and as such, this needs to be investigated more fully. Thus, this project attempted to better define the relationship between protein turnover and the efficiency of feed use through residual feed intake. The hypothesis was that animals with a lower protein turnover will be more efficient and hence, will have a decreased residual feed intake compared to animals with high protein turnover. Consequently, those animals with low residual feed intake (and low protein turnover) will have decreased meat tenderness and hence, eating quality.

Chapter 2

Protein metabolism of Angus heifers divergently selected for residual feed intake

CHAPTER 2: Protein metabolism of Angus heifers divergently selected for residual feed intake

2.1 Introduction

Protein turnover is energetically expensive (Oddy *et al.*, 1998). Protein synthesis requires much more energy than protein degradation. The synthesis of a peptide bond requires at least five times the energy of that required to break the same bond (McBride and Kelly, 1990, Oddy *et al.*, 1998). Protein turnover accounts for 15-20% of the metabolic rate (Waterlow, 1984). Protein synthesis and protein metabolism have been estimated to contribute as much as 50% of resting heat production (Webster, 1978).

The turnover of proteins has been implicated as a major source of inefficiencies that contributes to variation in residual feed intake. Richardson and Herd (2004) hypothesised from cattle divergently selected for residual feed intake, biological mechanisms such as protein turnover, tissue metabolism, and stress could account for 37% of the variation seen in residual feed intake of these animals. Following divergent selection for residual feed intake for half a generation in beef cattle, Richardson and Herd (2004) observed three trends regarding protein degradation and protein accretion in the whole body and the correlation with residual feed intake. Firstly, more efficient steers had a lower rate of protein degradation or more efficient mechanisms for protein degradation. This conclusion was drawn based on the observation that there a negative correlation between residual feed intake and the proportion of chemical protein in the whole body as a percentage of live weight at slaughter (Richardson *et al.*, 2001). Additionally, there was a negative correlation between residual feed intake and protein

gain. Secondly, there was a positive association between the sire EBV for residual feed intake and plasma urea concentration at the start of the residual feed intake feedlot test. As urea is an end product of protein degradation (as well as ammonia production in the rumen and the deamination of amino acids in the liver), this implies that high residual feed intake steers had a greater rate of protein degradation. The third trend from the data presented by Richardson and Herd (2004) was a positive correlation between blood aspartate amino transferase concentration and residual feed intake at both weaning and the start of the residual feed intake feedlot test. An elevated aspartate amino transferase concentration suggests a higher rate of liver protein catabolism in the progeny from high residual feed intake sires. The contribution of protein turnover, and the energy cost associated with the turnover of protein, towards the differences in animals further divergent in residual feed intake was assessed herein.

2.2 Materials and Methods

2.2.1 Experimental protocols

Sixteen (16) Angus beef heifers from an experimental population divergently selected for residual feed intake (RFI) for approximately 3-4 generations either for high RFI (low "efficiency"; n=8, average mid-point parental RFI EBV=0.64±0.07 kg/d) or low RFI (high "efficiency"; n=8, average mid-point parental RFI EBV=-0.78±0.26 kg/d) were used, based on parental EBVs calculated by BREEDPLAN (Graser *et al.*, 2005). Heifers were aged 277±14.2 days at induction into the Beef Research Unit at the University of New England and weighed 272.9±24.8 kg. Prior to experimentation, animals were adapted to an 'intermediate' feedlot ration comprising barley grain, chopped sorghum hay, Molofos® and minerals (Table 2.1). Half (n=8) of the replicate (4 high and 4 low RFI heifers) were assigned to a low feed intake (105% of

maintenance energy requirements) dietary treatment and the other half (n=8) of the replicate (4 high and 4 low RFI heifers) were assigned to a high feed intake (180% of maintenance energy requirements) that equated to approximately 90% of *ad libitum* feed intake (SCA, 1990). Once the animals had adapted to this feeding level for approximately 28 days, the measurements of interest were taken. The animals were swapped to the other level of feed intake after approximately 35 days and measurements taken again (after a 21 day adaption period) such that traits of interest were measured at both feeding levels for each animal. During the entire experimental period, the heifers were housed in individual pens in the Beef Research Unit at University of New England and had *ad libitum* access to water.

Table 2.1: Diet ingredients and composition

	Diet
Ingredients (%)	
Barley	50
Sorghum Hay	40.5
Molofos®	8
Lime	1
Bicarbonate Soda	0.5
Composition	
Crude Protein, %	14.33
Gross Energy, MJ/kgDM	16.68
Organic Matter Digestible Energy, MJ/kgDM	18.16

2.2.2 Protein metabolism and leucine kinetics

Infusions and plasma samples for protein metabolism were taken over the 24 hours following the measurement of heat production (Chapter 3). L-leucine-1-^{13}C was infused at a rate of 10µmol/kg $BW^{0.75}$/hr into the right external jugular catheter. The L-leucine-1-^{13}C was infused for 12 hours for the first four heifers to determine when the plateau in enrichment had been reached for L-leucine-1-^{13}C, α-^{13}C-ketoisocaproic acid (α-^{13}C-KIC) and $^{13}CO_2$ enrichments (Figure 2.1). Plateaus were obtained in L-leucine-1-^{13}C, α-

^{13}C-KIC and $^{13}CO_2$ enrichments by six hours and therefore subsequent infusions of 8 hours were used in the remaining heifers. Blood samples were taken into 10mL heparinised syringes for analysis of L-leucine-1-^{13}C, α-^{13}C-KIC enrichment and for the analysis of $^{13}CO_2$ enrichment (10mL). Samples were taken at -60, -30, 0, 120, 240, 300, 360, 390, 420, 450 and 480 minutes after the start of infusion. For the first four heifers, samples were collected for a further 240 minutes at 30 minute intervals. Blood samples were kept on ice until centrifuged at 1100g for 20 minutes at 5°C. Plasma was stored at -20°C until analysed.

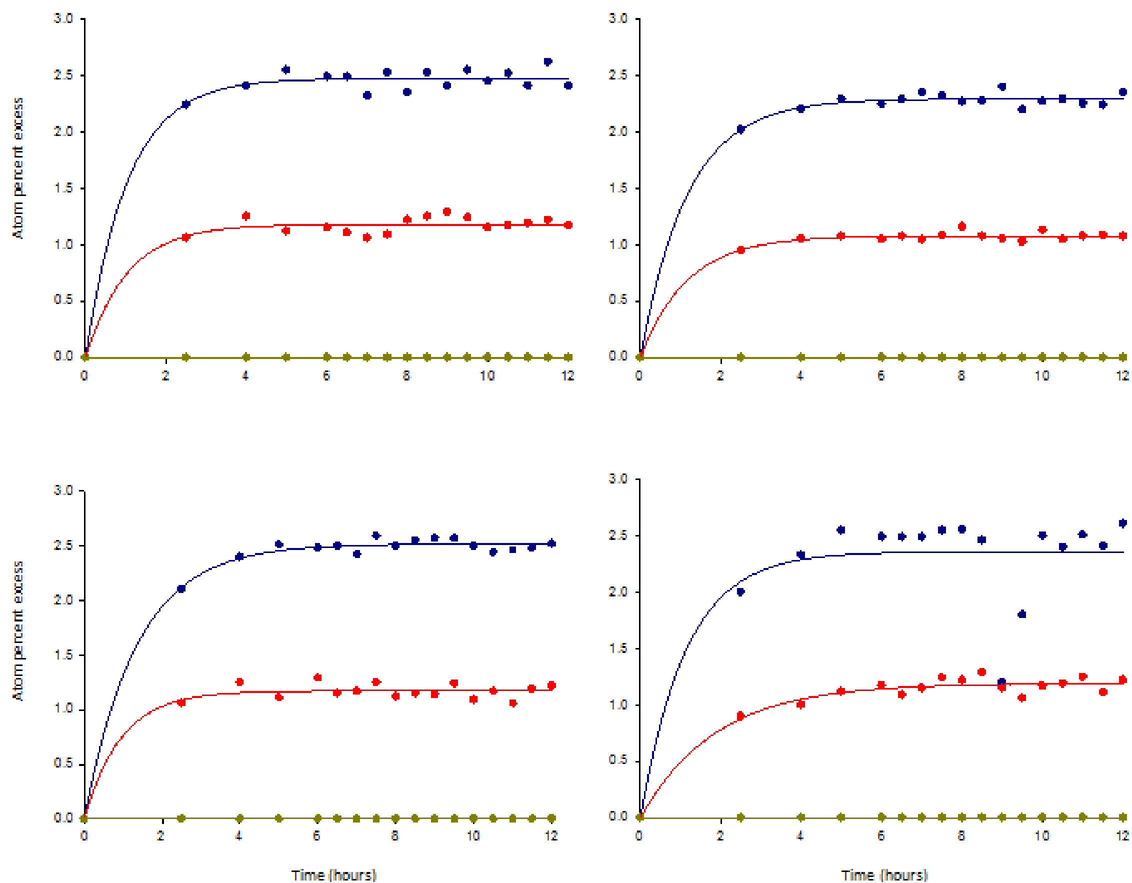

Figure 2.1: Enrichments (atom percent excess) of plasma leucine (●), α-ketoisocaproic acid (●) and carbon dioxide (●) of the first four animals infused for 12 hours with 10µmol/kg BW0.75/hr L-leucine-1-13C.

2.2.3 Determination of α-ketoisocaproic acid and leucine enrichment

The enrichment of α-ketoisocaproic acid (α-KIC) and leucine from plasma was determined by the method of Calder and Smith (1988). Plasma (0.8g) was deproteinised with 0.18mL of 35% sulfosalic acid, vortexed and centrifuged at 7800g for 10 minutes. The supernatant was applied to 1.5ml of AG50W-X8 100-200 mesh cation exchange resin and eluted with 1mL of deionised water. The eluant was collected and used for ketoacid enrichment. Following washing of the resin with an additional 4mL of deionised water, the amino acids were eluted with 2mL of 2M ammonium hydroxide and 1mL of deionised water. The amino acid fraction was lyophilised at -20°C overnight until dry.

N-*tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) derivatives of the ketoacid fraction were prepared as follows. One mL of 0.5% phenylenediamine was added to the ketoacid fraction, purged with nitrogen and heated at 90°C for one hour. After this had cooled, 2mL of ethyl acetate was added, agitated and centrifuged at 1150g for five minutes resulting in two separate phases. The organic (uppermost) layer was transferred into a culture tube containing 0.5g of sodium sulphate. The organic and inorganic layers were separated again with 2mL of ethyl acetate with the organic layer once again being transferred. The ethyl acetate was decanted into a 3-4mL vial and evaporated at 90°C under a gentle stream of nitrogen until approximately 0.5mL remained. The remaining volume was transferred to a 1mL v-vial and evaporated to dryness under the same conditions. This was then derivatised with 75µL of MTBSTFA:acetonitrile and heated at 90°C for 15 minutes. The derivatised sample was then transferred to an auto sampler vial that had been fitted with a 100µL glass inset.

MTBSTFA leucine derivatives were synthesised as follows. The freeze-dried samples were dissolved in 350µL of 0.1M hydrochloric acid. These were evaporated to dryness at 90°C under a gentle stream of nitrogen in a 1mL reaction vial. To this residue, 100µL of MTBSTFA: acetonitrile was added and heated at 90°C for 20 minutes. After cooling, the derivatised sample was transferred into an auto sampler vial fitted with a 100µL glass inset.

Leucine and α-ketoisocaproic acid (as MTBSTFA derivatives) were analysed on a Varian gas chromatograph mass spectrometer using a 1µL split (40:1) injection. The column used was an Alltech EC-1 (30m x 0.25mm x 0.25µ). The derivatives were injected at an injector temperature of 150°C. The oven temperature remained at 150°C for 0.1 minutes, increased 15°C per minute to 280°C and then held at this temperature for 20 minutes after injection. The retention time of the MTBSTFA derivatives were 5.60 minutes for α-ketoisocaproic acid and 5.26 minutes for leucine using a helium head pressure of 10psi.

2.2.4 Determination of 3-methyl-histidine

Urine was collected into a 20L drum using a No. 26 Folley catheter (1.2m, 30mL balloon) that was inserted into the bladder. Urine was acidified with 300mL of 5N sulphuric acid. Subsamples (5%) of the urine were frozen daily whilst the rest was discarded. Subsamples remained frozen at -20°C until analysis. Analysis of urinary 3-methyl-histidine (3MH) was made using the colorimetric method of Fitch *et al.* (1986), the fluorescamine derivatisation method of Wassner *et al.* (1980), and the *o*-phthalaldehyde / mecaptoethanol derivatisation method of Turnell and Cooper (1982).

However, none of the methods used appeared to be repeatable and sensitive enough for the quantification of 3-MH. For this reason, the results have not been presented herein.

2.2.5 Determination of carbon dioxide enrichment

^{13}C enrichment of CO_2 in blood was analysed by the acidification of whole blood and measuring the $^{13}CO_2$:CO_2 ratio. Blood samples were processed using a modified procedure described by Young (1968). Within two minutes of blood sampling, 10mL of whole blood was injected into a McCartney bottle containing two 2mm glass beads. A 12x75mm culture tube containing 2mL of CO_2 free 1M NaOH was placed inside the McCartney bottle which was sealed. Two (2) mL of CO_2 free 0.5M H_2SO_4 was injected into the blood sample through the cap and septum of the McCartney bottle. The acidified blood sample was mixed in the bottle by swirling until the blood had coagulated. This was incubated at room temperature for 24 hours prior to processing to allow the NaOH to absorb as much CO_2 as possible.

After 24 hours, the culture tubes were removed from the McCartney bottles and the contents of each tube transferred to a Bijou bottle. The culture tube was washed with 1mL of CO_2 free 5% (w/v) NH_4Cl. The CO_2 was precipitated as $BaCO_2$ with the addition of 0.5mL 20% (w/v) $BaCL_2.2H_20$ in the Bijou bottle. The $BaCO_2$ solution was quantitatively transferred to a Millipore® 25mL microanalysis filter holder fitted with 25mm Whatman No. 452 filter paper connected under vacuum to a Buchner funnel and washed with deionised water. The $BaCO_3$ precipitates were dried with several washes of acetone and transferred to a scintillation vials containing two 2mm glass beads and ground to a powder using a vortex mixer.

The $BaCO_3$ powder was analysed with an automated C analyser (NA 1500 Nitrogen/Carbon/Sulfur Analyser, Carlo Erba Instruments, Stada Rivoltana, Italy) interfaced with a mass spectrometer (TRACERMASS, Stable Isotope Analyser; Europa Scientific Ltd, UK) to determine the ^{12}C:^{13}C isotope ratio. The atom percent excess was calculated from the background sample taken prior to $NaH^{13}CO_3$ infusion.

2.2.6 *Calculation of parameters of leucine kinetics*

Parameters of leucine flux were calculated as described by Krishnamurti and Janssens (1988). Briefly, plasma leucine flux, leucine oxidation and protein synthesis and degradation were estimated from the following equations:

$$Leucine\ Flux\ (mmol/kgBM/day) = \left[\frac{100}{IE_{Leu}} - 1\right] \times RI$$

▯ 濅㊉re:

$kgBM$ = Kilograms body mass,

IE_{Leu} = Isotopic enrichment of plasma ^{13}C-leucine at plateau (atom % excess), and

RI = Rate of infusion of ^{13}C-leucine.

$$Leucine\ Oxidation\ (mmol/kg/day) = \frac{(IE_{CO_2}/0.81) \times RCO_2}{IE_{\alpha-KIC}}$$

Where:

IE_{CO_2} = Isotopic enrichment of plasma $^{13}CO_2$ at plateau (atom % excess),

RCO_2 = CO_2 production rate, and

$IE_{\alpha-KIC}$ = Isotopic enrichment of plasma $^{13}\alpha$- KIC at plateau (atom % excess).

$$Protein\ Synthesis\ (g/kg/day) = \frac{(Leucine\ Flux - Leucine\ Oxidation)}{Leucine\ concentration\ in\ protein} \times 0.131$$

Where:

0.131 is the conversion constant for leucine from mmol/kg/day to g/kg/day as the molecular weight of leucine is 131.2 g/mol.

$Leucine\ concentration\ in\ protein =$ 6.0% assuming a constant fraction of 60g leucine/kg of synthesised protein (Lobley *et al.*, 1980)

$$Protein\ Degradation = Protein\ Synthesis - Protein\ Accretion$$

Where:

$$Protein\ Accretion = Retained\ Nitrogen \times 6.25$$

Where:

$$Retained\ Nitrogen = Nitrogen\ Intake - Nitrogen\ Excretion$$

Muscle (lean) gain was estimated as containing 22% protein (Oltjen *et al.*, 1986). Therefore,

$$Muscle\ Gain = \frac{Protein\ Accretion}{0.220}$$

The heat production (HP) for protein synthesis (PS), protein degradation (PD) and protein turnover (PT) calculations were derived as follows:

$$HP\ for\ PS\ (MJ/day) = \frac{PS\ (g/day)}{110} \times 5 \times 0.078$$

Where:

the average molecular weight of amino acids in protein was assumed to be 110 g/mol (Oddy *et al.*, 1998), and the number of ATP molecules required for the synthesis of one gram of protein was assumed to be 5 (Milligan and McBride, 1985). The energy content of ATP was assumed to be 0.078 MJ/ATP (Oddy *et al.*, 1998).

$$HP\ for\ PD\ (MJ/day) = \frac{PD\ (g\ /day)}{110} \times 1 \times 0.078$$

Where:

the number of ATP molecules required for the degradation of a peptide bond was assumed to be 1 (Milligan and McBride, 1985).

$$HP\ for\ PT\ (MJ/day) = EE\ for\ PS + EE\ for\ PD$$

Where:

EE = Energy Expenditure.

Protein synthesis (PS), degradation (PD) and turnover (PT) were also calculated as to their contribution toward whole body heat production by dividing the heat production for protein synthesis, degradation and turnover (MJ/day) by whole body heat production (MJ/day) in chapter 3.

2.2.7 Statistical analysis

All statistical analyses were conducted using Proc MIXED in SAS 9.1. Tests of significance of fixed effects were calculated utilising type III sums of squares mixed models. Fixed effects fitted in the models included residual feed intake line (high, low), feeding level (105M, 180M) and the period of trait measurement (1st, 2nd) in the crossover design. Animal was fitted as a random term with live weight at the start of experimental periods fitted as a covariate. All interactions were tested in the maximal model with non-significant interactions being removed in order of least significance. This enabled the best linear unbiased estimates and standard errors to be extracted.

2.3 Results

Heifers in the residual feed intake lines did not differ from one another in leucine kinetics, protein metabolism or for the energy requirements for protein metabolism

($P<0.05$) (Tables 2.2, 2.3 and 2.4). Feeding level had the largest effects on leucine kinetics, protein metabolism and the energy requirements for protein metabolism (Table 2.2 and 2.4). During measurement period (Time) #2, there was a 15% increase in the contribution of protein synthesis and a 24% increase in the contribution of protein turnover (balance) to the heat production from protein metabolism of heifers, however, these were only trends ($P<0.1$).

Table 2.2: Main effects means and SEM over the treatment periods in leucine kinetics for high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

	RFI Line				Feeding Level			
	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
Leucine Kinetics								
Leucine APE	2.07	2.14	0.1	NS	2.27	1.99	0.09	0.07
α-KIC APE	1.88	1.83	0.13	NS	1.89	1.81	0.13	NS
CO2 APE	0.003	0.003	0.0004	NS	0.003	0.003	0.0004	NS
Leucine flux (mmol/hr)	35.74	34.02	1.66	NS	32.02	37.74	1.66	0.04
Leucine oxidation (mmol/hr)	0.30	0.33	0.04	NS	0.28	0.35	0.04	NS

NS = Not significant i.e. $P>0.10$; SEM = Standard error of the mean; APE = Atom percent excess; α-KIC=α-ketoisocaproic acid

The oxidation of leucine to α-KIC during measurement period #2 was 63% greater in high RFI heifers. However, this did not translate into an overall effect of RFI line on leucine oxidation (Table 2.2). Leucine flux was 18.0% greater in heifers fed at 180% ME_m than at 105% ME_m (Table 2.2). No RFI line effects were observed for the leucine kinetics (Table 2.1 and 2.3).

The increase in leucine flux of heifers fed at 180% ME_m, was associated with a 17.7% increase in protein synthesis over heifers fed at 105% ME_m ($P<0.05$) (Table 2.3). Protein degradation was 14.3% higher in heifers fed at 180% ME_m than heifers fed at 105% ME_m ($P<0.05$). The 3.4% difference between protein synthesis and degradation resulted in a 61.4% increase in protein balance and gain of the heifers fed at 180%

ME_m. While there was no significant difference between high and low RFI heifers in protein metabolism (Table 2.3), the low RFI heifers had a 5.1% increase in protein synthesis and 4.8% increase in protein degradation. This resulted in an 8.3% increase in protein gain and balance of the low RFI heifers as a result of the 0.3% between line difference in protein synthesis and protein degradation.

Table 2.3: Main effects means and SEM over the treatment periods in protein metabolism for high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

	RFI Line				Feeding Level			
	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
Protein Metabolism								
Protein Synthesis (g/day)	1847.45	1757.49	91.79	NS	1655.71	1949.23	86.67	0.04
Protein Degradation (g/day)	1684.27	1606.84	97.12	NS	1535.63	1755.48	93.2	NS
Protein Balance (g/day)	163.18	150.65	22.25	NS	120.08	193.75	21.35	0.04
Muscle Gain (g/day)	741.71	684.75	101.14	NS	545.8	880.66	97.06	0.04

NS = Not significant i.e. P>0.10; SEM = Standard error of the mean
Protein balance = protein synthesis – protein degradation

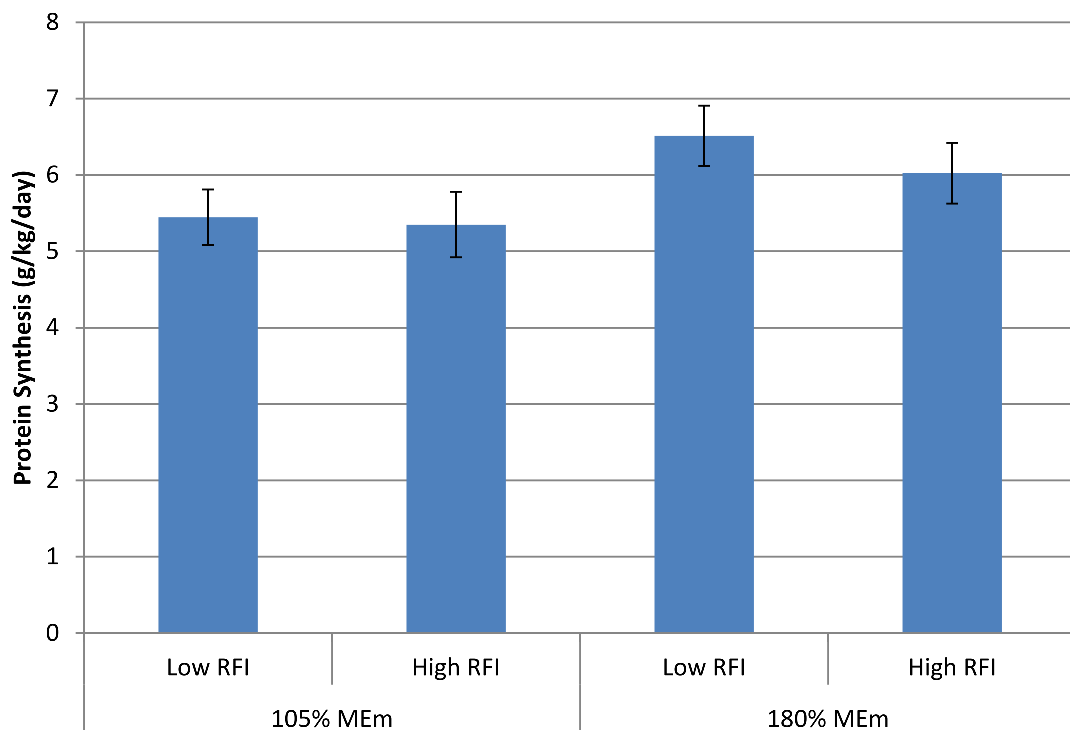

Figure 2.2: Least squares means for the RFI by feeding level interaction on protein synthesis adjusted for weight (covariate).

When live weight was taken into consideration, there was no RFI line by feeding level interaction for daily protein synthesis (Figure 2.2), daily protein degradation (Figure 2.3) or daily protein balance (Figure 2.4). However, when adjusted for weight, the increase of 16.4% in daily protein synthesis at 180% ME_m was significantly higher ($P<0.05$) than for heifers fed at 105% ME_m. Likewise, when adjusted for live weight, daily protein degradation was higher (13.0%; $P<0.10$) and daily protein balance was higher (60.9%; $P<0.05$) in heifers fed at 180% ME_m than heifers fed at 105% ME_m.

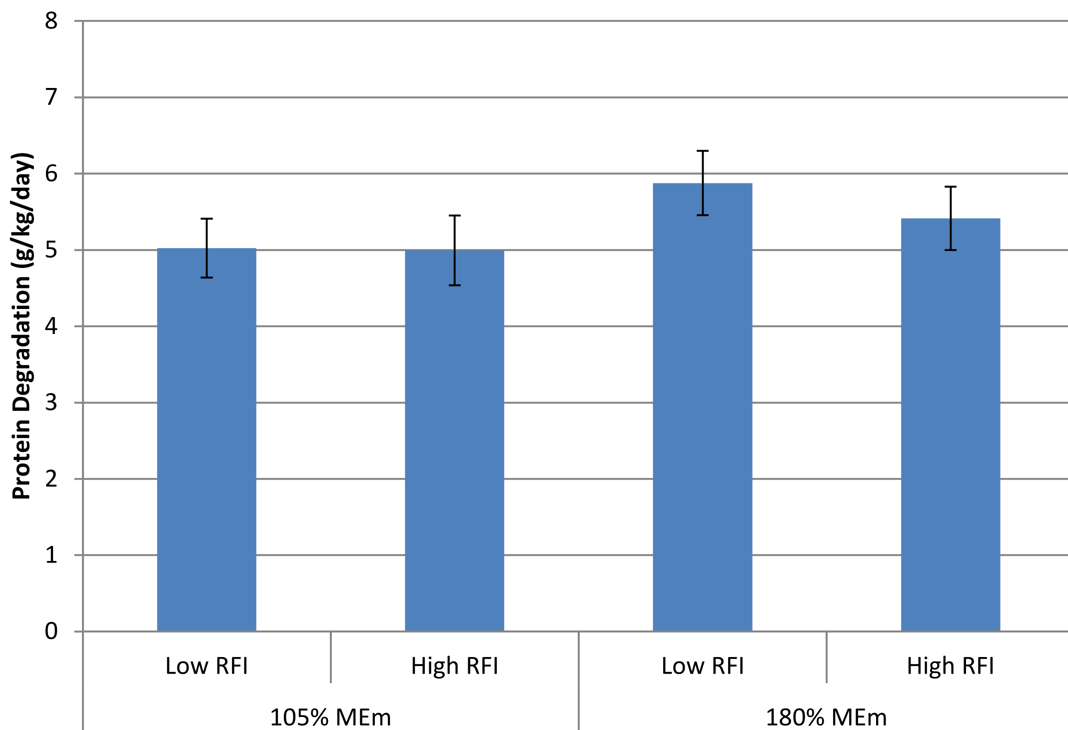

Figure 2.3: Least squares means for the RFI by feeding level interaction on protein degradation adjusted for weight (covariate).

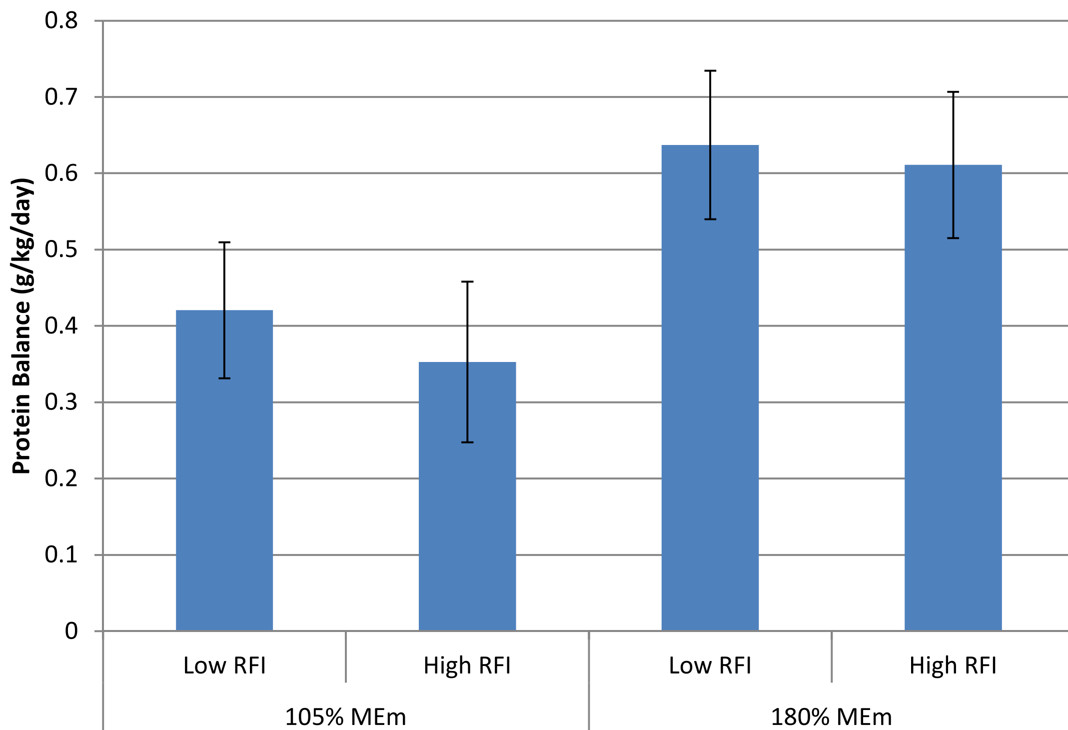

Figure 2.4: Least squares means for the RFI by feeding level interaction on protein balance adjusted for weight (covariate).

RFI line did not affect the contribution of protein metabolism to heat production ((P>0.05) (Table 2.4). Heat production for protein synthesis and degradation was greater in the low RFI heifers than the high RFI heifers but this was not significant (P>0.05). So there was a greater overall energy cost of protein turnover in the low RFI animals, although the "between line" difference was not significant.

Energy requirements for protein synthesis was 17.7% higher in heifers fed at 180% ME_m than heifers fed at 105% ME_m. However, the proportion of heat production for protein synthesis did not differ between feeding levels. Feeding level also did not impact the proportion of heat production that was required for protein degradation or protein turnover. Heat production for protein degradation was not affected by feeding level. However, the contribution of protein synthesis and degradation to protein

turnover resulted in a 17.4% increase in heat production of heifers fed at 180% ME_m over heifers fed at 105% ME_m.

Table 2.4: Main effects means and SEM over the treatment periods in the energetics of protein turnover for high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

	RFI Line				Feeding Level			
	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
Energetics								
HP for PS (MJ/day)	6.55	6.23	0.32	NS	5.87	6.91	0.31	0.04
HP as PS (%HP)	14.07	15.26	0.78	NS	15.04	14.3	0.76	NS
HP for PD (MJ/day)	1.19	1.14	0.07	NS	1.09	1.24	0.07	NS
HP as PD (%HP)	2.79	2.57	0.17	NS	2.79	2.57	0.16	NS
HP for PT (MJ/day)	7.74	7.37	0.39	NS	6.95	8.16	0.38	0.05
HP as PT (%HP)	18.05	16.65	0.95	NS	17.83	16.87	0.91	NS

NS = Not significant i.e. $P>0.10$; SEM = Standard error of the mean
HP = Heat production; PS = Protein synthesis; PD = Protein degradation; PT = Protein turnover
PT = PS + PD

2.4 Discussion

As protein metabolism contributes such a large proportion (15-25%) of basal heat production (Reeds *et al.*, 1998, Pym *et al.*, 2004, Waterlow, 2006), it would be reasonable to assume that if differences in heat production (HP) associated with residual feed intake existed, protein metabolism may contribute toward these differences. However, no differences were observed in protein metabolism (synthesis or degradation) between the residual feed intake lines although differences were found between feeding levels.

After half a generation of selection for residual feed intake, Richardson and Herd (2004) found evidence that indirect measures of protein metabolism may explain some of the differences in residual feed intake. These authors found that there were positive correlations between residual feed intake with blood urea and with aspartate amino

transferase as markers of whole body protein degradation and protein degradation of the liver, respectively. A negative correlation was also seen between whole body chemical protein, protein gain and residual feed intake. However, after 3.5 generations of selection for residual feed intake, the results herein on direct measures of protein metabolism indicate that there is no difference between residual feed intake lines in protein synthesis or protein degradation (Table 2.3).

Others have attempted to measure the extent to which differences in protein metabolism can explain the difference in residual feed intake, but have been unable to support this argument with their findings. For example, Castro Bulle and co-workers (2007) suggested that the fractional degradation rate of myofibrillar proteins, as measured by 3-methyl-histidine excretion, and maintenance energy requirements were correlated (r=0.76). However, neither the fractional degradation rate of myofibrillar proteins or maintenance energy requirements were significantly related with residual feed intake (Castro Bulle *et al.*, 2007, Sainz *et al.*, 2007). This parallels the results observed herein.

The experiments of Richardson *et al.* (2004) after half a generation of selection for residual feed intake also estimated protein degradation by indirect measures. They used the quantification of urinary 3-methyl-histidine (3MH) (an estimate of skeletal muscle protein degradation), creatinine (an indicator of total protein in the animal), and the 3MH:creatinine ratio (an estimate of protein degradation to correct for skeletal protein content). Notably, these indirect measures did not differ between residual feed intake lines. In the Richardson and co-workers (2004) trial, there were two periods of measurement. The animals underwent a residual feed intake test in the feedlot after which they underwent metabolism trials in an animal house. During their time in the

animal house, 3MH was positively correlated, but not significantly, with residual feed intake and feed conversion ratio (P>0.10). However, 3MH was not correlated over the whole trial in which both periods during the feedlot and animal house were used. In the animal house, creatinine was positively correlated (P<0.10) to the feed conversion ratio (FCR), but was negatively correlated, though not significantly over the whole experiment (P>0.10). The 3MH:creatinine ratio was negatively correlated in the animal house (P<0.05), but over the whole experiment, 3MH:creatinine was positively correlated with residual feed intake (P>0.10). These discrepancies between experimental locations may have been due to extra stress in the animal house.

Results that supported the hypothesis that protein metabolism may be involved in the differences in residual feed intake are those of McDonagh *et al.* (2001) with steers after a single generation of divergent selection for RFI. In these steers, they reported a difference in myofibril fragmentation index between the RFI lines, where the high RFI line had significantly greater levels of myofibril fragmentation. This was consistent with a decrease in calpastatin activity and hence, more m- and μ-calpain being available for post-mortem proteolysis in the high RFI line. However, in different animals, Baker *et al*. (2006) reported no difference in calpastatin activity between high and low RFI steers which is similar to observed elsewhere in this work (Chapter 5). The results from Baker *et al*. (2006), Castro Bulle *et al.* (2007), a feedlot trial (Chapter 5) and here indicate that protein metabolism may not be involved in the differences in feed intake observed between high and low RFI animals as purported by Richardson and Herd (2004).

It appears that animals fed at 105% ME_m grew much faster than expected (Chapter 3), suggesting that the diet quality was underestimated. Extrapolation from these data

suggests that the 105% ME_m feeding level really equated to 116.2% ME_m, and the 180% ME_m feeding level was equal to 188.3% ME_m. Lobley (1998) reviewed the effects of nutrition on protein metabolism and showed that below maintenance, protein synthesis is reduced and lower than protein degradation, such that there is a net loss of protein. However, as feed intake increases above maintenance, protein synthesis also increases above protein degradation such that there is a net gain of protein. However, changes in protein degradation, above and below maintenance energy requirements, are small compared to protein synthesis. Boisclair *et al.* (1993) found that the difference in protein synthesis of steers fed at 220% ME_m was 82% more than steers fed at 60% ME_m. The difference in protein degradation was only 15% greater in steers fed at 220% ME_m. This is supported herein as the difference in protein degradation was not significant between the feeding levels (13.0%) even though there was considerable variation. In contrast, protein synthesis was significantly different between feeding levels (17.7%), albeit the feeding level treatments were not as extreme as Boisclair *et al.* (1993).

2.5 Conclusions

After half a generation for selection for residual feed intake, Richardson *et al.* (2004) generated data to suggest that protein metabolism may explain some of the differences between residual feed intakes lines. However, after 3.5 generations of selection for residual feed intake in the same selection lines, protein metabolism was not observed to be different. Protein metabolism explained 37% of the variation in residual feed intake as put forward by the hypothesis of Richardson and Herd (2004). Nevertheless, there were other components that contribute toward heat production that may explain the difference in feed intake between residual feed intake lines. Consequently, the

hypothesis that heat production may contribute towards differences in residual feed intake was tested.

Chapter 3

Energy, nutrient balance and body composition of Angus heifers divergently selected for residual feed intake

CHAPTER 3: Energy, nutrient balance and body composition of Angus heifers divergently selected for residual feed intake

3.1 Introduction

In typical beef production systems, the cost of feed accounts for over half of the total cost of production. Accordingly, improvements in the efficiency of feed utilisation are a desired management objective. There is phenotypic variation in feed intake independent of variation in average weight and weight gain (termed residual feed intake, RFI), which is moderately heritable (Arthur *et al.*, 2001b, Arthur and Herd, 2008). Selection of beef cattle for high or low RFI measured shortly after weaning has been underway for almost a decade (Arthur *et al.*, 2001b, Arthur and Herd, 2008). The animals used were divergently selected for RFI for 3-4 generations were used to investigate possible biological mechanisms contributing to this trait.

In beef cattle divergently selected for RFI for 1 generation, up to 95% of the variation in RFI was attributed to differences in heat production rather than to energy retained in body tissues (Richardson and Herd, 2004). There is particular interest in the effects of selection for reduced RFI on the efficiency of energy use because it was originally hoped that selection would result in reduced maintenance energy requirements and therefore, feed requirements. The aim of this study was to assess the contribution of heat production (HP) in young beef cattle selected for and against RFI.

3.2 Materials and Methods

3.2.1 Animals

The animals used in this experiment and treatment of those animals were the same as those used in chapter 2. Infusions and plasma samples for protein metabolism were taken the following 24 hours from the measurement of heat production (HP). Animals selected for this trial were not different in age, weight or weight gain prior to the start of experimentation (described in chapter 2). The trial design and diets of the animals were as described in chapter 2.

3.2.2 Body Composition

Real time ultrasound scanning (RTUS) was performed by an accredited scanner for subcutaneous fat at the 13th rib and P8 rump sites, intramuscular fat and EMA. Internal body fat was measured using the technique of Ribeiro *et al.* (2008).

3.2.3 Heat production (HP)

CO_2 entry rate was determined (RCO_2, L/day) using $NaH^{13}CO_3$ and the results were used to determine the HP using a modified method of Li *et al.* (2008) with recommendations from Junghans *et al.* (2007). At 10am, an intravenous bolus of 17.5µmol $NaH^{13}CO_3$ per kg body mass dissolved in sterile saline was infused into the right external jugular catheter, and the mass of $NaH_{13}CO_3$ injected was determined. Blood sampling was through the left external jugular catheter. Blood samples (10mL) were collected in heparinised 10mL syringes at 1, 5, 10, 15, 20, 30, 60, 120, 240, 360, 720 and 1440 minutes from $NaH^{13}CO_3$ infusion. A sample for background enrichment of ^{13}C was taken prior to $NaH^{13}CO_3$ infusion.

Blood samples were processed using a modified procedure described by Young (1968) and in more detail in Chapter 2.2.4. Carbon dioxide entry rate (L/day) was calculated from the area under the double exponential decay curve of carbon atom percent excess versus time curve (Nolan and Leng, 1974). Heat production was estimated from these prediction equations:

$$HP\ (MJ/day) = 0.0163 \times RCO_2 + 3.92$$ (Appendix 3.1), and

$$HP\ (MJ/day) = 0.0096 \times RCO_2 + 2.41$$ (Adapted from Corbett *et al.*(1971)),

Where:

HP = Heat production

RCO_2 = Carbon dioxide entry rate (L/day)

3.2.4 Nitrogen and energy balance

Prior to feeding, the weight of the feed refused by each animal was determined. Faeces and urine were collected over a five-day period for each animal. For each animal, 5g of titanium dioxide was added to the feed as a digestibility marker seven days prior to, up to and including the collection. Faeces (300g) were sub-sampled daily over the five-day collection period and dried at 80°C until constant weight. Sub-samples of feed and refusals (~300g) were also dried at 80°C until constant weight during the same period. Once dry, the faeces and feed were ground until they could pass through a 1mm screen and were stored until analysed.

Feed, faeces and refusals (0.5g each) were analysed for nitrogen using a Leco N and C analyser with EDTA as an internal standard. Feed and faeces (0.5g each) were analysed for energy content using an IKA bomb calorimeter. Feed, faeces and refusals (2.5g each) were dried at 105°C for analysis of dry matter and analysed for organic matter by

ashing. The ashing procedure involved heating the feed and faeces at 250°C for two hours using a 5°C ramp per minute from 0°C. Feed and faeces were then heated at 600°C for five hours to completely combust the samples.

Urine was collected into a 20L drum using a No. 26 Folley catheter (1.2m, 30mL balloon) that was inserted into the bladder. Urine was acidified with 300mL of 5N sulphuric acid. Sub-samples (5%) of the urine were frozen daily, whilst the rest was discarded. Sub-samples remained frozen at -20°C until analysed. Urine (0.5g) was analysed for nitrogen using the Leco N and C analyser with EDTA as an internal standard. Urinary energy was estimated using the equation of 4.80 kcal (0.02008 MJ) per gram of nitrogen assuming that more than 90% of the nitrogen contained in urine is due to urea and the remaining 10% is comprised of nitrogenous (ammonia and uric acid) waste products (Elliott and Davison, 1975).

3.2.5 Determination of total titanium dioxide

The determination of titanium dioxide was done using a Kjeldahl digestion method of Myers *et al.* (2004). Faecal samples (0.5 g each) were digested in 13 mL concentrated sulphuric acid at 420°C for two hours in 400mL Kjeldahl digestion tubes using a potassium-copper Kjeldahl catalyst. After cooling, 10mL of hydrogen peroxide were added and made to a total weight of 100g with deionised water. This solution was then filtered through a No. 41 filter paper. Absorbance was measured on the UV spectrometer at 410nm. Absorbances were corrected from a standard curve comprising five samples within the 0-5mg range to calculate total titanium dioxide content in the faeces and refusals.

3.2.6 Statistical analysis

All statistical analyses were conducted using Proc MIXED in SAS 9.1. Tests of significance of fixed effects were calculated utilising type III sums of squares mixed models. Fixed effects fitted in the models included residual feed intake line (high, low), feeding level (105M, 180M) and the period of trait measurement (1st, 2nd) in the crossover design. Animal was fitted as a random term with live weight at the start of experimental periods fitted as a covariate. All interactions were tested in the maximal model with non-significant interactions being removed in order of least significance. This enabled the best linear unbiased estimates and standard errors to be extracted.

3.3 Results

3.3.1 Body composition

Animals selected for the trial were not different in age, weight or weight gain prior to start of experimentation. By the start of period 1 of experimentation, there was no difference between the high and low RFI lines in weight or P8 and rib fat depths ($P>0.05$) (Table 3.1). However, there was a significant difference in the estimated intramuscular fat percentage (IMF) as the low RFI heifers had 13.6% less IMF. Even though not significant, the low RFI heifers were 2.3% heavier, had 5.7% more eye muscle area (EMA), had 20.0% and 13.0% less fat depth at the rib and P8 depots at the start of period 1. By the start of period 2 of experimentation, there was a significant difference in weight, P8 and rib fat depth, EMA and intramuscular fat percentage ($P<0.05$). The low RFI heifers were still heavier had more EMA and less fat. When weight at the start of the measurement period was included as a covariate in the model, it was not found to affect any of the variates and was removed from analysis. The initial

value for each of the variates was fitted instead as covariates. These interacted with rib fat depth and there was a trend with IMF (*P*<0.10).

No interactions between the main effects were observed for the body composition traits. There were trends for RFI line x feeding level on changes in EMA over the measurement periods as well as an RFI line x measurement period (time) interaction for changes in weight and average daily gain (*P*<0.10).

Table 3.1: Raw means for age and body composition at the start of each measurement period for high and low residual feed intake heifers.

	Period 1				Period 2			
	Low RFI	SEM	High RFI	SEM	Low RFI	SEM	High RFI	SEM
Age (days)	307.75	4.48	300.88	4.63	359.75	4.48	352.88	4.63
Weight (kg)	291.44	12.92	276.31	6.33	328.25	13.01	304.75	4.99
P8 Fat Depth (mm)	5.00	0.76	5.75	0.59	6.63	0.68	8.38	0.63
Rib Fat depth (mm)	4.00	0.71	5.00	0.42	5.38	0.56	6.75	0.62
Eye Muscle Area (cm2)	48.88	1.22	46.25	1.70	54.38	0.80	49.63	1.85
Intramuscular Fat (%)	2.42[a]	0.18	2.80[b]	0.12	3.70	0.27	4.24	0.19

SEM = Standard error of the mean
[a,b] Mean with different superscripts differ significantly at P<0.05

Feeding level had the largest effect on the change in body composition traits during the measurement periods (Table 3.2). Heifers fed at 180% maintenance grew 65% faster, and deposited 451% and 413% more fat over ribs (12th-13th) and rump (P8), and had 227% more IMF than heifers fed 105% maintenance (Table 3.2). Interestingly, there was no statistical difference in EMA gain over the measurement periods between heifers fed at 180% and 105% of maintenance irrespective of RFI line.

As expected, there was no difference in changes in weight and average daily gain (ADG) between RFI lines (Table 3.2). This was expected because metabolic mid-weight (MMWT) and ADG of parents were fitted in the model to calculate RFI for the

selection of the parental matings. The low RFI heifers had lower rump fat (51%) and rib fat (56%) deposition (*P*<0.05), but not IMF deposition, than the high RFI heifers, regardless of feeding treatment. RFI line additionally had no effect on gain in muscle growth over the measurement periods as measured by changes in EMA.

Table 3.2: Main effects means and SEM for absolute changes over the treatment periods in weight and body composition for high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

	Treatment				Treatment			
	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
Production								
Weight Gain (kg)	24.19	26.16	1.76	NS	19.01	31.33	1.71	0.0002
Average Daily Gain (kg/day)	0.62	0.66	0.05	NS	0.48	0.80	0.05	0.0003
Body Composition								
Final Eye Muscle Area (cm^2)	55.35	53.20	1.39	NS	55.05	53.50	1.20	NS
Final Rib Fat Depth (mm)	5.60	7.34	0.49	0.03	5.93	7.00	0.39	0.02
Final P8 Fat Depth (mm)	6.67	9.08	0.69	0.03	7.44	8.31	0.56	NS
Final Intramuscular Fat (%)	3.92	4.49	0.19	NS	4.02	4.39	0.17	NS
Change Eye Muscle Area (cm^2)	4.60	4.40	0.92	NS	4.34	4.66	0.87	NS
Change Rib Fat Depth (mm)	0.78	1.60	0.28	0.05	0.43	1.94	0.23	<0.0001
Change P8 Fat Depth (mm)	0.88	2.00	0.37	0.05	0.56	2.31	0.36	0.005
Change Intramuscular Fat (%)	0.85	0.98	0.13	NS	0.56	1.27	0.13	0.002

NS = Not significant i.e. *P*>0.10; SEM = Standard error of the mean

3.3.2 Feed intake and digestibility

Weight was fitted as a covariate (to adjust for the effects that weight may have on feed intake and digestibility). No interactions were observed for traits of interest other than dry matter intake (DMI) and hence, metabolisable energy intake (MEI) and nitrogen intake (N intake) (Figure 3.1 & Table 3.3) on the RFI line by feeding level interaction. When the heifers were fed at 105% ME_m, there was no difference in feed intake (0.024±0.07kg per day) between the RFI lines. However, when fed at 180% of ME_m , the high RFI heifers ate significantly more than the low RFI heifers (5%; *P*<0.05). This difference equated to 0.353±0.07 kg per day. Note though that when the change in rib fat depth was fitted as a covariate, there were no significant differences in DMI, and

hence, there was no difference in MEI and N intake between high and low RFI heifers fed at 180% ME_m (P>0.10).

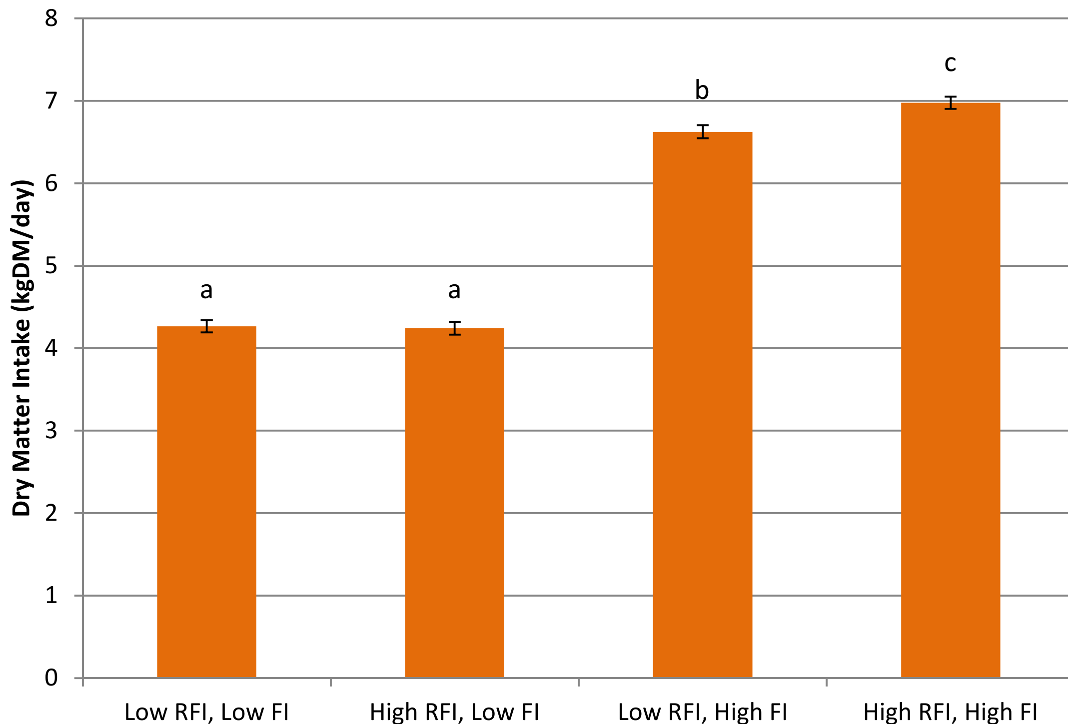

Figure 3.1: Least squares means for the RFI by feeding level interaction adjusted for weight (covariate). [ab] Superscripts differ significantly at P<0.05.

Without rib fat depth as a covariate, RFI line affected DMI and hence, MEI and N intake (Table 3.3). The high RFI line ate 0.169±0.05kg more than their low RFI counterparts irrespective of feeding level treatment (Table 3.4). This resulted in these high RFI animals consuming 3.7±1.2g and 2.7±0.9 MJ more protein and energy, respectively, than the low RFI line. This difference in feed intake did not result in differences in apparent dry matter digestibility (DMD) digestibility or organic matter digestibility (OMD) between the RFI lines. The association between feed intake and DMD and OMD was r=0.1 and r=-0.03, respectively. Nitrogen excretion in faeces and urine did not differ significantly between the RFI lines (P<0.05). Additionally, there was no association between nitrogen retained and RFI line.

Feeding level had the largest effects on digestibility as well as nitrogen and energy partitioning (Table 3.3). Animals fed at 180% ME_m consumed 38% more nitrogen (protein) and consequently, retained more as protein (38%). However, they also excreted more nitrogen in faeces (43%) and urine (33%) than animals fed at 105% ME_m. As expected, animals fed at 180% ME_m consumed more energy (37%) and consequently, excreted more energy as faeces (44%), urine (33%) and estimated methane (37%) than animals fed at 105% ME_m.

Table 3.3: Main effect means and SEM for feed intake, digestibility, nitrogen and energy partitioning of high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

	Treatment				Treatment			
	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
Dry Matter								
Intake (g/day)	5441	5610	0.053	0.047	4259	6796	0.052	<0.0001
DMD (%)	68.4	68.3	0.762	NS	69.8	66.9	0.691	0.006
OMD (%)	60.5	60.2	0.595	NS	61.5	59.1	0.528	0.003
Nitrogen Partitioning, (g/d)								
Intake	124.9	128.6	1.224	0.047	97.5	156.0	1.23	<0.0001
Faecal	38.8	41.8	0.986	NS	28.9	51.1	0.99	<0.0001
Urinary	60.8	62.8	3.579	NS	49.5	74.2	3.365	0.0001
Retained	25.3	24.5	3.199	NS	19.1	30.8	3.21	0.026
Energy Partitioning, (MJ/d)								
GE-Intake	90.9	93.6	0.891	0.047	71.0	113.5	0.895	<0.0001
ME-Intake	53.9	54.9	0.844	NS	43.2	65.5	0.723	<0.0001
Faecal	28.5	30.0	0.711	NS	21.1	37.4	0.714	<0.0001
Urinary	1.2	1.2	0.071	NS	1.0	1.5	0.066	0.0001
Methane*	7.3	7.5	0.071	0.045	5.7	9.1	0.071	<0.0001

DMD = dry matter digestibility
OMD = organic matter digestibility
NS = Not significant i.e. $P>0.10$; SEM = Standard error of the mean; GE = Gross Energy, ME = Metabolisable Energy
*Methane energy estimated as 0.08xGEI (MJ/day)

3.3.3 *Heat production*

CO_2 entry rate was the same for both RFI lines (Table 3.4). Hence, heat production was not different between RFI EBV and was not correlated with RFI line (r=0.04). Heat production per $kgBM^{0.75}$ was not correlated with RFI EBV (r=0.13). Heifers fed at 105% ME_m had 19.5% lower CO_2 entry rate than heifers fed at 180% ME_m (P<0.05). Consequently, they expended ~20% less energy) overall, and expended 22.4% less energy relative to their body mass than animals fed at 180% ME_m (P<0.05).

Table 3.4: Main effects means and SEM for CO2 entry rate and heat production of high and low RFI heifers fed at either 105% or 180% maintenance feeding levels.

		Treatment				Treatment			
	r_{RFI}	Low RFI	High RFI	SEM	Sig	1.05M	1.80M	SEM	Sig
RCO_2 (L/day)	0.04	4210.54	4562.43	189.69	NS	3883.26	4889.71	188.69	0.0026
HP (MJ/day)[1]	0.04	72.84	79.93	3.28	NS	67.18	84.59	3.25	0.0026
HP ($MJ/kgBM^{0.75}/day$)[1]	0.13	0.99	1.07	0.04	NS	0.91	1.15	0.04	0.0022
HP (MJ/day)[2]	0.04	42.83	46.21	1.82	NS	39.69	49.35	1.81	0.0026
HP ($MJ/kgBM^{0.75}/day$)[2]	0.14	0.58	0.63	0.02	NS	0.54	0.67	0.02	0.0023

r_{RFI} = phenotypic correlation between RFI and main effect
RCO_2 = CO_2 entry rate
HP = Heat production
NS = Not significant i.e. P>0.10; SEM = Standard error of the mean
[1] HP estimated from Appendix 3.1
[2] HP estimated from Corbett *et al.* (1971)

There was a diet by RFI line interaction (P<0.05) for gross energy intake (GEI) and metabolisable energy intake (MEI) (Figure 3.2). However, this was not the case for heat production (HP) (P>0.05). This GEI by RFI line interaction should not have existed in the experimental design as it was expected that by reducing feed intake to ~90% *ad libitum* requirements (180% maintenance) both low and high RFI animals would consume the same on both intakes. However, the *ad libitum* feed intake of the low RFI heifers was much less than expected i.e. feeding at 180% ME_m exceeded the intake

capacity of low residual feed intake heifers. There was no difference between high and low RFI lines fed at 105% ME_m in GEI, MEI or HP. When fed at 180% ME_m, the high RFI line had higher GEI and MEI ($P<0.05$) and consequently, had a higher HP. However, heat production (RCO_2) in the high RFI line was not significantly different from the low RFI line ($P>0.05$; Figure 3.2).

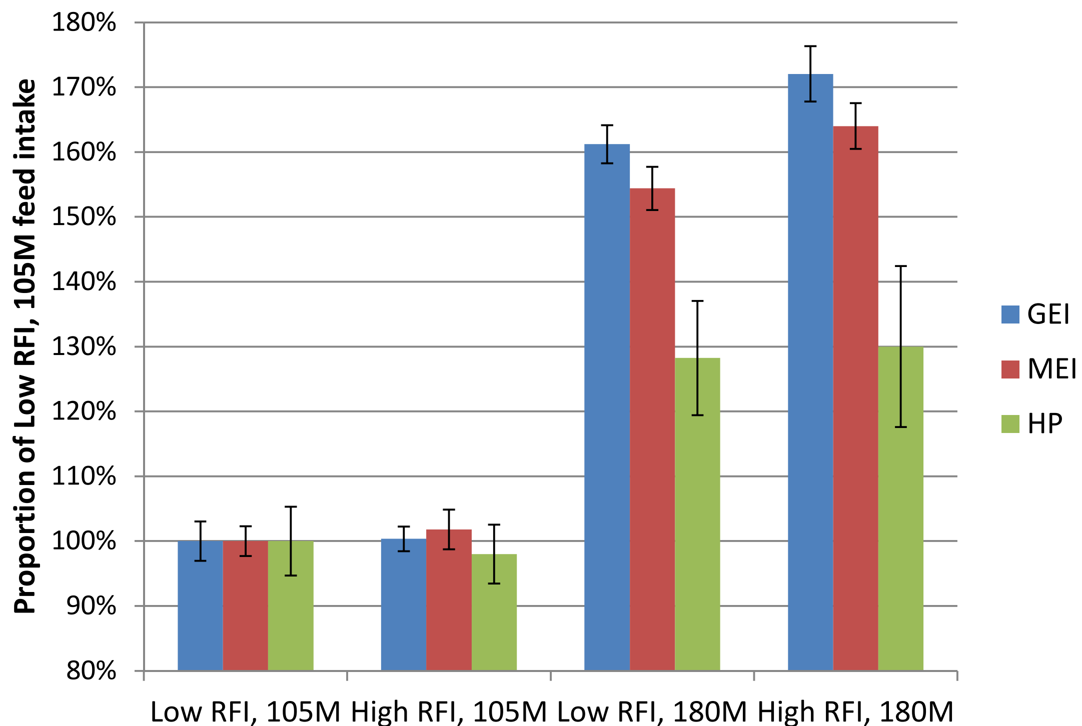

Figure 3.2: Least squares means for the RFI by feeding level interaction adjusted for weight (covariate). GEI = Gross Energy Intake; MEI = Metabolisable Energy Intake; HP = Heat Production; 105M= 105% ME_m; 180M = 180%ME_m.

3.4 Discussion

Animals utilised in this study did not undergo a post weaning RFI test as described by Archer *et al.* (1997) and Arthur *et al.* (2004a), but were the progeny of parents that had been selected utilising these tests. Prior to any major genetic selection (one generation) for residual feed intake, there was considerable evidence to suggest that energetic differences existed in this population of animals. The results from Trangie Research Station suggested that there are many different mechanisms to account for variation in

RFI of beef animals (Richardson and Herd, 2004). The largest of these mechanisms was purported to be in heat production (heat production) (Richardson *et al.*, 2004, Richardson and Herd, 2004). Interestingly, the authors did not observe differences in heat production between high and low RFI lines (Richardson *et al.*, 2001).

The results herein suggest that there are no detectable differences in heat production between RFI lines at 105% maintenance feeding levels or at 180% ME_m. Although there is substantial variation around the relationship, selection for RFI in this herd appears to have resulted in no measurable change in the efficiency of utilisation of feed energy. The current study was on a limited number of animals, and the measurements of heat production were by indirect methods. However, there is additional evidence that animals, which vary in RFI, do not differ in energy transactions. Lancaster (2008) reported similar results to those reported herein, in that at ME_m and during times of restricted feeding, heat production (as measured by heart rate) was similar between RFI phenotypes or was greater in low RFI beef cattle. Additionally, Lancaster (2008) found that when fed *ad libitum*, the low RFI animals had lower heat production (heart rate) than the high RFI animals. This is expected given the relationship between energy intake and heat production and the higher energy intake of the high RFI animals. Interestingly, the high RFI animals have lower heat production per unit of ME intake (inferred from the results) over all studies. This apparent anomaly goes unmentioned and undiscussed though it is probably consistent with an increase in fatness. Despite this, Lancaster (2008) still concluded that low RFI animals "will improve the energetic efficiency of beef production".

Gabarrou *et al.* (1997) reported when high and low RFI cockerels were deprived of feed, there was no statistical difference in heat production (HP). This led the authors to conclude that there was no difference in basal metabolic rate. Similar to the results herein and those of Lancaster (2008), when fed *ad libitum*, the high RFI cockerels had a significantly higher heat production that the low RFI cockerels. Between the lines, 25-36% of this difference was represented by a difference in physical activity, with the remaining 64-75% difference in heat production was accounted for by dietary induced thermogenesis.

Basarab *et al.* (2003) calculated that RFI was related to the composition of live weight gain and that some of the variation in RFI could be explained by variation in empty body fat gain. When RFI was adjusted for gain in ultrasound back fat thickness and marbling, Basarab *et al.* (2003) showed that cattle with negative RFI values had lower metabolisable energy intakes, lowered heat production (heat production) and retained less energy. Therefore, they concluded that a proportion of the metabolisable energy intakes of high RFI animals were accounted for by the differences in the composition of the gain. However, a much greater proportion could be attributed to differences in heat production. This relationship between metabolisable energy intake and heat production is exactly what would be expected from nutrition / energetic models (Oltjen *et al.*, 1986, Williams *et al.*, 1992, Williams and Jenkins, 1998).

Similarly, Castro Bulle and co-workers (2007) estimated that there was no difference in ME requirements for maintenance or in the net energetic efficiency of gain in beef steers of high and low RFI phenotypes. In contrast, Nkrumah *et al.* (2006) demonstrated that when feed intake was fixed, there was considerably greater heat production in high

RFI steers compared to their low RFI counterparts. This difference in heat production was greater than could be explained by differences in energy intake, retained energy or the heat increment of feeding.

Boddicker *et al.* (2011a) showed in pigs after 5 generations of selection for reduced RFI that when the low RFI (Selected) and Control (unselected) lines were fed at weight stasis, the Select line had a 20% less ME_m requirement than the Control line. However, when fed above maintenance, the authors suggested that the control line was more efficient at retaining energy consumed. Another study by Boddicker *et al.* (2011b) showed no difference in feed intake between the Select and Control lines when fed at weight stasis.

For the most part the literature suggests that animals with a low RFI phenotype do not have reduced maintenance requirements. Additionally, genetic selection for RFI as yet appears to have no effect on maintenance energy requirements across a range of species. All the same, there is literature implying that at constant weight and a constant weight gain, low RFI animals, when fed *ad libitum*, have reduced maintenance requirements, despite the lack of hard data to support these theories.

It has been documented that RFI is heritable and the phenotype of the animals has changed as intended with selection (Arthur *et al.*, 2001b, Arthur and Herd, 2008). What are the implications if there has been no change in the relationship between heat production and energy intake? Kennedy *et al.* (1993) and van der Werf (2004) elegantly demonstrated that selection of a trait, such as RFI (where Feed intake (FI) = Weight (MWt) + Production parameter (ADG) + RFI), is equivalent to selection on the

component traits. So if selection for RFI has resulted in a reduction in feed intake at constant weight and daily gain, as it is in this case, and by Arthur *et al.*, (2001a) and others, and there is no change in the relationship between energy intake and expenditure (this study), it follows that the energy content of gain (= fat content) must be less. This is exactly what has been observed in this work and by Richardson and Herd (2004), and inferred by the genetic and phenotypic correlations reported by Robinson and Oddy (2004).

Similar to the observations of others, the study herein shows large differences in the deposition of fat (primary subcutaneous) between the high and low RFI animals. This divergence in fatness following 3-4 generations of selection for RFI is much larger than that observed from the progeny selected for one generation of RFI (Richardson and Herd, 2004). Unlike previous studies on these animals (Richardson *et al.*, 2001), between RFI line variation was not seen in protein gains, as measured by eye muscle area or by nitrogen retention. Comparable to other investigations using these same genetics, there was no differentiation between the RFI lines in weight or average daily gain. This would be expected given that RFI is measured at constant weight and weight gain.

Past reports have shown RFI is correlated genetically and phenotypically with subcutaneous fatness. Arthur *el al.* (2001b) estimated very low genetic correlations of $r = 0.17$ and $r = 0.06$ for rib fat depth and P8 fat depth, respectively, after divergent RFI selection for one generation. Following further generations of selection for RFI in the same population, these genetic correlations were amended to $r = 0.68$ and $r = 0.71$ for rib fat depth and P8 fat depth, respectively (Arthur *et al.*, 2004b). These later

correlations are more in line with those found by others. Robinson and Oddy (2004) reported high genetic correlations between RFI and subcutaneous fatness at rib and rump (P8) sites of $r = 0.58$ and $r = 0.79$, respectively.

Mader *et al.* (2009) did not find significant correlations between back fat thickness or intramuscular fat and RFI in beef cattle. However, the RFI phenotype was correlated with trim and kidney fat ($r = 0.34$; P = 0.008). The lack of a relationship between RFI and intramuscular fat is in general agreement with the published literature (Nkrumah *et al.*, 2004, Castro Bulle *et al.*, 2007). Therefore, it may not be surprising that Mader et al. (2009) did not find a correlation between intramuscular fatness and RFI. What is surprising is that Mader *et al.* (2009) did not find a correlation between RFI and subcutaneous fatness, although there are other reports with similar results (Castro Bulle *et al.*, 2007). However, the majority of the literature on RFI would suggest that these two traits are correlated (Arthur *et al.*, 2001b, Basarab *et al.*, 2003, Richardson and Herd, 2004, Robinson and Oddy, 2004, Kelly *et al.*, 2010), especially when measured in gains over the RFI test period (Nkrumah *et al.*, 2004, Lancaster *et al.*, 2009, Kelly *et al.*, 2010). The non-significant relationship between subcutaneous fat and RFI reported by Mader *et al.* (2009) may have been due to the use of crossbreds in the study where there genuine differences in RFI due to breed differences are observed (Schenkel *et al.*, 2004, Crowley *et al.*, 2010). Schenkel *et al.* (2004) and Crowley *et al.* (2010) reported significant differences in RFI between breeds of bulls during performance testing. Interestingly, those breeds with lower RFIs were represented by breeds that were genetically leaner than those breeds with higher RFI (Robelin, 1986, Marshall, 1994). This may suggest that between breed differences in RFI may not be due to differences

in maintenance energy requirements alone but may be due to breed differences in fatness.

Robinson and Oddy (2004) concluded that direct selection for RFI may not be as effective at reducing RFI as direct selection for reduced fatness. In their data, there was a strong genetic relationship between subcutaneous fat and RFI (r = 0.72 and r = 0.48 for rump and rib fat, respectively). As the heritability of RFI was lower than that of subcutaneous fat (18% versus 42-45%) and given the high genetic correlation between fatness and RFI, selection for reduced RFI would be more effective if selection pressure were exerted on fatness instead. If selection pressure were on fatness rather than RFI, implementation may be faster than using the actual measurement of RFI. Fatness is a trait that is relatively easy to measure via ultrasound and certainly more cost effective that measuring RFI. This, however, brings its own complications, namely, if fatness were reduced, there may be implications for maternal productivity (e.g. conception rate, days to calving, etc.).

Many authors have suggested including fatness (usually subcutaneous) into the genetic (statistical) models to predict RFI (Basarab *et al.*, 2003, Schenkel *et al.*, 2004, van der Werf, 2004, Knott *et al.*, 2008, Kelly *et al.*, 2010). The data from these authors suggest that addition of fatness or components of body composition in the models to predict RFI may not explain all of the variation in RFI but will result in a reduction in the variance associated with RFI. Certainly, the study herein would suggest that after successive generations of selection for RFI, selection pressure has been exerted on subcutaneous fatness such that most if not all of the variation in RFI (hence, energy intake) in these animals may be explained by the greater deposition of energy as fat. Therefore, the

inclusion of fatness in models used to predict RFI is imperative to enable selection for RFI to change basal metabolism. This was confirmed in the current study when changes in rib fat depth were fitted as a covariate and there was no difference shown between RFI lines for DMI.

In pigs, Boddicker *et al.* (2011b) estimated that 87% of the difference in *ad* libitum feed intake between low RFI selection line and control line genotypes, which were 5 generations divergent, may be due to differences in carcass composition. Another report by Boddicker *et al.* (2011a) concluded that "most, it not all, of the differences in feed intake between the two lines were accounted for by the difference in carcass energy between the Select (low RFI) and Control lines". At *ad libitum* feed intake, the between line difference in net energy consumption was 53.6 MJ. However, the between line difference in retained energy was 80.4 MJ. This trend in greater energy retained versus energy intake in the Control lines over the Select line was the same across four feeding treatments (*ad libitum*, 75% *ad libitum,* 55% *ad libitum* and weight stasis). This difference between lines in retained energy was due to the fat content. The authors suggested that the Control line may be more efficient at retaining energy above maintenance levels as the difference in energy retained was greater than the difference in energy intake.

There was no difference in protein deposition as measured by eye muscle area and nitrogen retention between the RFI genotypes herein. In these cattle selection lines, correlations between RFI and eye muscle area are not significantly different from zero (Arthur *et al.*, 2001b, Richardson and Herd, 2004, Herd *et al.*, 2009). However, Richardson and Herd (2004) did report a significant change in eye muscle area over the

RFI test period between the high and low RFI genotypes, such that the low RFI animals had greater gains ($P<0.05$) in eye muscle area. This is consistent with other studies that show, at best, a low negative correlation between RFI and eye muscle area (Arthur *et al.*, 2001c, Basarab *et al.*, 2003, Nkrumah *et al.*, 2004, Schenkel *et al.*, 2004, Nkrumah *et al.*, 2007).

In addition, ADG was not significantly different between the RFI genotypes herein, which is consistent with published literature. As by design, a trait such as RFI (where feed intake (FI) = weight (MWt) + production parameter (ADG) + RFI) is adjusted for the component traits (i.e. ADG and MWt), there should be no difference in ADG between the phenotypes. It is encouraging that as intended, genetic selection for RFI based on parental phenotypes does not change one of the component trait (ADG) phenotype of the progeny.

No feeding level by RFI genotype interaction existed. Even though there was a feeding level by RFI genotype interaction for feed intake wherein the high RFI heifers fed at 180% ME_m consumed more than the low RFI heifers fed 105% ME_m, there was no evidence that these high RFI heifers grew faster. This supports the earlier hypothesis that high RFI heifers retain this 'extra' energy as fat. Feeding level did affect growth rate in that heifers fed more grew faster, as expected. However, the animals fed at 105% ME_m grew much faster than expected, suggesting that the diet quality was underestimated. Extrapolation from these data suggests that 105% ME_m feeding level really equated to 116.2% ME_m, and the 180% ME_m feeding level was equal to 188.3% ME_m.

At 105% ME_m, there was no difference in feed intake at constant weight and daily gain between RFI lines, suggesting no difference in basal energetic efficiency. When fed at 180% ME_m, the high RFI line ate 0.353 kg/day (3.5%) more than the low RFI line. This is consistent with other researchers who have concluded that residual feed intake is closely related to daily feed intake for phenotypic correlations (Arthur *et al.* 2001; Jensen *et al.* 1992; Kennedy *et al.* 1993; Basarab *et al.* 2003) and genetic correlations (Kennedy *et al.* 1993; Arthur *et al.* 2001). This study and others show that any perceived differences in "efficiency" of the trait can be attributed to the amount of energy consumed by the animal and the divergence of fat deposition in the genotypes or phenotypes. This implies that the additional energy intake (RFI) by the high residual feed intake animals was accounted for by the additional deposition of energy (fat), such that energy intake = energy deposition + heat production, where energy intake has a positive linear relationship with heat production, as expected from nutritional models. These results are from a small number of animals, but if true, then the differences in energy intake can be explained as the heat increment of feeding and energy deposition differences.

No relationship between RFI and energy intake or expenditure was observed at 105% ME_m. However, there was a divergence in heat production ($P>0.05$) and intake ($P<0.05$) between RFI lines at 180% ME_m. Due to the increasing coefficient of variation in measurement of heat production above basal maintenance levels, it is hard to determine whether evaluating more animals would result in a statistical difference in heat production between RFI lines at 180% ME_m. Regardless, the results herein suggest no deviation in the well-established relationship between energy intake and expenditure.

Roberts *et al.* (2007) reported that RFI of heifers under restricted feeding (80% *ad libitum,* ~160% ME_m) had a lower variance (Var_{RFI} = 0.004) than heifers fed *ad libitum,* (~200% ME_m, Var_{RFI} = 0.088). Back extrapolation from these data would suggest that at 75% of *ad libitum* feed intake (~150% ME_m), there would be no variance associated with RFI and hence, no difference in "efficiency" *per se*. They concluded that the variation in appetite would contribute much more to variation in RFI of heifers with a higher feed intake, and that it may be useful to measure RFI at restricted feeding to reduce the variation in RFI associated with appetite. Herd *et al.* (2006) reported in Angus cows that the variance in RFI at near-maintenance conditions was not associated with RFI at *ad libitum* as heifers, or with RFI at *ad libitum* conditions as mature cows, or with the RFI estimated breeding value (EBV) as measured during post weaning conditions. Essentially, the conclusion is that there is no variance in RFI at maintenance and hence no difference in maintenance requirements between high and low RFI animals. This suggests that when feed is available, the low RFI animals will eat less at a constant weight and weight gain but will not eat any differently from the high RFI animals when feed is restricted. These are the same conclusions as those drawn here. Similarly, Silverstein (2006) noted that genetic differences in RFI of rainbow trout were only expressed under apparent *ad libitum* conditions and not when feed intake was limited. This implies that there is much greater variation in actual feed intake, and hence appetite, than in RFI.

This begs the question: If there is very little variation in feed intake at maintenance, is the RFI of an animal likely to be the same at different stages of maturity? In growing and finishing Santa Gertrudis steers, Brown (2005) reported a moderate phenotypic correlation between RFI during the growing phase (291.1±33.79 to 395.4±39.03 kg) and

RFI during the finishing phase (431.4±42.7 to 513.9±51.2 kg) of r_p=0.47. This correlation was stronger than those observed for feed conversion ratio (FCR; r_p=0.22 P<0.05) or partial efficiency of growth (PEG; *r*=0.29 P<0.05). Arthur *et al.* (2001d) reported a moderate phenotypic correlation r_p=0.43 and a high genetic correlation r_g=0.75 between the RFI of Charolais bulls measured at 12 month and 18 months. These correlations were also stronger than the correlations for FCR (r_p=0.06 and r_g=0.42 for phenotypic and genetic correlations, respectively). Arthur *et al.* (1999) and Archer *et al.* (2002) showed an across breed phenotypic and genotypic correlations between post weaning RFI and mature cow RFI (4-4.5 year olds) of r_p=0.36 and r_p=0.40 (r_g=0.98), respectively. Herd *et al.* (2006), using the same genetic resource as the animals herein, demonstrated moderate phenotypic correlations of r_p=0.39 and r_p=0.29 between the post weaning and mature (4 year old) RFI tests and between the RFI EBV and mature cow RFI test. These results indicate that RFI is moderately repeatable across stages of maturity and has a strong genetic component, signifying that RFI may be a good measure of feed conversion efficiency whilst having no change in weight for age, average daily gain and mature cow weight. However, it must be noted that only 9-22% of the variance in post weaning RFI can be explained by variation in mature RFI. This implies that RFI measurement at post weaning and maturity are different traits. The implication of this is that measurement of post weaning RFI will have very little impact on the breeding herd "efficiency" per se.

Richardson *et al.* (1996) calculated that a 1% difference in dry matter digestibility had the potential to reduce daily feed intake by 2.3% in rapidly growing animals. In young bulls and heifers that differed in RFI, Richardson et al. (1996) found a 1% (P=0.10) difference in dry matter digestibility (Low RFI = 68.1% and high RFI = 67.1%, P=0.10)

that accounted for 14% of the variation in RFI. Whilst in that study, dry matter digestibilities between RFI genotypes did not differ, others have reported differences in digestibility. Krueger *et al*. (2008) reported dry matter digestibilities of 73.1% and 70.5% in low and high RFI Brangus heifers. Brown (2005) showed significant moderate phenotypic correlation of -0.32 between RFI and dry matter digestibility ($P<0.05$) as measured by the acid insoluble ash method. This equated to 70.80%, 66.05% and 66.42% dry matter digestibility in high, medium, and low RFI steers, respectively. Whilst these three references are the only ones available showing differences in dry matter digestibility, if true, this could offer a possible mechanism for differences in RFI. However, the results herein showed no difference in dry matter digestibility despite the two RFI genotypes differing by 3.5% in actual feed intake at 180% ME_m.

3.5 Conclusions

In summary, the results from this study provide evidence that most if not all of the variation in heat production could be accounted for by the amount of energy consumed. Heat production per unit of metabolisable energy-intake did not differ between the selection lines. There was evidence for differences in fat deposition; the high-RFI animals retained more energy in fat. The energy transactions herein suggested that there was no difference in efficiency of energy utilisation between the RFI lines. As this study and others have shown, any perceived differences in efficiency of RFI can be attributed to the amount of energy consumed by the animal and the divergence of fat deposition in the genotypes or phenotypes. However, as only a small number of animals were sampled in this trial, further physiological and biochemical evaluation is necessary before firm conclusions can be drawn. Given the practical limitations for the measurement of the components of residual feed intake (that is, actual feed intake,

weight and average daily gain), it is no surprise that selection pressure is extended to feed intake and composition of gain (relative proportion of fat and lean). Unfortunately, basal or underlying metabolic rate, the trait to be minimised, appears at this stage to be unaltered. The concerns of selection pressure on feed intake and fat deposition associated with selection for residual feed intake should be addressed. More work needs to be undertaken to understand the full consequences of selection for RFI and before the trait can be properly implemented within industry. However, at this time, it is clear that there are unlikely to be benefits in terms of improved energy efficiency and a reduction in energy costs due to maintenance, the largest cost of feed in a production system.

Chapter 4

Regulation of appetite and indicators of energy balance in Angus heifers divergently selected for residual feed intake

CHAPTER 4: Regulation of appetite and indicators of energy balance in Angus heifers divergently selected for residual feed intake

4.1 Introduction

Appetite regulation is a complex process that involves multiple signals integrating at the arcuate nucleus in the mediobasal hypothalamus (Sartin *et al.*, 2011). These signals take the form of orexigenic (appetite stimulating) and anorexigenic (appetite suppressing) peptides from peripheral sources, such as adipose tissue reserves and products of digestion, as well as responses to the ingestion and presentation of food. Orexigenic regulation of appetite is controlled by the stimulation of neuropeptide Y (NPY) and agouti-related peptide (AgRP), whereas anorexigenic regulation of appetite is controlled by the stimulation of cocaine and amphetamine-related transcript (CART) and proopiomelanocortin (POMC) (Sartin *et al.*, 2011). The hypothalamus uses these signals to manage energy homeostasis when feed availability and quality allow.

There are two systems responsible for the immediate monitoring of an animal's energy status (Black *et al.*, 2011). These are the adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycine (mTOR), which have the role of monitoring cellular and organismal energy status through the ratio of adenosine monophosphate (AMP):adenosine triphosphate (ATP) (Inoki *et al.*, 2012). The AMP:ATP ratio is hard to determine experimentally. However, there are other substrates in circulation that enable the estimation of energy balance. Two of these are glucose and non-esterified fatty acids (NEFA). Circulating glucose has been found to be

positively correlated with energy balance (Reist *et al.*, 2002). Whereas, circulating concentrations of NEFA are negatively correlated with energy status (Emery *et al.*, 1992). Reist *et al.* (2002) suggested that NEFA is likely to be the best indicator of energy balance (r_p = -0.69) as it is the first indicator of lipolysis of adipose tissue, at least in ruminants.

Orexigenic and anorexigenic control of appetite is primarily stimulated by the actions of three hormones: ghrelin, insulin and leptin. Ghrelin is considered the "hunger hormone" and has orexigenic effects (Wertz-Lutz *et al.*, 2006). Ghrelin's orexigenic effect is via the stimulation of *NPY* and *AgRP* gene expression in the hypothalamus to produce the NPY and AgRP peptides (Kojima and Kangawa, 2005). Insulin has orexigenic and anorexigenic control of appetite via the suppression of NPY and activation of POMC, respectively (Roche *et al.*, 2008). Whereas, leptin reduces feed intake by stimulating the anorexigenic neurons, CART and POMC, and also suppressing the orexigenic NPY and AgRP neurons (Ahima, 2005).

The results herein (chapters two and three) provide evidence that most, if not all, of the variation in feed intake between high and low RFI heifers can be accounted for by the heat increment of digestion and body composition. However, high and low RFI animals differed significantly in actual feed intake. A potential explanation of this difference could be variation in the energetic status and appetite between high and low RFI animals and therefore, this possibility was explored herein.

4.2 Materials and Methods

4.2.1 Animals

The animals used in this experiment and treatment of those animals were the same as those used in chapter 2. Infusions and plasma samples for protein metabolism were taken the following 24 hours from the measurement of heat production (HP). Animals selected for this trial were not different in age, weight or weight gain prior to the start of experimentation as described in chapter 3. The dietary composition of animals fed in this study is described in chapter 2. Animals in this study were not measured at both time periods as in chapters 2 and 3, but only during the second time period.

Blood samples (10mL) were collected in heparinised 10mL syringes at -30, -10, 0, 10, 20, 30, 45, 60, 90 and 120 minutes relative to time of feeding. Blood sampling was taken through a catheter that had previously been implanted into the left external jugular (Chapter 2). Blood samples were kept on ice until centrifuged at 1100g for 20 minutes at 5°C. Plasma was stored at -20°C until analysed. Plasma was analysed for glucose, insulin, non-esterified fatty acids (NEFA) and ghrelin by Professor Jim Mcfarlane (The University of New England, Armidale, NSW, Australia). Leptin was assayed by double-antibody RIA by Mrs. Margret Blackberry (The University of Western Australia, Perth, WA, Australia) using the method described by Blanch *et al.* (2000).

4.2.2 Statistical analysis

All statistical analyses were conducted using Proc MIXED in SAS 9.1 (1989). Tests of significance of fixed effects were calculated utilising type III sums of squares mixed models. Fixed effects fitted in the models included residual feed intake line (high, low), feeding level (105M, 180M) in the crossover design as well as time relative to feeding

(-30, -10, 0, 10, 20, 30, 45, 60, 90 and 120 minutes). Unlike the measurements in chapters 2 and 3, animals were not measured at both time periods, but only during the second time period. Animal live weight at the start of experimental periods was fitted as a covariate. All interactions were tested in the maximal model with non-significant interactions being removed in order of least significance. This enabled the best linear unbiased estimates and standard errors to be extracted.

4.3 Results

Plasma metabolite concentrations (glucose, NEFA, ghrelin, insulin and leptin) did not differ significantly between low and high RFI heifers (Table 4.1). However, the high RFI heifers did have lower plasma ghrelin at 180% ME_m ($P<0.01$). No other RFI group by feeding level interactions were significant. Both insulin and ghrelin differed between feeding levels (Table 4.1). Plasma insulin was ~60% greater in heifers fed at 180% ME_m ($P<0.10$). Whereas, plasma ghrelin was 20% less in heifers fed at 180% ME_m ($P<0.05$). Glucose, NEFA and leptin did not differ significantly between heifers fed at 105% and 180% ME_m.

Table 4.1: Best linear unbiased estimates of plasma metabolite concentrations for low and high RFI heifers and feeding levels of 105% ME_m and 180% ME_m.

	Glucose (mmol/L)	NEFA (µmol/L)	Ghrelin (pg/mL)	Insulin (µIU/mL)	Leptin (ng/mL)
RFI Group					
Low	4.66 ± 0.12	161.36 ± 23.92	379.33 ± 25.89	2.46 ± 0.45	1.76 ± 0.23
High	4.59 ± 0.13	141.94 ± 22.27	330.88 ± 25.90	2.35 ± 0.41	1.70 ± 0.21
Feeding Level					
105% ME_m	4.69 ± 0.12	172.14 ± 23.92	393.80^a ± 25.89	$1.86^†$ ± 0.41	1.62 ± 0.23
180% ME_m	4.56 ± 0.11	131.15 ± 22.27	316.40^b ± 24.00	$2.95^†$ ± 1.41	1.83 ± 0.21

[ab] Means within rows with difference superscripts differ significantly at $P<0.05$
[†] Means within rows differ significantly at $P<0.10$

4.3.1 Glucose

Glucose was not different between RFI genotype animals (Table 4.1; Figure 4.1). Additionally, there was no effect of feeding treatment on plasma glucose in that heifers fed at 105% and 180% ME_m did not differ in plasma glucose concentration (Table 4.1; Figure 4.2). No effect was observed on the interaction of RFI and feeding level. However, the interaction between RFI genotype and feeding level was significantly different for the decrease in glucose concentration from 30 minutes prior to feeding to 120 minutes after feeding (P<0.05;).

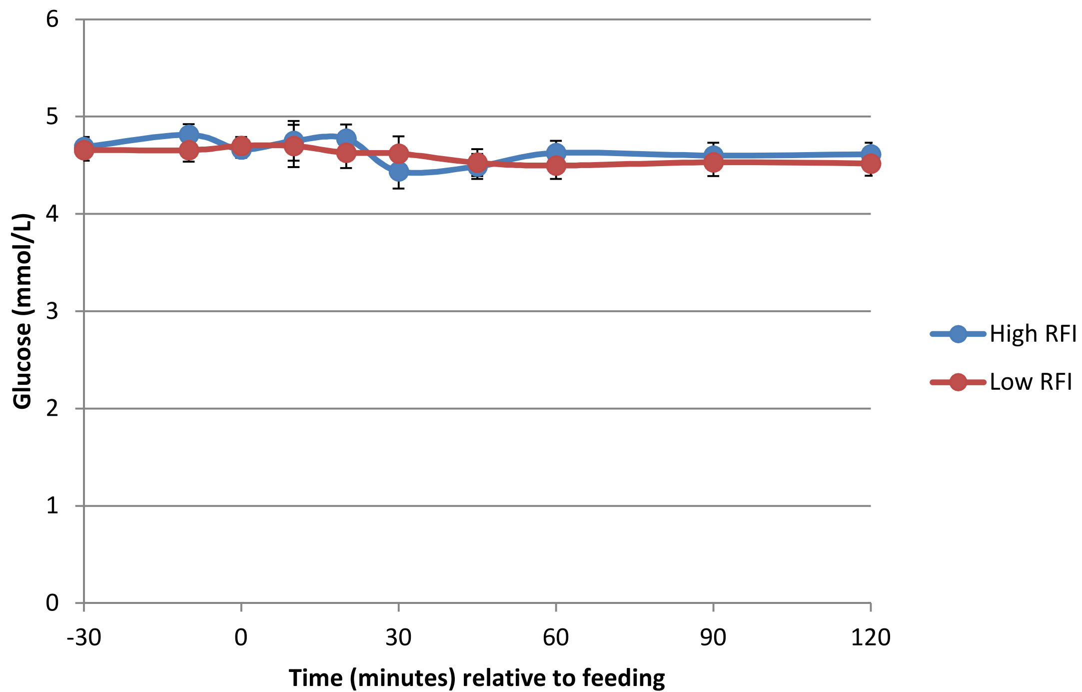

Figure 4.1: Plasma glucose concentration of high and low RFI heifers during feeding.

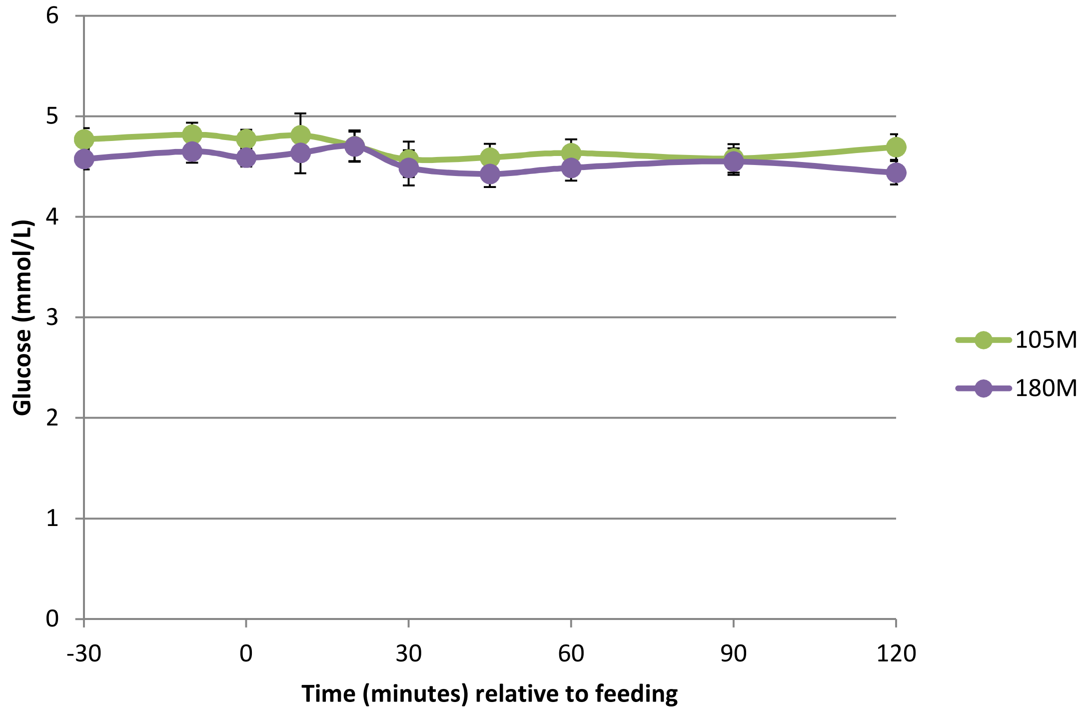

Figure 4.2: Plasma glucose concentration in heifers fed at 105% and 180% ME_m during feeding.

4.3.2 NEFA

Plasma non-esterified fatty acids (NEFA) were not significantly different between high and low RFI genotype animals (Table 4.1 and Figure 4.3). However, at all time-points relative to feeding level, plasma NEFA concentrations were greater in low RFI heifers compared with high RFI heifers (P>0.10; Figure 4.3). Plasma NEFA showed a sharp increase in concentration just prior to feeding followed by a decline to 120 minutes subsequent to feeding (Figures 4.3 and 4.4).

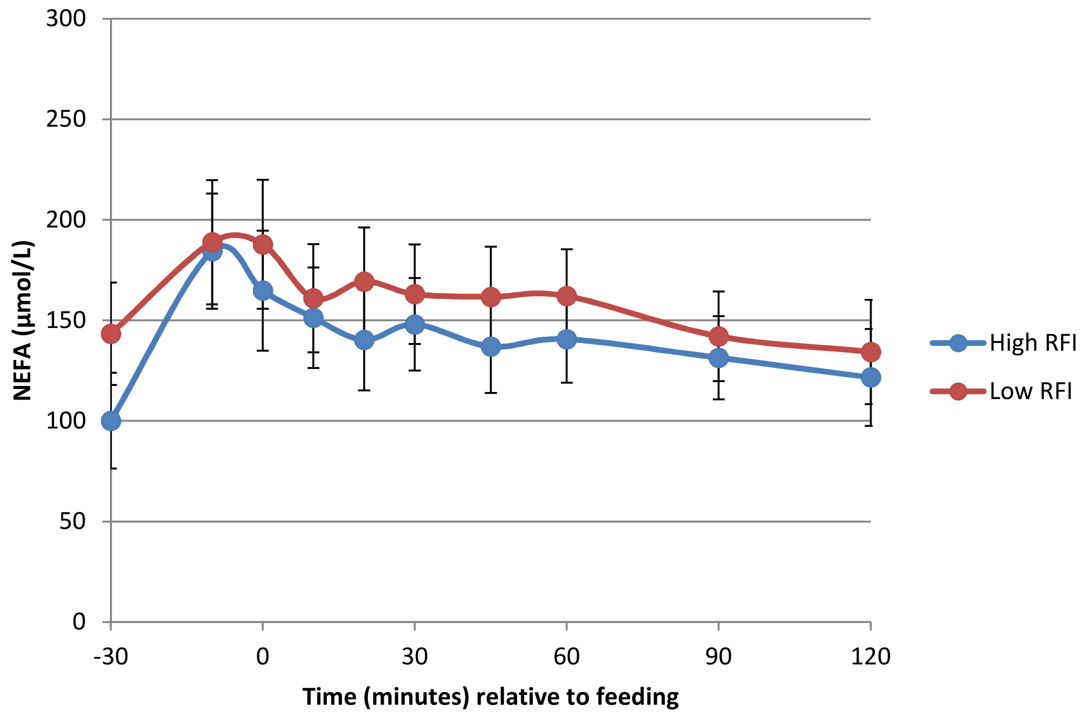

Figure 4.3: Plasma NEFA concentration of high and low RFI heifers during feeding.

Plasma NEFA concentrations was not significantly different between feeding levels of heifers (Figure 4.4). However, NEFA concentrations tended ($P<0.10$) to be greater in heifers fed at 105% ME_m until 30 minutes after feeding (Figure 4.4).

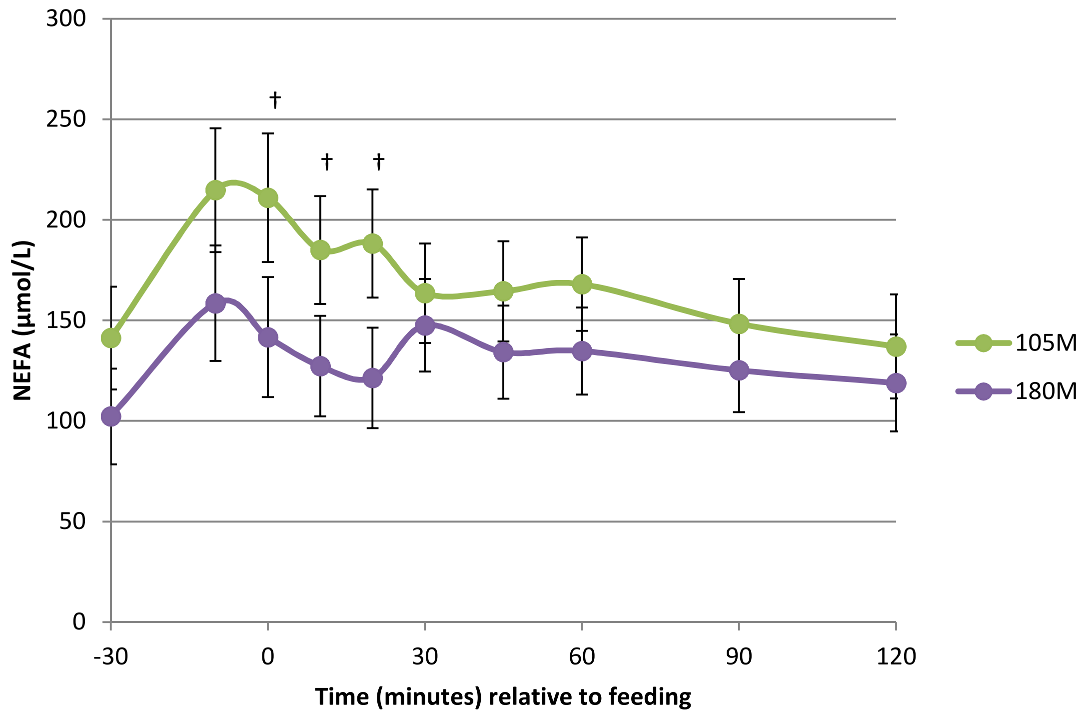

Figure 4.4: Plasma NEFA concentration in heifers fed at 105% and 180% ME_m during feeding. † = P<0.10.

4.3.3 Ghrelin

Plasma ghrelin concentrations were not significantly different between high and low RFI genotype heifers (P>0.05; Table 4.1). Nevertheless, high RFI heifers had significantly lower concentrations of plasma ghrelin at 30 minutes prior to feeding (Figure 4.5). Overall, the high RFI heifers had lower concentrations of circulating ghrelin than low RFI heifers, suggesting that this genotype was more satiated (Figure 4.5). There seems to be no real relationship between time relative to feeding and ghrelin concentrations other than a slight downward trend associated with 30-45 minutes until 2 hours after feeding in heifers of both RFI genotypes (Figure 4.5).

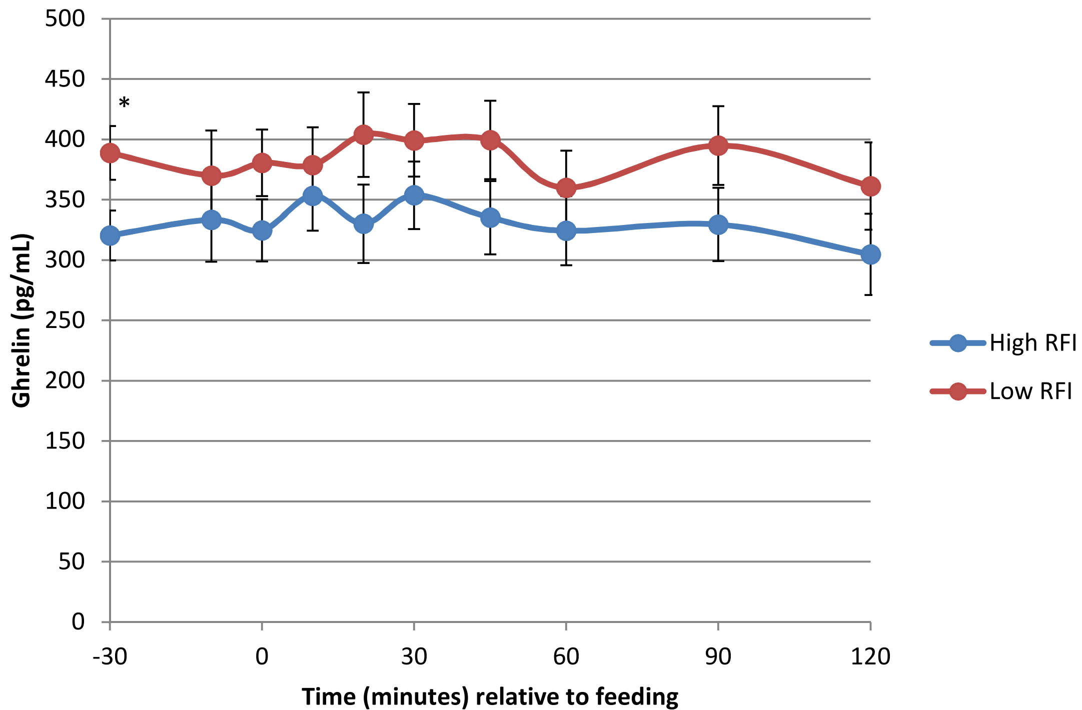

Figure 4.5: Plasma ghrelin concentration of high and low RFI heifers during feeding.* = P<0.05

Feeding level significantly affected the concentrations of plasma ghrelin ($P<0.05$; Table 4.1 and Figure 4.6). Heifers fed at 180% ME_m had significantly lower concentrations of plasma ghrelin at feeding and up to 90 minutes after feeding time (Figure 4.6) than heifers fed at 105% ME_m. This suggests that heifers fed at higher levels were more satiated than those fed essentially at maintenance. Whilst the relationship between time relative to feeding and plasma ghrelin concentrations is not strong, it appears this is linear in heifers fed at 180% ME_m. This relationship for heifers fed at 105% ME_m showed a tendency to increase prior to feeding to a maximum at 30 after feeding with a decline up to 120 minutes after feeding (Figure 4.6).

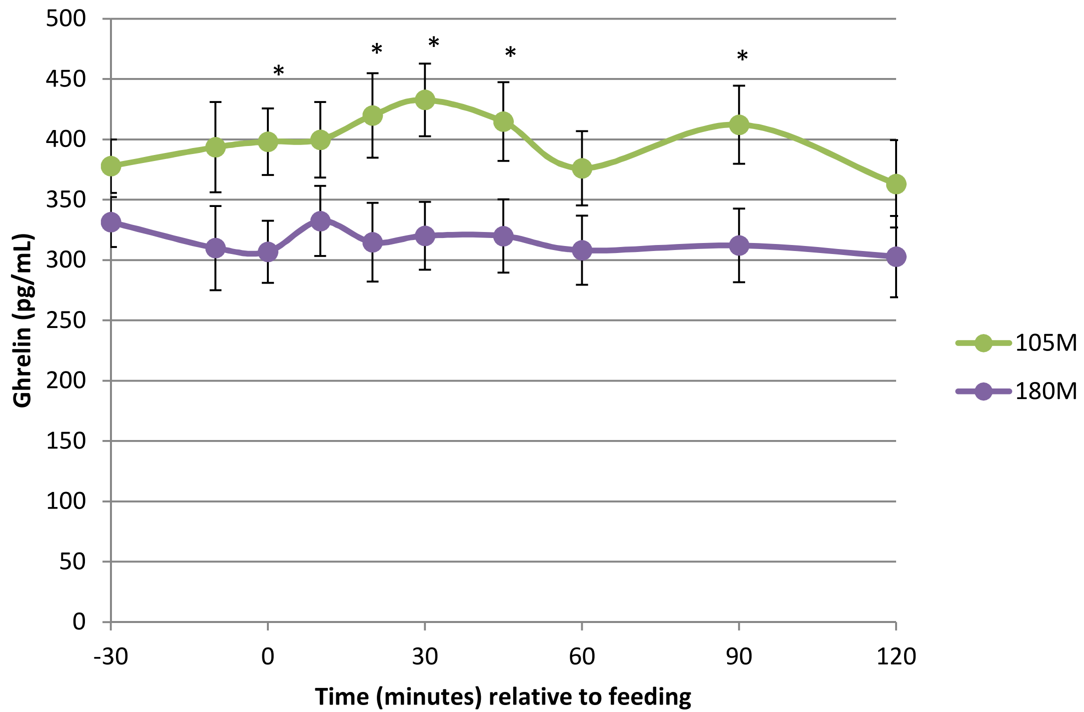

Figure 4.6: Plasma ghrelin concentration in heifers fed at 105% and 180% ME_m during feeding. * = P<0.05.

Whilst there was no overall effect of ghrelin concentrations between high and low RFI genotype heifers, the high RFI heifers fed at 180% ME_m had significantly lower concentrations of circulating ghrelin ($P<0.05$; Figures 4.7 and 4.8). This trend was observed at most time points relative to feeding, suggesting that the high RFI heifers fed at 180% ME_m were more satiated than all other RFI by feeding level treatments (Figure 4.8).

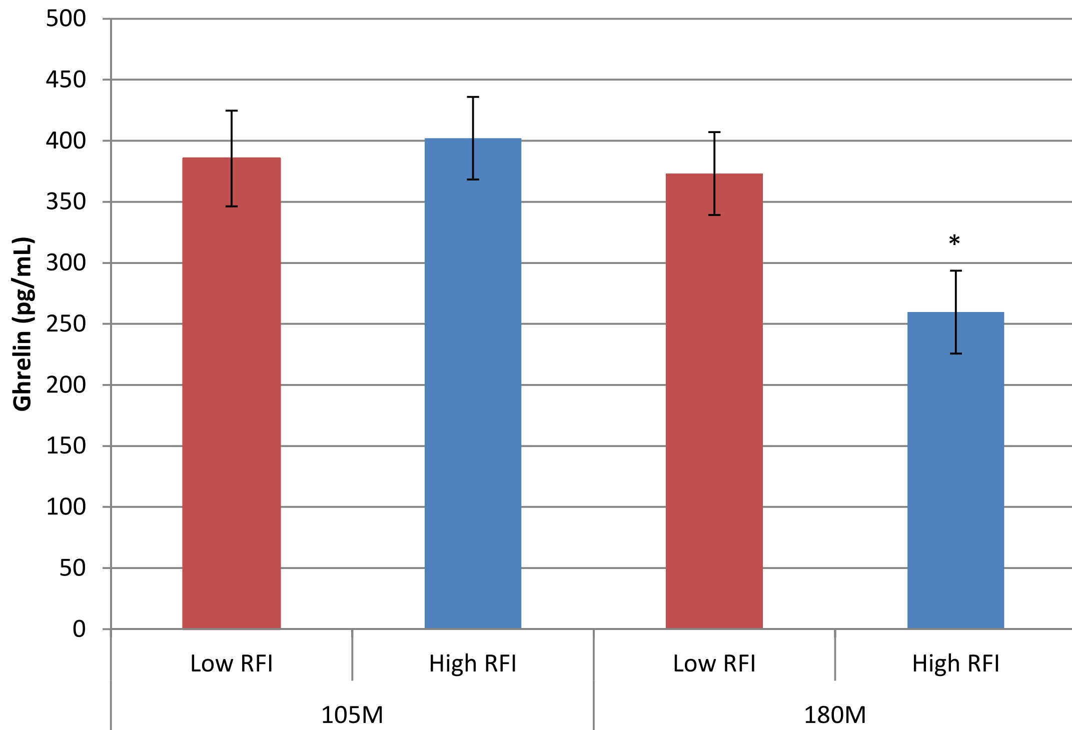

Figure 4.7: Average plasma ghrelin concentrations over time of high and low RFI heifers fed at 105% and 180% ME_m. * = P<0.05

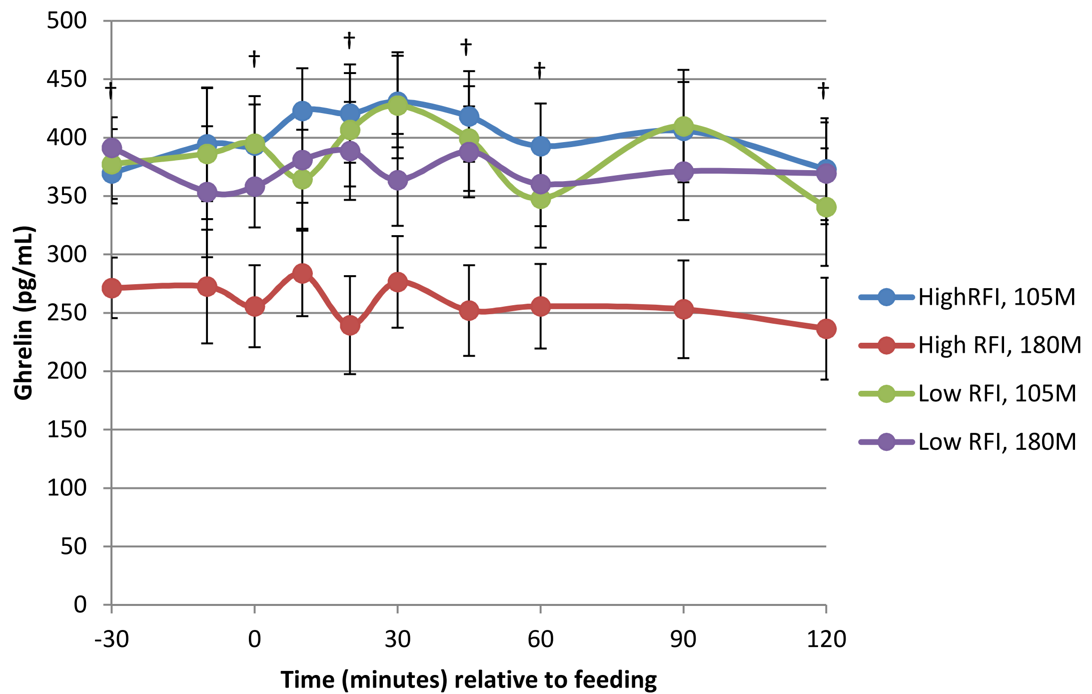

Figure 4.8: Plasma ghrelin concentration of high and low RFI heifers fed at 105% and 180% ME_m during feeding. † = P<0.10.

4.3.4 Insulin

Plasma insulin concentration was not significantly different between high and low RFI genotype heifers (Table 4.1). However, low RFI heifers had significantly higher ($P<0.01$) plasma insulin at 30 minutes after feeding (Figure 4.9). The small standard errors associated with this time subsequent to feeding suggest that this is not an outlier associated with the high RFI genotype heifers. Additionally, at both 105% and 180% ME_m feeding levels, there was a decrease associated with this feeding time point (Figure 4.10). Plasma insulin increased at the presentation of feed just prior to feeding, decreased to a low at 30 minutes post feeding but had reached a similar level two hours after feeding to 30 minutes prior to feeding (Figures 4.9 and 4.10).

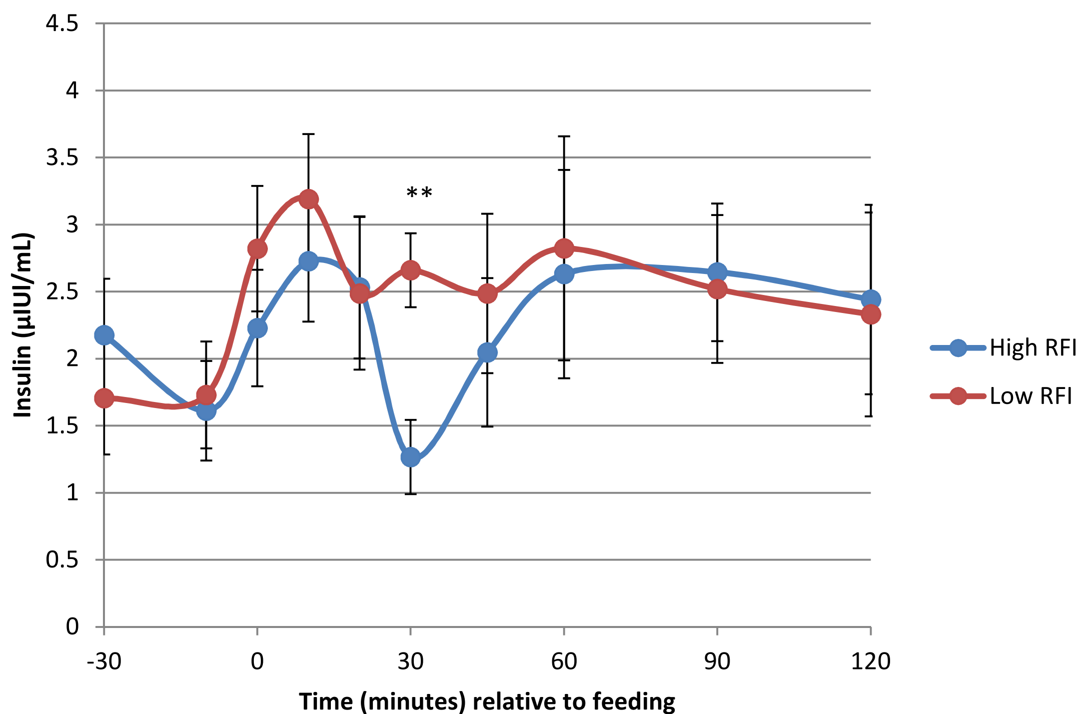

Figure 4.9: Plasma insulin concentration of high and low RFI heifers during feeding. ** = P<0.01.

Plasma insulin tended (P<0.10) be lower in heifers fed at 105% ME_m than at 180% ME_m (Table 4.1). Insulin was significantly lower (P<0.05) in heifers fed at 105% ME_m than heifers fed at 180% ME_m at 30 minutes prior to feeding, at feeding and at 20 to 30 minutes subsequent to feeding. However, there were no differences between heifers fed at 105% and 180% ME_m from 45 to 120 minutes post feeding (Figure 4.10). Additionally, plasma insulin concentrations at 30 minutes prior to feeding and two hour subsequent to feeding were not different, although there was a trend for this to be greater at two hours post feeding in heifers fed at 105% ME_m (Figure 4.10) than prior to feeding.

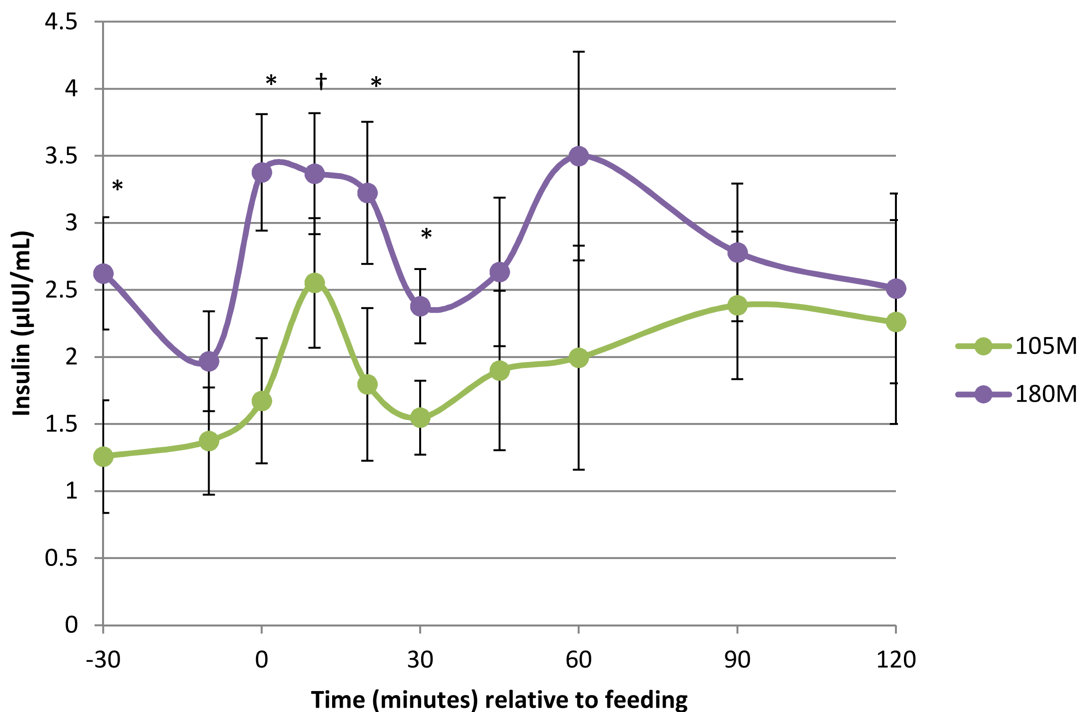

Figure 4.10: Plasma insulin concentration in heifers fed at 105% and 180% MEm during feeding. * **= P<0.05;** † **= P<0.10.**

4.3.5 Leptin

Plasma leptin concentrations were not different between high and low RFI genotype heifers (Table 4.1 and Figure 4.11). Additionally, there was no effect of feeding treatment on plasma leptin in that heifers fed at 105% and 180% ME_m did not differ in plasma leptin concentration (Table 4.1; Figure 4.12). However, heifers fed at 180% ME_m had higher plasma leptin concentrations at all time periods relative to feeding than heifers fed at 105% ME_m. No effect was observed between the interaction of RFI and feeding level on leptin levels.

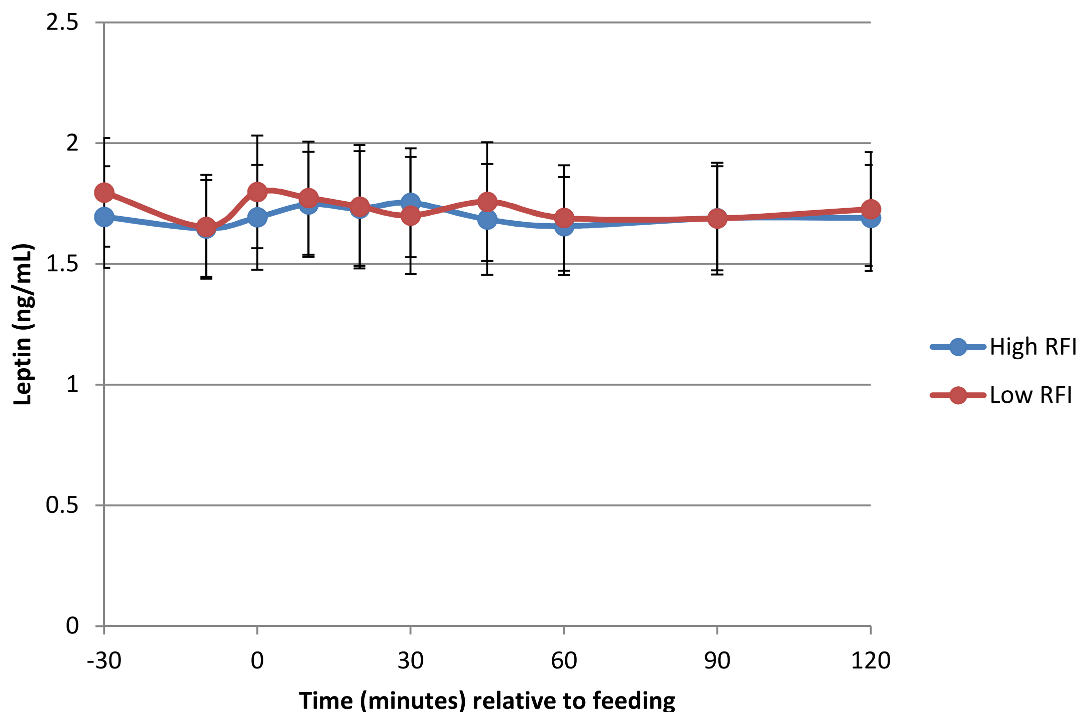

Figure 4.11: Plasma leptin concentration of high and low RFI heifers during feeding.

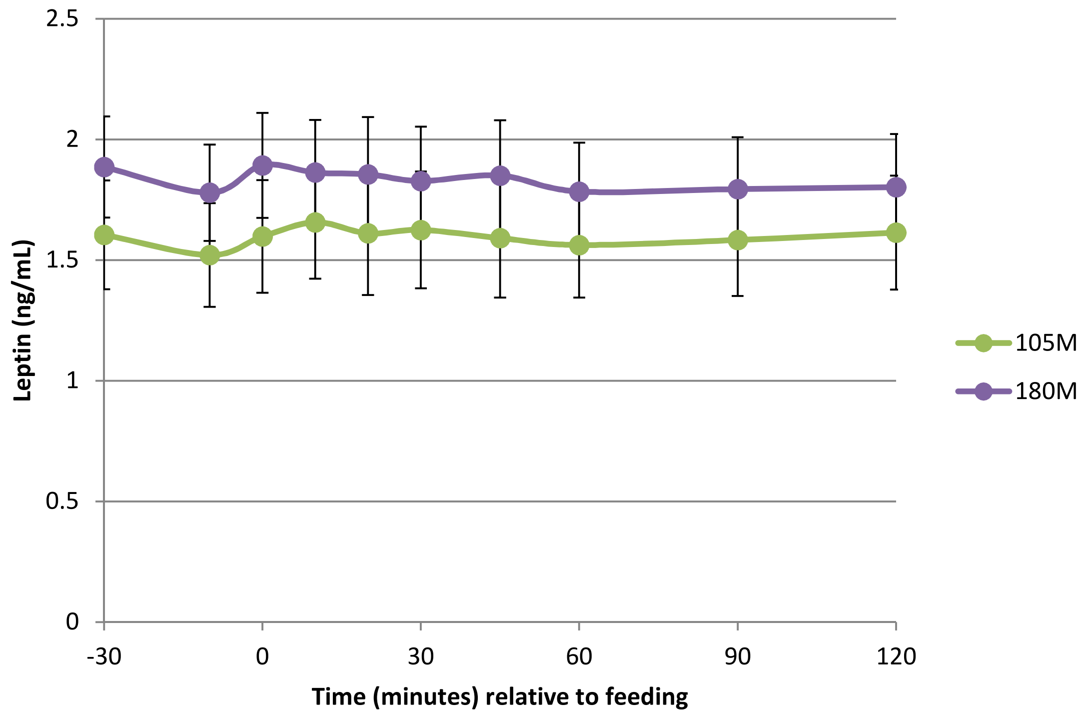

Figure 4.12: Plasma leptin concentration in heifers fed at 105% and 180% MEm during feeding.

4.4 Discussion

4.4.1 Energy Status

4.4.1.1 Glucose

Circulating glucose, among other roles, is involved in the regulation of appetite and a signal of energy status. Blood glucose in ruminants is primarily (>90%) derived from gluconeogenesis with little absorbed from the gut due to microbial usage in the rumen (Otchere *et al.*, 1974). The precursors of gluconeogenesis in the liver are the products of volatile fatty acid fermentation, particularly propionate (Young, 1977). Gluconeogenesis is reduced during prolonged periods of reduced energy intake, as a result of insufficient propionate production (Chilliard, 1999). As such, circulating glucose is positively correlated with energy balance (Reist *et al.*, 2002). Additionally, circulating glucose is affected by body condition score (Adams *et al.*, 1987) and is

likely to impact the ability of insulin to act anorexigenically dependent on the nutritional status of the animal (Roche *et al.*, 2008).

Neither feeding level nor RFI genotype impacted glucose although glucose was correlated with feed intake in animals fed at 180% ME_m (r_p = -0.44). However, glucose was not correlated with RFI line or was different between feeding level, indicating that glucose is a poor predictor of energy balance in ruminants that are fed above maintenance. Similar to this study, both Richardson *et al.* (2004) and Laurence (2010) found no difference between RFI lines in glucose, although Richardson *et al.* (2004) showed a positive correlation between glucose at the start of a 72 day test for RFI and actual RFI over the test period.

4.4.1.2 NEFA

Circulating concentrations of non-esterified fatty acids (NEFA) are negativly correlated with energy status (Emery *et al.*, 1992). Reist *et al.* (2002) suggested that NEFA are likely to be the best indicator of energy balance in ruminants (r_p = -0.69) as they are the first indicator of lipolysis of adipose tissue, followed by glucose (r_p = 0.46). NEFA that are mobilised from adipose tissue undergo β-oxidation in the liver to produce acetyl-CoA, which is oxidised to form ATP in the tri-carboxylic acid cycle. However, excess acetyl-CoA that cannot be oxidised in the tri-carboxylic acid (TCA) cycle is used to form ketone bodies such as β-hydroxybutyrate. β-hydroxybutyrate is not as good an indicator of negative energy balance as plasma NEFA concentration as only excess acetyl-CoA is used in its formation.

Whilst not significantly different, the low RFI heifers had greater concentrations of circulating NEFA than high RFI heifers. The fact that circulating NEFA were greater suggests that the low RFI heifers were in a more negative energy status than the high RFI heifers, suggesting that there may be some differences with stress responses between high and low RFI animals. The difference in circulating NEFA was 11% greater at 105% ME_m and 13% greater at 180% ME_m in the low RFI heifers indicating that the low RFI animals were mobilising more fatty acids from adipose tissue for energy. This is consistent with the NEFA differences between heifers fed at 105% ME_m and those fed at 180% ME_m. The results of Richardson *et al.* (2004) from early work in this population conflict with the results herein. They showed that low RFI animals had significantly more circulating triacylglycerols and less β-hydroxybutyrate than the high RFI animals. This suggests that the low RFI animals are in a positive energy balance as they were synthesizing fatty acids and storing these fatty acids as triacylglycerols rather than mobilising fatty acids. Unfortunately, NEFA concentrations were not measured in these animals (Richardson *et al*, 2004). The lower circulating β-hydroxybutyrate though suggests that there was not an excess of NEFA (product of triacylglycerols) to form ketone bodies such as β-hydroxybutyrate. Related to this, Laurence (2010) showed that on low nutrition, NEFA were only greater in high RFI cows than low RFI cows in the third trimester of pregnancy, but were not different on a high plane of nutrition. Laurence (2010) also showed that β-hydroxybutyrate was greater in animals on a low plane of nutrition than those on a high plane of nutrition, but this was not consistently different between RFI genotypes. These results may differ from this study in that the high RFI cows had greater adipose tissue reserves and therefore, could mobilise more NEFA for metabolism and the β-hydroxybutyrate differences were directly influenced by the degree of adiposity rather than negative energy balance *per se*. Another key point

is that there appears to be an interaction between RFI genotype and nutrition on NEFA and triacylglycerol concentrations in the blood. In the study by Richardson, *et al* (2004), the animals were *ad libitum* grain fed. In the study by Laurence (2010), the animals were *ad libitum* grass fed and the animals herein were predominantly grain fed on 2 levels of nutrition. This makes direct comparisons across these studies difficult.

4.4.2 Appetite Regulation

4.4.2.1 Ghrelin

Ghrelin is considered the "hunger hormone", has orexigenic effects (Wertz-Lutz *et al.*, 2006) and is produced primarily by the abomasum in ruminants (Roche *et al.*, 2008). Ghrelin's orexigenic effects (via the stimulation of NPY and AgRP in the hypothalamus) are well defined in monogastric animals, and are dependent on the status of the animal (e.g. pregnant, lactation status) (Kojima and Kangawa, 2005, Roche *et al.*, 2008). Although the effects of ghrelin in ruminants are less well defined, experiments on growing, non-pregnant and non-lactating animals consistently show that ghrelin has orexigenic responses on feed intake.

Wertz-Lutz *et al.* (2006) showed a 3-6 fold difference in circulating concentration of ghrelin in fed vs fasted steers. In the experiment here, there was a 24% difference in circulating ghrelin between heifers fed at 105% and 180% ME_m. Although no differences were observed between high and low RFI groups, there was an interaction between RFI and feeding level, indicative of high RFI heifers fed at 105% ME_m being more satiated. However, the results herein (Chapter 3) showed that when fed at 180% ME_m, the high RFI line ate 0.353 kg/day (3.5%) more than the low RFI line, which had

reached *ad libitum* at this feeding level. This suggests that there must be other (than ghrelin) anorexigenic feedback mechanisms controlling feed intake in these animals.

4.4.2.2 Insulin

Other than insulin's well defined roles in energy homeostasis, insulin has a direct effect on the orexigenic and anorexigenic centres of the hypothalamus, as well as indirect effects on leptin to modulate feed intake (Roche *et al.*, 2008). Circulating insulin has been found to be proportional to body adipose reserves (McCann *et al.*, 1992, Caldeira *et al.*, 2007) and has been shown to provide a adipose signal to the hypothalamus for the long term regulation of feed intake (Schwartz *et al.*, 1992, Schwartz *et al.*, 2000). Orexigenic and anorexigenic control of appetite by insulin is via the suppression of NPY and activation of POMC (Roche *et al.*, 2008). The lower insulin levels between the animals being fed 105% ME_m versus 180% ME_m indicate that animals should be more orexigenic on the 105% ME_m diet as expected.

The differences were observed in circulating insulin 30 minutes post-feeding between RFI lines indicate the high RFI animals should have been more satiated immediately post-feeding. As the high RFI animals ate more on the 180% ME_m diet, however, it again suggests that there are other appetite feedback mechanisms controlling feed intake in these animals.

4.4.2.3 Leptin

Kennedy *et al.* (1953) first suggested that a substance was produced by adipose tissue which acted on the hypothalamus to control food intake. They proposed that the effect

of orexigenic or anorexigenic signals to the hypothalamus was primarily 'lipostatic' or 'adipostatic'. It is now recognised that leptin is the main signal produced from adipose tissue that regulates feed intake (Roche *et al.*, 2008). Circulating leptin concentrations are positively correlated with adipose tissue mass (Blache *et al.*, 2000). Leptin reduces feed intake by stimulation of the anorexigenic neurons, CART and POMC, and also decreasing the activity of orexigenic NPY and AgRP neurons, as reviewed by Ahima (2005). Additionally, leptin inhibits the central orexigenic actions of melanin-concentrating hormone and orexins.

Richardson *et al.* (2004) showed RFI was positively correlated with leptin (r_p = 0.39; P<0.05) and was consistent with the degree of fatness in steers after one generation of divergent selection for RFI. After subsequent selection for RFI in the same population, Laurence (2010) showed that there was no association between leptin and RFI in cows over multiple calvings, even though the high RFI cows were an average of more than 30% fatter, as measured by P8 fat depth, than the low RFI cows during the same time. Similarly, in this work, there was no difference in leptin between high and low RFI heifers that were more divergent in RFI and fatness (based on EBVs) than those used by Richardson *et al.* (2004) and Laurence (2010). Additionally, the results herein showed no correlation between leptin and P8 fat depth, even though the high RFI heifers were 27% fatter than the low RFI heifers during this period (Chapter 3 section 3.3.1). Laurence (2010) showed there was a correlation between fatness and leptin in genetically high fat and low fat cows (based on P8 fat EBV's) that were 22% different phenotypically in P8 fatness. It would appear that in this experimental population, selection for RFI has resulted in weaker feedback mechanisms from the adipose tissue

to reduce the feed intake in high RFI animals. Certainly in this population, the reasonably defined relationships between adipose tissue deposits and leptin do not exist.

4.5 Conclusions

The mechanisms controlling appetite in the Trangie Angus selection lines remain unknown. It would appear that the low RFI heifers had a reduced sensitivity to circulating ghrelin and hence, have a reduced feed intake compared to high RFI heifers. Additionally, the less fat low RFI heifers may be more stressed and certainly appear to be mobilising adipose tissue to produce NEFA as an energy source. In high RFI animals, there may be weaker negative feedback mechanisms from fatness to reduce feed intake similar to those observed in obese humans. The levels of insulin, ghrelin and leptin cannot account for differences in the feed intake of the high and low RFI animals herein. However, although these are the most obvious hormones for controlling appetite, feed intake is a very complex system and there are a host of other hormones, factors and pathways that may differ between the lines. Therefore, these animals would be a useful resource to study factors controlling appetite in ruminants.

Chapter 5

Carcass composition and meat quality traits of long fed feedlot finished Angus steers divergently selected for residual feed intake

CHAPTER 5: Carcass composition and meat quality traits of long fed feedlot finished Angus steers divergently selected for residual feed intake

5.1 Introduction

Improving profitability of beef production has traditionally been achieved through increasing output values, such as growth rate and fertility (Arthur *et al.*, 2001b), with little focus on reducing input costs. Feed is the largest recurring input cost in a feedlot operation. Therefore, use of sires that are known to be genetically low-RFI animals by the beef industry offers the potential for commercial cattle producers to breed steer progeny that will eat less during feedlot finishing with no effect on average daily gain. However, the opportunity to improve profitability in the feedlot is dependent not only on the existence of genetic variation in RFI, but also on the magnitude of the genetic correlations with other key production traits. For feedlot cattle, these traits include carcass and meat quality traits, many with tight market specifications and penalties for non-compliance. Positive genetic correlations between RFI and subcutaneous fat depth have been reported in young Angus bulls and heifers (Arthur *et al.*, 2001b) and in feedlot steers from Australian temperate and tropically-adapted genotypes (Robinson and Oddy, 2004, Barwick *et al.*, 2009), suggesting that breeding for low RFI is accompanied by lower levels of subcutaneous fatness.

Evidence confirming that selection on RFI can change feed intake whilst maintaining production but accompanied by change in body composition has been reported by Herd *et al.* (2003b). Divergent selection based on RFI measured on young Angus bulls and heifers resulted in steers of low-RFI parents having lower feed intake in a research

feedlot, with no compromise in growth performance, and hence, an improved feed conversion ratio (FCR), compared to steers from high-RFI parents. Subcutaneous fat depth measured on the live animal and on the carcass was lower in the steers of low-RFI parents, whereas the cross-sectional area of the eye-muscle (*M. longissimus thoracis et lumborum*; EMA) on the live animal and carcass weight as a percentage of pre-slaughter weight (dressing percentage) was greater.

Residual feed intake, also termed net feed intake (NFI), is the measure of feed efficiency that has been adopted by the major beef cattle breeds in Australia for the purpose of genetic improvement. Genetic merit for RFI is described by estimated breeding values (EBV) for post-weaning NFI and feedlot NFI (BREEDPLAN, 2010). The aim of this experiment was to evaluate, under the management conditions of a large commercial feedlot, growth, feed efficiency and carcass characteristics of steers bred to be genetically divergent in RFI from Angus bulls and cows with known EBVs for post-weaning RFI.

5.2 Materials and Methods

5.2.1 Cattle Breeding

The steers used in this experiment were bred at the NSW Department of Primary Industries Agricultural Research Centre, Trangie, NSW. Two research herds were in the breeding program at Trangie. The majority of the animals used in this experiment were progeny from the feed efficiency research herd described in Arthur *et al.* (2001b). A smaller number (n=98) of the cattle used in this experiment were progeny of cows from an Angus Society of Australia Progeny Test Program that had been conducted at the Trangie Research Centre. The sires and dams had EBVs for post-weaning RFI

calculated by the Animal Breeding and Genetics Unit (University of New England, Armidale, NSW), using each animal's own post-weaning RFI-test information as well as information on relatives. The sires and dams were classified by their RFI-EBV and mated in 2005 to produce offspring genetically-divergent for RFI. The calves were born June-September 2006. The RFI-EBV for the each progeny was calculated as the mid-parent RFI-EBV, being the average of the RFI EBVs of both parents.

5.2.2 Cattle management and measurements

Male calves were castrated at approximately four months of age and managed together with their dams as a single herd until weaning in mid-February 2007. The steers continued to be managed together as a single mob and all received the same health treatments and access to improved pasture and feed supplements until feedlot entry. The steers were vaccinated (UltraVac® 5in1, Bovilis®MH and Pestiguard™) to reduce the risk of bovine respiratory disease once in the feedlot.

At a date specified by the feedlot, those steers that had attained a weight of 400kg live weight were purchased by the feedlot. At this date, of the 271 steers weaned, one died, two were excluded because of poor leg structure, six were excluded because of uncertain parentage, and 42 failed to attain the specified induction weight. These 42 steers contained roughly equal proportions of high, medium and low RFI-EBV candidate animals. The remaining 216 steers sold to the feedlot were drafted into three groups, being of low RFI-EBV (mid-parent RFI-EBV ≤-0.3kg/day; N=73), medium RFI-EBV (mid-parent RFI-EBV >-0.3 to 0.14 kg/day; N=73), and high RFI-EBV (mid-parent RFI-EBV ≥0.16kg/day; N=70). The low RFI-EBV steers were the progeny of 14 sires, the medium RFI-EBV steers were the progeny of 14 sires (8 in common with the

low RFI-EBV group), and the high RFI-EBV steers were progeny of 9 sires (1 in common with the medium RFI-EBV group; no sire had progeny in all 3 groups).

Before departure from the Trangie Research Centre, the steers were scanned using ultrasound by an accredited technician to measure subcutaneous fat depth between the 12/13 ribs (ribfat) and cross-sectional area of the *M. longissimus thoracis et lumborum* or “eye-muscle”. Animals were trucked from the Research Centre to the feedlot where they were inducted the morning after arrival. Induction involved a 5-in-1 vaccination (Ultravac™), an anthrax vaccine (Living Spore Sterne Strain - Pfizer) and being treated with Dectomax®, Bovilis® and Rhinoguard®.

The experiment started at an average age of 447 ± 17 (sd) days and an average weight of 439 ± 31kg. The steers were treated the same as other commercial animals in the feedlot except that they were weighed at day 35 and day 113. The cattle had access to feed until removed from the pens to be weighed (days 35 and 113) or slaughtered. Each group of steers was fed in a separate pen but on the same ration. The three pens were each located at the end of adjacent cul-de-sac rows, and were of similar size and orientation to the sun. Dividing the RFI groups to provide replication was not possible.

The steers were fed a starter ration for the first 16 days before changing to two intermediate rations for another 16 days, followed by a finisher ration for the remaining time in the feedlot. The steers were fed for a total of 251 days. Cattle were fed up to four times per day, with experienced pen riders determining when and how much to feed, with a view to minimizing wastage but ensuring that all animals had *ad-libitum* access to feed throughout the day. The amount of feed placed into each pen from the

delivery truck was recorded. The diets were a ‘wet ration’ based on barley with silage and roughage. The finisher diet consisted of 57% grain, 15% silage and 18% roughage with 65% dry-matter (DM), 12.1% crude protein and 12.3 MJ metabolizable energy (ME)/kg dry matter (DM).

Four steers were removed from the pens and treated for health reasons (2 low RFI-EBV; 2 high RFI-EBV). Once they were removed, they could not be returned to their original pens as they no longer met antibiotic-free market specifications. A further four steers (3 low RFI-EBV: 1 medium RFI-EBV) were sold early by feedlot management to fill other market requirements. These 8 steers were not included in the final data set analysed.

After 251 days on feed, the steers from the three pens were walked to the abattoir (~500 meters) adjacent to the feedlot and slaughtered on the same day. After stunning and exsanguation, the carcasses were weighed and 14kg added for blood loss (the usual weight of blood loss from steers of this weight recorded in the abattoir) to determine the “final” weight for each steer. Carcasses were split, suspended by the Achilles tendon and each side weighed before being chilled overnight.

The next morning, the left-side was quartered between the 7th and 8th ribs and the exposed surface measured by an accredited assessor following standard procedures (AUS-MEAT, 2005). Traits measured were rib fat and EMA (between 7th and 8th ribs), degree of ossification as assessed by the extent of calcification of the cartilage in the sacral and dorsal vertebrae (MSA ossification – 100 to 590 by units of 10), AUS-MEAT marble score (AUS_MS; 1 (nil) to 9 (abundant) by 0.1 unit scale), Meat Standards Australia marble score (MSA_MS; 100 to 1100 by units of 10), fat colour (from 0 (near

white) to 4 (dark cream) by units of 1), meat colour (1A (pale pink) to 1C (dark pink); 2 (pale red); 3 (red)) and pH (units of 0.01). The rib fat and EMA measurements (between 12th and 13th ribs) taken on the animals by ultrasound scanning just prior to feedlot entry were taken as induction values.

The carcasses were boned out the day following slaughter. After boning, a sample (155 mm) of the cube roll (incorporating the *M. longissimus dorsi*, *Spinalis dorsi* and *Semi-spinalis dorsi* muscles) was taken. Directly following bone-out, a 70 mm thick slice was frozen at minus 20ºC (1 day aged treatment) and a further 70 mm thick slice was stored at 4ºC for a further 6 days (7 days aged treatment) before being frozen at -20ºC for meat quality analysis. The remaining 15 mm thick slice was frozen at -20 to examine potential associations between genetic variation in RFI and phenotypic data for different fat depots via image analysis and chemical intramuscular fat extraction. A further 50 g of muscle from the *M. longissimus dorsi* was frozen for assessment of calpastatin activity.

Preparation of samples and measurement of peak force, compression cooking loss, pH and meat colour were performed as described by Perry *et al*. (2001). Briefly, samples were thawed overnight and a cooking block (~200g) was removed from the *longissimus dorsi* portion of the cube roll from each ageing treatment. The samples were then returned to the chiller where the freshly cut surface were allowed to bloom for 60 mins. Meat colour was measured on the bloomed surface with a Minolta Chroma Meter (colour space = L*a*b*). Following this, pH was measured and the samples were returned to the chiller. After the samples had reached 4ºC, they were weighed and cooked in individual bags in a 70ºC water bath for 60 mins after which they were

cooled rapidly in running water for 30 mins. Following cooling, the samples were re-weighed for assessment of cooking loss and returned to the chiller until objective tenderness measurements were assessed the following day. Peak force and compression measurements were performed on a Lloyd Instruments LRX Materials Testing Machine fitted with a 500N load cell with the mean of six measurements for peak force and compression used.

The quantification of calpastatin activity was performed on meat from 10 animals randomly selected from each of the low medium and high RFI-EBV groups as described by Shackelford *et al*. (1994). Briefly, 10 g of the *M. longissimus dorsi* was homogenised in 30ml of post-rigor extraction buffer. This was centrifuged at 3500g and dialysed overnight with a Tris-EDTA buffer. The supernatant was heated in a water bath at 95ºC for 15 mins to denature the calpain enzymes. After cooling in an ice bath for 15 minutes, the supernatant was centrifuged again and filtered through glass wool. The supernatant was assayed in 100 μL increments to 500 μL and made up to 1 mL with the Tris-EDTA elution buffer. To this, m-calpain, an assay media containing casein and $CaCl_2$ was added and incubated for 1 hour at 25ºC in a waterbath. The reactions were stopped with 2 mL of 5% trichloroacetic acid. m-calpain activity was determined at A_{278} with calpastatin activity/gram determined from the equations of Koohmaraie *at el.* (1990).

To examine fat deposition (subcutaneous, intramuscular and intermuscular fat), a 15mm thick slice of the cube roll was used for calculation of seam fat area via image analysis and measurement of intramuscular fat content (IMF%) via chemical extraction according to the protocol described by Siebert *et al.* (2006). For image analysis, all

samples were photographed and images stored as separate jpeg files with a resolution of 180dpi. Subsequent image manipulation involved using Adobe® Photoshop® CS2 to trim the seam fat from the surrounding muscles and saved as individual images, again at 180dpi. The seam fat area (SF) was measured using a Matlab R2007a, an interactive software program.

5.2.3 Statistical analysis

Because of the differences in energy density between the three rations initially fed and the finisher ration, the weight of feed delivered into each pen each day was arithmetically adjusted to being equivalent to a ration with 12MJ ME/kg DM. The adjusted weight of feed delivered into a pen was divided by the number of animals in the pen to calculate feed offered on a per head basis. Daily feed intake (DFI) by individual animals from the feed bunks could not be measured. Feed management and recording in the feedlot is known to increase apparent day-to-day variation in feed intake per head. Analysing the data as means for consecutive three-day blocks reduced the day-to-day variation whilst still allowing underlying trends in feed consumption to be apparent (Figure 5.1).

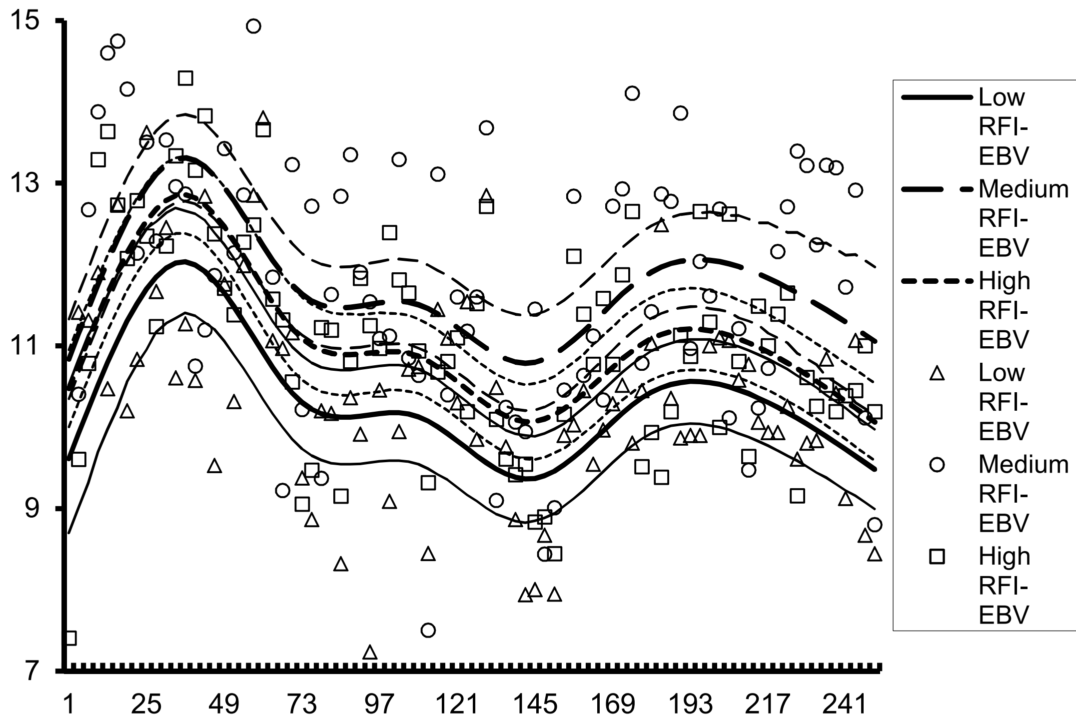

Figure 5.1: Cubic smoothing splines for consecutive three-day means for adjusted daily feed intake (adjDFI-3 day bloc) the high low RFI-EBV (low mid-parent RFI-EBV), medium RFI-EBV and high RFI-EBV (high mid-parent RFI-EBV) pens of Angus steers (± 2 se). Y-axis = kg feed intake, x-axis = days on feed

Trends in DFI for each pen over time and differences between pens were apparent (Figure 5.1). Curves in the form of splines were fitted to the data and analysed using ASREML (Gilmour *et al.*, 1999) in the model:

Y ~ mu + Pen*Day + spline(Day,8) + Pen.spline(Day,8)

where Y is DFI and Day is a covariable. In these analyses, DFI and Day represent the means of sequential groups of 3 days (*ie.* Day 1 = average days 1-3, Day 4 = average days 4-6, *etc.*). This model examined differences between pens, variation over the duration of the trial (*i.e.* over Day), and variation between pens over the duration of the trial. A random model fitting cubic smoothing splines with 8 knot points – first a single spline that describes all the variation in the 3 pens, then 3 separate splines (one for each pen) was used for non-linear variation in DFI across time. The standard error (SE) about

the predicted DFI for each pen for each day was multiplied by 2 to give an approximate 95% confidence interval for each spline.

The average daily growth rate of the steers was highest over the first 35 days in the feedlot (1.38kg/day; Appendix 5.1) and declined in the subsequent periods to 1.23kg/day over days 35-113 and to 0.92kg/day over days 114-251, and became less variable as shown by the reduction in standard deviation (SD) over the time periods (0.57 *v* 0.28 *v* 0.20). Daily weight gains calculated over a 35 day period, without more frequent intervening weights, and the corresponding derived feed efficiency traits, are less accurate than 70 day measurements (Archer *et al.*, 1997). For this experiment, the first 35-day period was regarded as an adaptation period, and evaluation of feedlot performance by the three groups was made for three periods: period 1 from day 35 to 113 (68 days); and period 2 from day 114 to 251 (137 days), and over the entire feedlot period from day 0 to 251 (251 days).

Without individual-animal feed intake data, it was not possible to calculate individual animal FCR or RFI. Pen DFI was divided by ADG of the steers in the pen to calculate FCR for each pen. The equations of SCA (1990) were used to predict the expected DFI for each pen for each period, using the average of the initial and end weights for steers in the pen and ADG over the period. Calculated in this way, the mean of predicted DFI for the three groups under-estimates the mean of observed DFI for period day 35 to 113, day 114 to 251 and day 1 to 251. In the latter period, this was 2.91kg/day, presumably due to “the wet” feeding of these cattle that differed from the experiments which the SCA (1990) equations are derived. The predicted DFI plus 2.91 was deducted from the

observed mean DFI for each pen to calculate RFI for each time period. This resulted in the mean RFI for the three pens over the 251-day period being zero.

The data set analysed was for 208 steer progeny from 26 different sires. All traits were analysed as if continuous in nature. For this purpose, meat colour was coded thus: 1A=1, 1B=1.3, 1C=1.7, 2=2, 3=3. Differences between mean values for traits recorded for the three RFI groups were determined using the general linear model (GLM) procedure in SAS (1989). Average age at induction of steers in the medium RFI-EBV group was 452 ±21 days compared with 445 ±16 and 444 ±13 days for steers in the low RFI-EBV and high RFI-EBV groups, respectively. Means for the three groups are presented as best linear unbiased estimates and standard errors determined in the models with age included as a covariate and group as the only fixed effect. Regressions for the traits against mid-parent RFI-EBV were calculated in models with age at induction included as a covariate and regression coefficients significantly different from zero were taken as evidence for genetic association. Percent change in each trait per kg/day change in mid-parent RFI EBV was calculated by dividing the regression coefficient by the mean of that trait.

5.3 Results

This trial was un-replicated at pen level, in that the three RFI groups were divided into separate pens. Replication was not possible due to constraints by the feedlot, however, the three pens were each located at the end of adjacent cul-de-sac rows, and were of similar size and orientation to the sun. The average daily growth rate of the steers was greatest over the first 35 days in the feedlot (1.38kg/day; Appendix 5.1), perhaps reflecting some ongoing re-alimentation after transport and induction. Average daily

growth rate declined in the subsequent periods to 1.23kg/day over days 35-113 and to 0.92kg/day over days 114-251. Daily feed intake declined from 11.3kg/day for days 35-113 to 10.8kg/day over the final days 114-251. The slowing in growth rate was accompanied by smaller reduction in feed consumed resulting in deterioration in FCR from 9.2 to 11.7, and in RFI from -0.88 to -0.70kg/day in the latter period.

The high RFI-EBV steers were the lightest group at induction and at each occasion when weighed thereafter (Table 5.1). They had the slowest growth rate during the period days 35 to 113, but grew equally as fast as the low RFI-EBV steers, and faster than the medium RFI-EBV steers in the period of days 114 to 251. There was no difference in growth rate between the low RFI-EBV and high RFI-EBV steers over the full 251-day period. The association for weights between periods and daily weight gain within periods with mid-parent RFI-EBV was weak, and mostly not different from zero ($P>0.05$), only tending to significance for daily weight gain over the full 251 day period, and weight at day 113 ($P=0.1$). The percentage change in these weights with a 1-unit change in mid-parent RFI-EBV were all less than 3%. The negative values for the regression coefficients for the latter two traits provide evidence for a weak association with genetic variation in RFI. Positive RFI-EBV was associated with slightly slower growth rate in the early feedlot period.

Modelling DFI over time showed the temporal patterns of feed intake were similar for the three groups (Figure 5.1). There was a significant pen effect (i.e. line effect; $P<0.001$), but no significant interaction between pens and time ($P>0.05$) (that is, all the splines had a similar shape over time). The final model with a spline curve fitted for each pen accounted for 18.3% of the variation in DFI for the 3 pens. The 95%

confidence intervals for each spline indicated that over the feedlot period, DFI by the low RFI-EBV steers was lower than the medium RFI-EBV steers, with DFI by the high RFI-EBV steers being intermediate between the low RFI-EBV and medium RFI-EBV groups (Figure 5.1).

Table 5.1: Mid-parent RFI-EBV, age, weight, average daily gain (ADG), feed intake and feed efficiency in the feedlot for Angus steers in high, medium and low RFI-EBV groups based on mid-parent RFI-EBV. Values are means (± se; LS-means for weights and daily gains) and regression coefficients (± se) for the trait with mid-parent RFI-EBV and percentage changes of traits.

	RFI-EBV group			Regression coefficient	Percent Change (% trait/unit RFI-EBV)
	Low	Medium	High		
Number of animals	68	72	68		
Mid-parent RFI EBV (kg/day)	-0.52 ± 0.02^{a}	-0.09 ± 0.02^{b}	0.63 ± 0.02^{c}		
Age at induction (days)	445 ± 2^{a}	452 ± 2^{b}	444 ± 2^{a}		
Weight at induction (kg)	434 ± 4^{a}	451 ± 4^{b}	430 ± 4^{a}	-1.1 ± 4.2	0.2
Weight at Day 35 (kg)	583 ± 4^{a}	593 ± 4^{a}	570 ± 4^{b}	-7.9 ± 4.4†	1.6
ADG Days 35 to 113 (kg/day)	1.22 ± 0.03^{a}	1.26 ± 0.03^{a}	1.19 ± 0.02^{b}	-0.02 ± 0.04	1.3
Weight at day 113 (kg)	583 ± 4^{a}	593 ± 4^{a}	570 ± 4^{b}	-9.2 ± 4.8†	1.6
ADG days 114 to 251 (kg/day)	0.94 ± 0.02^{a}	0.88 ± 0.02^{b}	0.94 ± 0.02^{a}	0.01 ± 0.03	0.7
ADG days 1 to 251 (kg/day)	1.11 ± 0.02^{a}	1.06 ± 0.01^{b}	1.07 ± 0.02^{a,b}	-0.03 ± 0.02†	2.6
Final weight (kg)	713 ± 5^{a}	714 ± 5^{a}	701 ± 5^{b}	-8.2 ± 5.6	1.2
Feed intake Days 35 to 113 (kg/day)‡	10.5	11.8	11.6		
Feed intake Days 114 to 251 (kg/day)‡	10.1	11.5	10.6		
Feed intake Days 1 to 251 (kg/day)‡	10.4	11.8	11.1		
FCR Days 35 to 113 (kg/kg)‡	8.6	9.4	9.7		
FCR Days 114 to 251 (kg/kg)‡	10.8	13.1	11.3		
FCR Days 1 to 251 (kg/kg)‡	9.4	11.1	10.4		
RFI Days 35 to 113 (kg/day)‡	-1.7	-0.6	-0.4		
RFI Days 114 to 251 (kg/day)‡	-1.7	0.7	-1.1		
RFI Days 1 to 251 (kg/day)‡	-0.8	0.7	0.1		

Means within rows with different superscripts differ significantly (P<0.05).
†denotes regression coefficient differing from zero at P<0.1.
ADG = average daily gain; FCR = feed conversion ratio, DFI = daily feed intake
‡DFI, FCR and RFI are simple group means and could not be statistically compared as individual animal data were not available.

The low RFI-EBV group grew as fast or faster than either the medium RFI-EBV or high RFI-EBV groups over each period (Table 5.1). Overall, they consumed less feed, providing evidence that they were the most feed efficient group over the periods from day 35 to 113, day 114-251 and over the full 251 days in the feedlot. Compared to the high RFI-EBV steers, the low RFI-EBV steers had a 12% lower FCR over the first period, 5% lower FCR over the second period, and 11% lower over the full 251 days in the feedlot. This was the function of having a 4% higher growth rate and a 6% lower feed intake (Table 5.1) The RFI of the low RFI-EBV steers was 1.34kg/day lower than the high RFI-EBV steers over the first period, 0.60kg/day lower over the second period, and 0.90kg/day lower over the full 251-day period.

Subcutaneous rib fat depth at feedlot entry was lowest in the low RFI-EBV steers and eye-muscle area was greater in the medium RFI-EBV steers (Table 5.2). Rib fat and rib fat gain had significant positive regression coefficients with mid-parent RFI-EBV, but eye-muscle area did not have this relationship. 1-unit in mid-parent RFI-EBV was associated with an observed 30% change in rib fat depth, and only a 0.4% change in EMA. After slaughter, the carcasses of the low RFI-EBV steers were heavier and had a greater dressing percentage than the high RFI-EBV steers, but did not differ in EMA. The differences in carcass weight and dressing percentage were consistent with the significant negative regression coefficients with mid-parent RFI-EBV, but the observed changes in carcass weight and dressing percentage associated with a 1-unit change in mid-parent RFI-EBV was less than 2%. When measured after slaughter, rib fat depth was less in the low RFI-EBV steers than the high RFI-EBV steers, while the reverse was observed for seam fat area, which was larger in the low RFI-EBV steers. The two fat traits had a positive and a negative regression coefficient, respectively, with mid-

parent RFI-EBV such that genetically lower RFI cattle had lower rib fat depth and greater seam fat. The observed change associated with a 1-unit change in mid-parent RFI-EBV was greater in rib fat depth (26%) than for seam fat (9%). Additionally, rib fat gain over the feedlot period was 24% per unit change in mid parent RFI-EBV in that the high RFI-EBV steers gained 2.6mm more fat than the low RFI-EBV steers. AUS-MEAT marble score, MSA marble score and chemical IMF% did not differ between the low RFI-EBV and high RFI-EBV steers, but there was a trend for IMF% to be associated with lower mid-parent RFI-EBV (P=0.1). The changes in the three intramuscular fat traits associated with a 1-unit change in mid-parent EBV were all less than 5%.

Table 5.2: Subcutaneous rib fat depth and eye-muscle area taken by ultrasound scan prior to induction and carcass traits for Angus steers in high, medium and low feed intake groups based on mid-parent RFI-EBV. Values are LS-means (± se) and regression coefficients (± se) for the trait with mid-parent RFI-EBV and percentage changes of traits.

	RFI-EBV group			Regression coefficient	Percent Change (% trait/unit RFI-EBV)
	Low	Medium	High		
Rib fat at induction (mm)	6.7 ± 0.2[a]	7.4 ± 0.2[b]	9.2 ± 0.2[c]	2.3 ± 0.2*	30
EMA at induction (cm^2)	63.5 ± 0.7[a]	66.6 ± 0.6[b]	63.9 ± 0.7[a]	0.3 ± 0.8	0.4
Hot carcass weight (kg)	417 ± 3[a]	420 ± 3[a]	406 ± 4[b]	-8 ± 4*	1.9
Dressing percentage (%)	58.5 ± 0.2[a]	58.9 ± 0.2[a]	58.0 ± 0.2[b]	-0.4 ± 0.2*	0.7
EMA on carcass (cm^2)	76.1 ± 0.4[a]	78.6 ± 0.4[b]	76.1 ± 0.4[a]	-0.3 ± 0.5	0.3
Rib fat depth on carcass (mm)	15.6 ± 0.6[a]	17.6 ± 0.6[b]	20.7 ± 0.6[c]	4.7 ± 0.7*	26
Rib fat gain (mm)	8.9 ± 0.6[a]	10.2 ± 0.6[ab]	11.5 ± 0.6[b]	2.4 ± 0.7*	24
Seam fat (cm^2)	24.6 ± 0.7[a]	25.8 ± 0.7[a]	22.0 ± 0.7[b]	-2.1 ± 0.9*	8.8
Ausmeat marble score	3.0 ± 0.1[a]	3.6 ± 0.1[b]	3.0 ± 0.1[a]	-0.1 ± 0.1	2.1
MSA marble score	477 ± 11[a]	569 ± 11[b]	463 ± 12[a]	-19 ± 15	3.8
IMF (%)	14.3 ± 0.4[a]	15.6 ± 0.4[b]	13.5 ± 0.4[a]	-0.7 ± 0.4†	4.9
Fat colour	1.20 ± 0.05[a]	1.09 ± 0.05[a]	1.37 ± 0.05[b]	0.17 ± 0.06*	14
Meat colour code	2.10 ± 0.05[a]	1.73 ± 0.04[b]	2.00 ± 0.05[a]	-0.03 ± 0.06	1.5
Ossification	139 ± 1[a]	142 ± 1[b]	143 ± 1[b]	4.0 ± 1.4*	2.8

Means within rows with different superscripts differ significantly ($P<0.05$)

* denotes statistically-significant regression coefficient ($P<0.05$). †at $P<0.1$

EMA=area of eye-muscle

Fat colour, as assessed on the sectioned carcass, was lower (whiter) for the low RFI-EBV steers compared to the high RFI-EBV steers, but meat colour did not differ (Table 5.2). There was a significant positive regression coefficient for fat colour, but not meat colour, with mid-parent RFI-EBV. Change in fat colour was 14% per unit mid-parent RFI-EBV compared to less than 2% for meat colour. Ossification was least in the low RFI-EBV steers and had a positive regression coefficient with mid-RFI-EBV, implying a genetic association, but the change in ossification per unit mid-parent RFI-EBV was less than 3%.

Table 5.3: Ultimate pH, calpastatin activity and meat quality characteristics of the M. longissimus dorsi from Angus steers in high, medium and low feed intake groups based on mid-parent RFI-EBV. Values are LS-means (± se) and regression coefficients (± se) for the trait with mid-parent RFI-EBV

	RFI-EBV group			Regression coefficient	Percent Change (% trait/unit RFI-EBV)
	Low	Medium	High		
Ultimate pH	5.53 ± 0.01[a]	5.49 ± 0.01[b]	5.53 ± 0.01[a]	0.00 ± 0.01	0.1
Calpastatin activity	2.38 ± 0.23[a]	2.25 ± 0.27[a]	3.14 ± 0.24[b]	0.55 ± 0.27*	21.5
Peak force day 1 (kg force)	3.48 ± 0.08[a]	3.29 ± 0.08[a]	3.15 ± 0.08[b]	-0.29 ± 0.10*	-8.8
Compression day 1 (kg force)	1.23 ± 0.02[a]	1.12 ± 0.02[b]	1.15 ± 0.02[b]	-0.06 ± 0.03*	-5.3
Cook loss day 1 (%)	11.5 ± 0.1	11.2 ± 0.1	11.3 ± 0.1	-0.20 ± 0.16	-1.8
L* day 1	38.9 ± 0.4[a]	40.5 ± 0.4[b]	39.4 ± 0.4[a]	0.36 ± 0.45	0.9
a* day 1	27.0 ± 0.3	27.1 ± 0.3	27.6 ± 0.3	0.70 ± 0.36†	2.6
b* day 1	13.2 ± 0.2	13.4 ± 0.2	13.5 ± 0.2	0.29 ± 0.26	2.2
Peak force day 7 (kg force)	3.01 ± 0.07	3.01 ± 0.07	2.87 ± 0.07	-0.16 ± 0.08†	-5.2
Compression day 7 (kg force)	1.21 ± 0.03[a]	1.10 ± 0.02[b]	1.11 ± 0.02[b]	-0.08 ± 0.03*	-6.6
Peak force ageing (kg force)‡	0.47 ± 0.06[a]	0.28 ± 0.06[b]	0.27 ± 0.06[b]	-0.15 ± 0.07*	-44
Compression ageing (kg force) ‡	0.03 ±0.02	0.02 ± 0.02	0.04 ±0.02	0.02 ± 0.03	63
Cook loss day 7 (%)	11.3 ± 0.1[a]	10.8 ± 0.1[b]	11.0 ± 0.1[ab]	-0.26 ± 016†	-2.4
L* day 7	40.1 ± 0.4[a]	41.7 ± 0.4[b]	40.3 ± 0.4[a]	-0.09 ± 0.44	-0.2
a* day 7	28.1 ± 0.3	28.3 ± 0.3	28.7 ± 0.3	0.38 ± 0.35	1.3
b* day 7	13.9 ± 0.2	14.2 ± 0.2	14.1 ± 0.2	0.13 ± 0.27	0.9

Means within rows with different superscripts differ significantly (P<0.05)
* denotes statistically-significant regression coefficient (P<0.05). †at P<0.1
‡ ageing calculated as the difference between peak force or compression values at day 1 and day 7.
L* = Minolta lightness colour value
a* = Minolta red-green colour value
b* = Minolta yellow-blue colour value

Compared with either the low or medium RFI-EBV steers, the meat of high RFI-EBV steers was more tender as measured by peak force after 1 day of ageing. This is not consistent with an increase in calpastatin activity of these animals as there was a 21.5% increase in calpastatin activity per unit increase in RFI-EBV (Table 5.3). However, the difference in peak force between the RFI-EBV groups was reduced for the ageing rate calculated as the difference in peak force between days 1 and 7. The difference in peak force ageing rate between the high and low RFI-EBV groups was significantly different in that the low RFI-EBV group was 0.20kg greater than high RFI-EBV group, resulting in a -44% change in peak force per unit mid-parent RFI-EBV. The compression ageing rate, calculated as the difference between days 1 and 7, was not significantly different between RFI-EBV groups. However, the meat from both medium and high RFI-EBV steers was less "chewy" than meat from the low RFI steers as measured by compression at 1 day and this difference was greater at day 7.The Minolta lightness colour value (L*) was higher (lighter) in both days 1 and 7 for the medium RFI-EBV group than the either the high or low RFI-EBV groups.

5.4 Discussion

This experiment demonstrated that a reduction in RFI had a favourable impact on the performance of Angus steers in a large commercial feedlot by reducing feed consumed with no adverse effects on final weight. The mean difference in mid-parent EBV for post-weaning RFI of 1.18 kg/day between the low RFI-EBV and high RFI-EBV groups was associated with a 10% improvement in FCR and 0.9 kg/day reduction in RFI sustained over the 251 days in the feedlot. The advantage in FCR and RFI was greater in the early period in the feedlot (day 35 to 113) than in the latter period (day 114 to

251); there being a reduction in FCR from 12% to 5% and in RFI from 1.34 to 0.60 kg/day. A 10% reduction in FCR translates directly to a 10% reduction in the amount of feed consumed per kilogram of weight gained. A 0.9 kg/day reduction in RFI is a saving of 226 kg of feed eaten to achieve the same growth and weight outcomes. That the low RFI-EBV steers had a lower feed intake and improved FCR without compromise to growth performance is consistent with other studies using Angus steers from the Trangie high and low post-weaning RFI selection lines (for example, Herd *et al.* 2003). Previous experiments were typically conducted over 70 to 100 days and involved fewer animals. In this experiment, conducted in a large commercial feedlot, the low RFI-EBV steers showed a sustained reduction in feed intake compared to that previously measured in the research feedlot.

The average growth rate of the steers was highest over the first 35 days in the feedlot and declined in the subsequent periods of day 35-113 and day 114-251, and became less variable as shown by the reduction in standard deviation over the time periods. Further, the advantage in feed efficiency between the low RFI-EBV and high RFI-EBV groups reduced over time on feed. This is as expected from the experience of the feedlot managers who had predicted that variation in growth performance and differences in FCR between groups of cattle would become proportionately less the longer they were on feed whilst they deposited more fat.

Whether reduced RFI has a positive or negative impact on feedlot profit will also depend on meeting the market specifications for carcass traits including fatness. Before the steers entered the feedlot, the high RFI-EBV group were carrying significantly more subcutaneous fat over the 12/13th ribs (2.5mm) than the low RFI-EBV group, and

finished the feedlot period with 5.1mm more subcutaneous fat at the 5/6th rib site on the carcass than the low RFI-EBV steers (Table 5.2). This resulted in the high RFI-EBV group having a 2.6mm increase in rib fat gain over the feedlot period. The association between rib fat depth and genetic differences in RFI became stronger between induction and feedlot exit, as evidenced by the regression coefficients of rib fat depth on mid-parent RFI-EBV increasing from 2.3 mm/kg/day at induction to 4.7 mm/kg/day at feedlot exit. The low RFI-EBV steers had 16mm of rib fat at slaughter, and had at least the same level of intramuscular or marbling fat and similar marbling scores as the high RFI-EBV steers. Further, the low RFI-EBV steers had dressing percentages that were higher than the high RFI-EBV steers. Being less fat, the low RFI steers would be expected to have a greater yield of retail beef with no loss of marbling grade. However, this was not due to lower subcutaneous fat levels as subcutaneous fat is positively related to dressing percentage. The low RFI-EBV steers had a greater dressing percentage presumably because they had a smaller visceral mass and gut contents as a function of lower feed intake.

In yearling Angus bulls and heifers evaluated for post-weaning RFI, Arthur et al (2001b) reported a significant phenotypic correlation (r_P=0.14) and genetic correlation (r_g=0.17) between rib fat and RFI. The genetically-low RFI-EBV steers were leaner over the ribs than the genetically-high RFI-EBV steers in this experiment, which is consistent with the measured difference in rib fat depth between young steers from the Trangie high and low RFI-selection lines reported by McDonagh *et al.* (2001). However, after 1 generation of selection for high and low RFI, Richardson *et al.* (2001) concluded that fatness is not the key driver of difference in RFI in the same animals. Consistent, strong phenotypic and genetic associations between rib fat depth and RFI in

feedlot steers from the major cattle breeds have been reported in Australia. For example, in tropically-adapted and temperate breeds, Robinson and Oddy (2004) and Barwick *et al.* (2009) reported r_p=0.11 and 0.21 and r_g=0.48 and 0.40, respectively. In a recent review, Herd and Arthur (2009) concluded that the association between fatness and RFI may depend on the age of animals, with the association becoming stronger in older lot fed animals.

The regression coefficients indicate the measured change in the traits that was observed with a one kilogram/day increase in mid-parent RFI EBV (Table 5.2). The percentage change enables a comparison of the magnitude of the effect on each trait from the genetic change in RFI under the conditions of this experiment. The association between RFI EBV and the fat traits implies that selection for lower RFI would decrease fatness. However, the change in rib fat thickness (subcutaneous fat depot) was much larger than the change in seam fat area (intermuscular fat depot), and the changes in intramuscular fat content were negligible. This suggests that while breeding to improve (decrease) RFI could reduce fatness, the magnitude of the effect differs between the adipose depots.

The extent of ossification within vertebrae has been used as an indicator of skeletal maturity, with higher values indicating greater maturity, and being weakly associated with less tender meat as assessed by consumer sensory panels (Watson *et al.*, 2007). The ossification score was greater for the high RFI-EBV steers than for the low RFI-EBV steers, and was positively correlated with mid-parent RFI-EBV. This suggests that the high RFI-EBV steers were closer to attaining skeletal maturity even though they were, on average, the same age and were slightly lighter in weight than the low RFI-

EBV steers. The size of the association is small with a less than 3% change in ossification score for a unit of mid-parent EBV-RFI.

Australian meat consumers have a preference for whiter fat (fat colour = 0) and for meat that is light red in colour (meat colour = 1B or 1C; (Egan *et al.*, 2001)). Fat colour was greater (less white in colour) on the cut section of the high RFI-EBV steer carcasses and positively correlated with mid-parent RFI-EBV. There was a 14% difference in fat colour associated with a unit of mid-parent EBV-RFI, considerably less than the 100% difference that would equate to a unit of fat colour. However, this did reflect that more of the low RFI-EBV steers carcasses graded fat colour 0 to 1 as compared to carcasses of the high RFI-EBV steers. This may have been due to the high RFI-EBV steers accumulating more β-carotene from pasture than the low RFI-EBV steers as they were fatter at feedlot induction. Meat colour did not differ between the low RFI-EBV and high RFI-EBV steers, nor was it correlated with mid-parent RFI-EBV, and with a mean colour of 1B would be acceptable to Australian consumers.

Tenderness is the single most important factor contributing to the evaluation of a satisfactory eating experience of beef by Australian consumers (Egan *et al.*, 2001). Tenderness as measured by compression at days 1 and 7 was lower in the low RFI-EBV steers meat samples. This may indicate that muscle from the low RFI-EBV has greater connective tissue content or that the structure of the connective tissue is stronger (i.e. has more crosslinks). Peak force of 1 day aged meat samples was less from the high RFI-EBV steers and negatively correlated with mid-parent RFI-EBV. After ageing for 7 days, this difference in peak force had almost disappeared as meat from the low RFI-EBV group aged faster. With more ageing, it would be expected there would be no

difference in tenderness as measured by peak force between the RFI-EBV groups. This suggests that meat from the low RFI-EBV steers must have a faster rate of post-mortem proteolysis. The decreased calpastatin activity of the low RFI-EBV steers corresponds with this observation and would result in more uninhibited m-calpain and μ-calpain being available for post-mortem proteolysis. These results suggest that protein metabolism may not be involved in the differences in feed intake observed between high and low RFI animals as observed by Richardson and Herd (2004) and as reported in chapter 2. Regardless of whether or not there are differences in tenderness or ageing rate between the RFI-EBV groups, it must be noted that the level of tenderness was acceptable and that these differences are small and unlikely to be identified by the consumer. However, as on-going selection for RFI takes place, this may be of concern for the future, though with more ageing, it is unlikely that this will affect the consumer.

These results are in contrast to those seen by McDonagh *et al.* (2001) in the Trangie steers resulting from a single generation of divergent selection for RFI. They reported no change in tenderness as measured by peak force or compression in samples that had been aged for 1 or 14 days, similar to that found by Brown (2005) and Baker *et al*. (2006). However, they did find a difference in the myofibril fragmentation index between the RFI lines, where the high RFI line had significantly greater levels of myofibril fragmentation at 1 and 14 days of ageing. This was consistent with their observed decrease in calpastatin activity in the high RFI line and hence, more m- and μ-calpain was available for post-mortem proteolysis in the high RFI line. Baker *et al*. (2006) reported no difference in calpastatin activity between high and low RFI steers. To date, there are no consistent reports of calcium dependent protease system activities in high and low RFI animals.

5.5 Conclusions

This experiment demonstrated that a reduction in post-weaning RFI had a favourable impact on the performance of Angus steers in a large commercial feedlot by reducing feed consumed with no adverse effects on final turnoff weight. Each low RFI-EBV steer consumed on average 2.60t of feed compared to 2.87t by the medium RFI-EBV and high RFI-EBV steers; this saved the feedlot 0.27t or $53 (at $200/tonne) of feed per animal with no compromise in weight gain. The feed efficiency benefit was sustained for 251 days and showed that genetic improvement of RFI will reduce feed costs in a large commercial feedlot. The low RFI-EBV steers finished with less subcutaneous fat measured at the 7/8th rib, which may impact on meeting market specifications, but there was no effect on fat colour, meat colour, marbling scores, IMF% and skeletal maturation. Dressing percentage and seam fat were higher in the low RFI-EBV steers. Together, this would be expected to result in a greater yield of retail beef with no reduction in visual meat quality or marbling grade. Breeding to reduce RFI, and therefore, reducing a major cost of production in the feedlot, may change distribution of carcass fat but the consequences may not be as severe as previously thought as not all fat depots appear to be equally affected. Meat tenderness may be slightly reduced, but with longer ageing periods, this is unlikely to be a problem.

Chapter 6

Responses to selection for RFI

CHAPTER 6: Responses to selection for RFI

6.1 Introduction

The original results from the RFI Angus selection lines at the Trangie Research Station suggest that there are many different mechanisms to account for variation in RFI of beef animals (Richardson and Herd, 2004). The mechanism accounting for most of the variation was purported to be differences in heat production (Richardson and Herd, 2004). Up to 85% of the differences between the RFI lines could be attributed to component traits that contribute to heat production and hence, maintenance energy requirements (Richardson *et al.*, 2004, Richardson and Herd, 2004). However, as clearly indicated in the results herein (Chapter 3), after 3.5 generations of selection for RFI herein, there were no discernable differences in heat production. The conclusion is that most if not all of the differences in energy intake between the RFI lines after 3.5 generations of selection for RFI can be explained by differences in the composition of gain. This leads to two important questions: 1) Are the results after 3.5 generations of selection for RFI to be expected to change the energetics of these animals? 2) Is this observation to be expected from growth/nutrition/energetic models?

6.2 Materials and Methods

The animals utilised herein represent progressive divergence in RFI from animals derived from the Trangie Research Station RFI selection lines (Arthur *et al.*, 2001a). This divergence in RFI is representative of steers 1 generations divergent in RFI from Richardson *et al.* (2001), steers 2.5 generations divergent in RFI as reported in Egarr *et*

al. (2009) and in Chapter 5 herein, as well as heifers 3.5 generations divergent in RFI as reported in chapters 2, 3 and 4 herein.

6.2.1 Animals: Steers divergent in RFI for 1 generation

The data from steers 1 generation divergent in RFI have been described elsewhere (Richardson *et al.*, 2001, Richardson and Herd, 2004, Richardson *et al.*, 2004). Briefly, the steers were the F_1 progeny of bulls and heifers that underwent a 120 day RFI test. The top and bottom 50% of heifers for RFI were mated to the top and bottom 5% of bulls from the test to generate the animals used herein and designated as 1 generation divergent in RFI. The most divergent steers (for RFI - based on sire RFI-EBV) (n=33) were used, 16 low and 17 high RFI animals.

After a three week acclimation period at Tullimba Research Feedlot, group 1 steers had their feed intakes recorded for 70 days before being recorded for a further 72 days at the University of New England's Beef Research Unit. Steers in group 2 had feed intake recorded in the feedlot for 106 days and 32 days in the beef research unit. At the start of the feedlot period and at the end of the Beef Research Unit period, ultrasound scans were made to determine rib (Rib-Fat) and P8 (Rump-Fat) fat depths as well as eye muscle area. This enabled the calculation of changes in fatness and lean growth.

Empty body protein weight (EBPW) and empty body fat weight (EBFW) were calculated from the equations derived by Richardson *et al.* (2001).

$$EBPW\ (kg) = 0.117 \times LW + 84.1 \times EMA - 74.3 \times \frac{Rib\ Fat}{LW} + 11.3 \times \frac{P8\ Fat}{LW}$$

$$EBFW\ (kg) = 0.233 \times LW - 95.8 \times \frac{EMA}{LW} + 331 \times \frac{Rib\ Fat}{LW} + 36.2 \times P8\ Fat$$

Where:

EBPW is empty body protein weight, EBFW is empty body fat weight, EMA is eye muscle area and LW is live weight.

The steers were slaughtered at approximately 14 months (live weight ~426 kg) at the Food Science Australia abattoir (Cannon Hill, Brisbane). Weights of all "fat free" organs, internal and external, were recorded at slaughter. After boning out these steers, the carcass fat and lean were combined and minced and analysed for chemical fat and protein. The data and literature estimates of the fat and protein content of external organs (head, hide, tail and hooves) were used in the calculation of energy retained as fat and protein.

Energy retained (RE) as fat (RE_{fat}) and protein ($RE_{protein}$) were calculated as follows:

$$RE_{fat}\ (MJ/day) = Change\ EBFW\ \times 39.3$$

$$RE_{protein}\ (MJ/day) = Change\ EBPW\ \times 23.6$$

Where:

The change in either EBFW or EBPW is the growth in fat or protein during the trial period.

The values of 39.3 and 23.6 (MJ/kg) were used as the energy densities of retained fat and protein, respectively, as MJ/kg (ARC, 1980).

Heat production (HP), the heat production of gain (HPG) and maintenance heat production (HPM) were calculated as follows:

$$HP\ (MJ/day) = MEI - RE_{fat} - RE_{protein}$$

$$HPG\ (MJ/day) = \left(\frac{RE_{fat}}{k_f} - RE_{fat}\right) + \left(\frac{RE_{protien}}{k_p} - RE_{protein}\right)$$

$$HPM(MJ/day) = HP - HPG$$

Where:

MEI is the metabolisable energy intake (ME-Intake). RE_{fat} and $RE_{protein}$ are the retained energy in fat and protein, respectively. Maintenance heat production (HPM) is the heat production that cannot be explained by the energy retained and the efficiency by which this energy is retained. k_f and k_p are the efficiency of utilisation of ME used for fat (k_f) and protein (k_p) synthesis, respectively. k_f and k_p are 0.70 and 0.20, correspondingly (Geay, 1984). As such, the HPM is due to the energy requirements for maintenance, activity and that lost from the heat increment of feeding. This estimate is different from the residual heat production reported by Richardson *et al.* (2001) due to an error in their calculations. Richardson *et al.* (2001) had used the equation below in their estimates of residual heat production.

$$Residual\ HP\ (MJ/day) = HP - \frac{RE\ as\ Fat}{0.7} - \frac{RE\ as\ Protein}{0.2}$$

However, this equation (given the equation for HP above) wrongly takes into account the energy retained as fat and protein gains twice by using HP instead of MEI, because these are already accounted for in HP (see HP equation above). Hence, the values for HPM used herein have been recalculated from their data. The values of 0.7 and 0.2 are the generalised efficiency of ME use for fat (k_f) and protein (k_p) synthesis, respectively (Geay, 1984).

6.2.2 Animals: Steers divergent in RFI for 2.5 generations

The data from steers 2.5 generations divergent in RFI have been also described (see Chapter 5). Briefly, the steers (n=216) used herein were the progeny of sires and dams were classified by their RFI-EBV and mated in 2005 to produce offspring genetically-divergent for RFI. The calves were born June-September 2006. The RFI-EBV for the each progeny was calculated as the mid-parent RFI-EBV, being the average of the RFI EBVs of both parents. The steers were fed in three groups based on their RFI-EBV, being of low RFI-EBV (mid-parent RFI-EBV ≤-0.3kg/day average; N=73), medium RFI-EBV (mid-parent RFI-EBV >-0.3 to 0.14 kg/day average; N=73), and high RFI-EBV (mid-parent RFI-EBV ≥0.16kg/day average; N=70). The steers were fed for a total of 251 days on a diet consisting of 57% grain, 15% silage and 18% roughage and contained 65% dry-matter (DM), 12.1% crude protein and 12.3 MJ metabolisable energy (ME)/kg dry matter (DM).

6.2.3 Animals: Heifers divergent in RFI for 3.5 generations

The animals used for this comparison have been previously described (see Chapters 2, 3, and 4). Briefly, sixteen (16) Angus beef heifers divergently selected for RFI for approximately 3-4 generations either for high RFI (low "efficiency"; n=8, average mid-point parental RFI-EBV=0.64±0.07 kg/d) or low RFI (high "efficiency"; n=8, average mid-point parental RFI EBV=-0.78±0.26 kg/d) were used, based on parental EBVs. Prior to experimentation, animals were adapted to an 'intermediate' feedlot ration that contained 50% grain, 40.5% roughage, 8% Molofos® (consisting mostly of molasses, urea and some minerals) and 1.5% minerals with 14.3% crude protein and 9.9 MJ metabolisable energy (ME)/kg dry matter (DM).

Half (n=8) of the replicate (4 high and 4 low RFI) were assigned to a 105% ME_m and the other half (n=8) of the replicate (4 high and 4 low RFI) were assigned to 180% ME_m. Once the animals had adapted (approximately 28 days) to this feeding level, the animals were weighed and ultrasound scanned. The measurements of interest were taken over a 35 day period before the heifers were weighed and scanned again. The feeding level treatment was switched and after a 21 day adaption period, they were weighed and scanned. Trait measurements were taken over a 42 day period before being weighed and scanned for the final time. The feeding level treatment was switched so that each animal had measurements of interest taken at both feeding levels. During the entire experimental period, the animals were housed in individual pens in the Beef Research Unit at University of New England.

Composition of empty body weight was determined at the start and at the end of the feedlot period for the steers 2.5 generations divergent in RFI and at the start and end of each measurement period for the heifers 3.5 generations divergent in RFI. Empty body protein and fat weight was predicted from weight, ultrasound rib fat depth and average daily gain as described by Williams and Jenkins (1998). This enabled energy retained as fat and protein to be calculated (as above) as well as heat production and HPM (as above).

The collection of results from the single trait selection for RFI at Trangie Research Station (Table 6.1 to 6.4) represents three different generations of selection for RFI, 1 generations (Tables 6.1 and 6.2), 2.5 generations (Table 6.3) and 3.5 generations (Table 6.4) of selection for RFI. The difference between the analyses is whether the progeny

are sorted as either low or high RFI animals based on either their sires RFI (Table 6.1: genetic difference) or their own RFI (Table 6.2; phenotypic differences).

6.2.4 Modelling RFI in the "Davis Growth" model

RFI differences were simulated using the model of steer growth and composition from Oltjen *et al.* (1986) during a typical 70-day RFI test. To determine the outcome without changing the energetics of the animal, three scenarios utilising different feed intakes were run using the "Davis Growth" model of Oltjen *et al.* (1986). The feed intakes used were low feed intake (10% below medium feed intake), medium feed intake and high feed intake (10% above medium feed intake). Data outputs from the model were based on feed intake, growth and the composition of growth, and the energetics over the 70 day test period.

The equations of Standing Committee on Agriculture, Ruminants Subcommittee (SCA) (1990) were used to predict the expected feed intake for each group for each period, using the average of the start and end weights for animals simulated in each feed intake group and average daily gain (ADG) over the period. Calculated in this way, the mean of the predicted feed intake for the three groups under-estimates the mean of actual feed intake by 3.45 kg/day when calculated from SCA (1990). The predicted daily feed intake (plus 3.45 kg/day) was deducted from the actual feed intake for each feed intake group to calculate RFI for each time period. This resulted in the mean RFI for the three groups over the simulated 70 day feedlot period being zero. Growth over the 70-day test was partitioned into empty body protein and fat weights. This enabled energy retained as fat and protein to be calculated (as above) as well as heat production and HPM (as above).

6.2.5 *Statistical analysis*

All statistical analyses were conducted using Proc MIXED in SAS 9.1 (SAS, 1989). For the analysis of steers 1 generation divergent in RFI, tests of significance of the fixed effects were calculated utilising type III sums of squares mixed models. Means for RFI group (low, high) were presented as best linear unbiased estimates and standard errors determined in the models with RFI group as the only fixed effect.

For the analysis of steers 2.5 generations divergent in RFI, tests of significance of the fixed effects were calculated utilising type III sums of squares general linear models. Means for the three groups were presented as best linear unbiased estimates and standard errors determined in the models with age (similar trait to induction weight) included as a covariate and RFI group as the only fixed effect.

For the analysis of heifers 3.5 generations divergent in RFI, tests of significance of the fixed effects were calculated utilising type III sums of squares mixed models. Fixed effects fitted in the models included RFI line (high, low), feeding level (105M, 180M) and the period of trait measurement (1st, 2nd) in the crossover design. Animal was fitted as a random term with live weight at the start of the experimental periods fitted as a covariate. All interactions were tested in the maximal model with non-significant interactions being removed in order of least significance. This enabled the best linear unbiased estimates and standard errors to be extracted.

6.3 Results

6.3.1 Single trait RFI selection for 1 generation: steers

When the steers selected for 1 generation for RFI were grouped based on their sire RFI (genetic differences; Table 6.1), the sires of these steers were 0.95 kg/day different in RFI ($P<0.05$). As these sires were randomly mated to cows with no RFI records, the resulting 0.31 kg/day difference in RFI of the progeny is less than that of their sires, as would be expected. This difference in RFI resulted in a 4.40 MJ/day greater difference in ME-intake of the high RFI steers ($P<0.05$) with no significant difference in weight gain over the trial or the weight at the start of the trial. A difference in ME-intake without a difference in weight or weight gain was expected when selecting for RFI as weight is considered in the statistical models of RFI selection.

Whilst there was no difference in the weights at the start of the experiment or the weight gain over the experiment between high and low RFIs steers, the composition of the gain was different (Table 6.1). The high RFI steers, although consuming more energy compared to their low RFI counterparts, deposited less protein. As a result, the low RFI steers gained 0.03 cm^2/day (25% more in eye muscle area ($P<0.05$). There was no difference in subcutaneous fat deposition as rib fat depth was the same between the high and low RFI steers. However, there was a difference in the total body fat (EBFW) with the low RFI animals depositing less fat than their high RFI counterparts. These differences in body composition resulted in the low RFI steers depositing 0.02 kg/day more protein (18.2%; $P<0.05$) and 0.03 kg/day less fat (11.5%; $P<0.10$) as empty body protein weight and empty body fat weight, respectively.

The difference in body composition between the RFI lines resulted in differences in the calculated energy retention and expenditure (Table 6.1). Whilst the low RFI steers consumed 4.41 MJ/day less energy (5.1%), they deposited 0.40 MJ/day more protein and 1.17 MJ/day less fat than the high RFI steers. The overall difference in energy retained between the high and low RFI steers was 0.79 MJ/day (6.2%) primarily due to the greater fat deposition compared to protein in the high RFI genotype steers as fat is a more energy dense than protein. This resulted in the low RFI steers having a 3.63 MJ/day (4.9% lower heat production than the high RFI steers ($P<0.05$). However, as the efficiency of deposition of protein is 3.5 times less than fat (k_p=0.20 and k_f=0.70, respectively), and the low RFI steers gained more protein with respect to fat, the heat production of gain (HPG) was 1.06 MJ/day (7.3%,) greater ($P<0.10$). Even though the HPG was greater in low RFI steers due to more protein deposition, the low RFI group had a much lower HP. As a result, the heat production of maintenance (HPM) of these steers was 4.66 MJ/day (7.9%) less than the high RFI steers ($P<0.05$). Thus, the difference in ME-intake between these lines of steers could not be due entirely to differences in body composition.

Table 6.1: Main effects means and SEM for energetics of *ad libitum* feed intake and body composition of Angus steers genetically differing in RFI fed for approximately 140 days. Assigned to low and high RFI groups based on sire RFI-EBVs.

	Low RFI (n=16)	High RFI (n=17)	Difference+ (%)
ME-Intake (MJ/day)	86.36 ± 1.53[a]	90.76 ± 1.48[b]	5.1
Sire RFI (kg/day)	-0.30 ± 0.04[a]	0.65 ± 0.04[b]	
Individual RFI (kg/day)	-0.15 ± 0.09[a]	0.16 ± 0.09[b]	
Start Weight (kg)	283.74 ± 6.78	291.84 ± 6.57	2.9
Mid Weight (kg)	353.48 ± 6.63	359.91 ± 6.44	1.8
Weight Gain (kg/day)	0.99 ± 0.03	0.97 ± 0.03	-2.0
Change Rib Fat Depth (mm/day)	0.05 ± 0.01	0.05 ± 0.00	0.0
Change EBFW (kg/day)	0.26 ± 0.02	0.29 ± 0.02	11.5
Change EMA (cm^2/day)	0.12 ± 0.01[a]	0.09 ± 0.01[b]	-25.0
Change EBPW (kg/day)	0.11 ± 0.01[a]	0.09 ± 0.01[b]	-18.2
RE as Fat (MJ/day)	10.17 ± 0.67	11.34 ± 0.65	11.5
RE as Protein (MJ/day)	2.53 ± 0.12	2.15 ± 0.11	-15.0
RE as Fat and Protein (MJ/day)	12.70 ± 0.65	13.49 ± 0.63	6.2
Heat Production (MJ/day)	73.64 ± 1.52	77.27 ± 1.47	4.9
Heat Production of Gain (MJ/day)	14.50 ± 0.49	13.44 ± 0.48	-7.3
Heat Production of Maintenance (MJ/day)	59.15 ± 1.16[a]	63.81 ± 1.51[b]	7.9

+ Difference between high versus low RFI = High RFI/Low RFI - 1
Means within rows with different superscripts differ significantly (P<0.05)
RE = Retained energy
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

When these same steers were grouped based on individual RFI measurements, there were more low RFI steers than high RFI steers, (n=19 vs. n=14), and the difference in RFI between lines was 0.67 kg/day (Table 6.2) rather than 0.31 kg/day (Table 6.1). This difference in RFI resulted in the low RFI steers consuming 7.76 MJ/day (9.1%) less than the high RFI steers (P<0.05), whilst having no difference in weight at the start of experimentation or weight gain over the period. The actual RFI was only moderately correlated with the sire RFI (*r*=0.35); the sire RFI was positive in both lines and not statistically different between RFI lines (Table 6.2).

Table 6.2: Main effect means and SEM for energetics of *ad libitum* feed intake and body composition of Angus steers following 1 generation of selection for RFI and fed for approximately 140 days. Assigned to high and low RFI based on own RFI measurements.

	Low RFI (n=19)	High RFI (n=14)	Difference[+] (%)
ME-Intake (MJ/day)	85.33 ± 1.18[a]	93.09 ± 1.38[b]	9.1
Sire RFI (kg/day)	0.09 ± 0.11	0.33 ± 0.14	
Individual RFI (kg/day)	-0.25 ± 0.06[a]	0.37 ± 0.05[b]	
Start Weight (kg)	286.74 ± 6.57	289.51 ± 7.66	1.0
Mid Weight (kg)	356.04 ± 6.09	357.09 ± 7.09	0.3
Weight Gain (kg/day)	0.94 ± 0.02[a]	1.03 ± 0.02[b]	9.6
Change Rib Fat Depth (mm/day)	0.05 ± 0.01	0.05 ± 0.01	-0.0
Change EBFW (kg/day)	0.27 ± 0.02	0.28 ± 0.02	3.7
Change EMA (cm^2/day)	0.10 ± 0.01	0.09 ± 0.01	-9.2
Change EBPW (kg/day)	0.09 ± 0.00	0.11 ± 0.01	22.2
RE as Fat (MJ/day)	10.52 ± 0.63	11.12 ± 0.73	5.7
RE as Protein (MJ/day)	2.22 ± 0.11	2.48 ± 0.13	11.7
RE as Fat and Protein (MJ/day)	12.74 ± 0.60	13.61 ± 0.70	6.8
Heat Production (MJ/day)	72.59 ± 1.22[a]	79.47 ± 1.42[b]	9.5
Heat Production of Gain (MJ/day)	13.41 ± 0.44[a]	14.70 ± 0.52[b]	9.6
Heat Production of Maintenance (MJ/day)	59.18 ± 1.39[a]	64.77 ± 1.62[b]	9.5

[+] Difference between high versus low RFI = High RFI/Low RFI - 1
Means within rows with different superscripts differ significantly (P<0.05)
RE = Retained energy
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

Even though there was a significant difference in RFI (and hence ME-intake), there was no difference in the composition of gain between RFI lines if grouped based on their own RFI (Table 6.2). However, the high RFI steers gained 0.09 kg/day more weight (9.6%) than the low RFI steers (P<0.05). No difference was observed in lean body content as measured by eye muscle area or empty body protein gain between the RFI groups. Additionally, no difference was observed in body fat gains as measured by rib fat depth or empty body fat weight gains between RFI lines. This resulted in there being no observable difference in the energy retained as fat or protein over the test period. As a result of a larger difference in ME-intake (Table 6.1 versus Table 6.2) and no difference in the energy retained as protein or fat, the difference in heat production and

HPM was greater. Due to the lower deposition of protein by the low RFI steers, the HPG was 1.29 MJ/day less (9.6%, P<0.05). However, due to a much greater difference of the low RFI steers producing 6.88 MJ/day (9.5%) less heat, these steers had a 5.59 MJ/day (9.5%) less HPM than the high RFI steers (P<0.05). This can be explained by the 9.1% difference in feed intake.

6.3.2 Single trait RFI selection for 2.5 generations: steers

Following 2.5 generations of selection for RFI, the difference in mid-parent RFI estimated breeding value (RFI-EBV) between low and high RFI-EBV steers equated to 1.15 kg/day (P<0.05; Table 6.3). This difference resulted in the low RFI-EBV steers having an 8.61 MJ/day (6.7%) lower ME-intake even though they grew 0.05 kg/day (4.5%) faster than the high RFI-EBV steers. As would be expected, there was no difference in the starting weights of the high and low RFI-EBV steers. However, the low RFI-EBV steers did finish the feedlot period at heavier weights (P<0.05), due to their greater weight gains.

Even though the low RFI-EBV steers had lower ME-intakes, there was no difference between the RFI-EBV lines in the deposition of lean muscle as measured by changes in eye muscle area (Table 6.3). However, modelling body composition showed these steers gained more lean mass as estimated by empty body protein weight in that they deposited 0.01 kg/day (9.9%) more protein than the low RFI-EBV steers (P<0.05). Although the low RFI-EBV steers deposited more lean muscle, they deposited less fat than the high RFI-EBV steers. Deposition of fat was greater in the high RFI-EBV steers, which gained more rib fat depth and empty body fat weight of 0.01 mm/day

(25%) and 0.10 kg/day (30.3%;), respectively, than their low RFI-EBV counterparts (P<0.05).

Table 6.3: Main effect means and SEM for energetics of *ad libitum* feed intake and body composition of Angus steers following 2.5 generations of selection for RFI and fed in a commercial feedlot for 251 days. Assigned to RFI group based on mid-parent RFI-EBVs.

	Low RFI	Medium RFI	High RFI	Difference+ (%)
ME-Intake (MJ/day)	127.92	145.14	136.53	6.7
Mid-parent RFI-EBV (kg/day)	-0.52 ± 0.02[a]	-0.10 ± 0.02[b]	0.63 ± 0.02[c]	
Start Weight (kg)	434.16 ± 3.65[a]	450.75 ± 3.54[b]	430.62 ± 3.65[a]	-0.8
Mid Weight (kg)	573.14 ± 3.90[a]	584.01 ± 3.79[b]	564.69 ± 3.90[b]	-1.5
Weight Gain (kg/day)	1.11 ± 1.02†	1.07 ± 0.02	1.06 ± 0.02†	-4.5
Change Rib Fat Depth (mm/day)	0.04 ± 0.00[a]	0.04 ± 0.00[ab]	0.05 ± 0.00[b]	25.0
Change EBFW (kg/day)	0.33 ± 0.02[a]	0.39 ± 0.02[ab]	0.43 ± 0.02[b]	30.3
Change EMA (cm^2/day)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.0
Change in EBPW (kg/day)	0.12 ± 0.00[a]	0.11 ± 0.00[b]	0.11 ± 0.00[b]	-9.9
RE as Fat (MJ/day)	13.12 ± 0.88[a]	15.28 ± 0.88[ab]	16.78 ± 0.89[b]	27.9
RE as Protein (MJ/day)	2.78 ± 0.05[a]	2.62 ± 0.05[b]	2.51 ± 0.05[b]	-9.7
RE as Fat and Protein (MJ/day)	15.90 ± 0.89[a]	17.91 ± 0.88[ab]	19.29 ± 0.90[b]	21.3
Heat Production (MJ/day)	112.02 ± 0.89[a]	127.23 ± 0.88[b]	117.90 ± 0.90[c]	5.3
Heat Production of Gain (MJ/day)	16.71 ± 0.36	17.08 ± 0.36	17.26 ± 0.36	3.3
Heat Production of Maintenance (MJ/day)	94.34 ± 2.12[a]	109.71 ± 2.11[b]	100.41 ± 2.14[c]	5.0

Means within rows with different superscripts differ significantly (P<0.05)
† Means within rows differ significantly (P<0.10)
+ Difference between high versus low RFI = High RFI/Low RFI - 1
‡ ME-Intake and the Heat Increment of Feeding are simple means and could not be statistically compared as individual animal data were not available.
RE = Retained energy
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

As a consequence of the differences in body composition between the low and high RFI-EBV steers, there were also differences in the energetics of these animals. The low RFI-EBV steers deposited less fat and consequently, retained 3.66 MJ/day (27.9%) less energy as fat than the high RFI-EBV animals (P<0.05). However, due to their greater deposition of protein, they retained 0.27 MJ/day (9.7%) more energy as protein (P<0.05). As the low RFI-EBV steers retained less energy overall, the difference in heat production between the lines was reduced. Despite this, the fat gains of the high RFI

steers was higher and resulted in them depositing 3.39 MJ/day (21.3%;) more energy as fat and protein than the low RFI steers ($P<0.05$). The low RFI-EBV steers consumed 8.61 MJ/day less energy and their heat production was 5.88 MJ/day (5.3%) less than the high RFI-EBV steers ($P<0.05$). There was no difference between RFI lines in the HPG. However, taking into account the efficiency of deposition of fat and protein and due to the greater energy retention of the high RFI-EBV steers through gains in fatness, the HPM was 4.73 MJ/day different (4.96%) between the RFI EBV lines ($P<0.05$). The high RFI steers had a higher requirement for maintenance, activity and heat increment associated with the increase in ME-intake.

6.3.3 Single trait RFI selection for 3.5 generations: heifers

After 3.5 generations of selection for RFI, the difference in mid-parent RFI-EBV of the heifers was 1.42 kg/day between the high and low RFI heifers (Table 6.4). When the feed intake of these heifers was restricted at 105% ME_m, there was no difference between high and low RFI heifers in feed intake (by definition as they were fed on a per weight basis). However, when fed at 180% ME_m (~90% *ad libitum*), the low RFI-EBV heifers ate 3.46% less, resulting in a 2.24 MJ/day difference in ME-intake between the RFI-EBV lines ($P<0.05$) (Table 6.4). This shows a reduction in appetite of these animals. As would be expected, there was no difference in weight at the start of the experiment or weight gain during the experiment between the RFI-EBV lines ($P>0.10$). This was true whether fed at either 105% or 180% ME_m, although weight gain was different between the feeding level treatments. The aim of feeding approximately 90% expected *ad libitum* (180% ME_m) feed intake was that both RFI-EBV lines would eat the same amount. Nevertheless, the low RFI-EBV heifers had a lower *ad libitum* feed intake than expected and hence, ate less than the high RFI-EBV heifers.

Heifers fed at 105% ME_m were not different in body composition changes over the experimental period (Table 6.4). When fed at 180% ME_m, there was no difference between the RFI-EBV lines in the growth of lean muscle over this period, as measured by changes in eye muscle area and empty body protein weight. However, the high RFI-EBV heifers gained 200% (0.04 mm/day) more fat than the low RFI-EBV heifers at this feeding level ($P<0.05$). This resulted in the high RFI-EBV heifers depositing 27.3% (0.09 kg/day) more empty body fat weight than the low RFI-EBV heifers ($P<0.05$).

There was no difference in body composition of heifers fed at 105% ME_m, and thus, energy retained as fat and protein between the RFI-EBV lines was similar (Table 6.4). Due to the similarity in ME-intake, there was no difference in heat production or HPM. Because the high RFI-EBV heifers fed at 180% ME_m deposited more fat, they retained 24.6% more energy as fat (3.42 MJ/day, $P<0.10$). However, even though the fat deposition was not significant at $P<0.05$, this was sufficient to counteract the higher ME-intake of these heifers and consequently, there was no significant difference in heat production or HPM between the RFI lines at this feeding level either.

Table 6.4: Main effect means and SEM for energetics of feed intake and body composition of high and low RFI heifers fed at either 105% of 180% maintenance feeding levels, the result of 3.5 generations of selection for RFI. Assigned to RFI group based on mid-parent RFI-EBVs.

	105% ME_m			180% ME_m			P-Value		
	Low RFI	High RFI	Difference[+] (%)	Low RFI	High RFI	Difference[+] (%)	Nutrition	Genotype	Nutrition x Genotype
ME-Intake (MJ/day)	42.64 ± 0.91	42.91 ± 0.95	0.6	64.83 ± 0.94^a	67.07 ± 0.91^b	3.5	**0.0001**	**0.0027**	**0.0223**
Mid-parent RFI-EBV (kg/day)	-0.78 ± 0.26^a	0.64 ± 0.70^b		-0.78 ± 0.26^a	0.64 ± 0.7^b			**<0.0001**	
Start Weight (kg)	305.35 ± 9.66	285.87 ± 9.66	-6.4	314.13 ± 9.66	295.34 ± 9.66	-6.0	**0.0029**	0.1763	0.8924
Mid Weight (kg)	313.09 ± 9.80	294.97 ± 9.80	-5.8	330.57 ± 9.80	311.34 ± 9.80	-5.8	**<0.0001**	0.1973	0.7010
Weight Gain (kg/day)	0.40 ± 0.08	0.49 ± 0.08	22.0	0.89 ± 0.08	0.88 ± 0.08	-1.1	**0.0002**	0.6058	0.5349
Change Rib Fat Depth (mm/day)	0.01 ± 0.01	0.02 ± 0.01	100.0	0.02 ± 0.01^a	0.06 ± 0.01^b	200.0	**0.0089**	**0.0846**	**0.0417**
Change EBFW (kg/day)	0.12 ± 0.05	0.15 ± 0.06	25.0	0.33 ± 0.06	0.42 ± 0.05	27.3	**0.0003**	0.8689	**0.0615**
Change EMA (cm2/day)	0.09 ± 0.04	0.11 ± 0.04	22.2	0.15 ± 0.09	0.14 ± 0.04	-6.7	0.4731	0.9677	0.1717
Change EBPW (kg/day)	0.08 ± 0.01	0.09 ± 0.01	12.5	0.13 ± 0.01	0.16 ± 0.01	23.1	**0.0005**	0.3469	0.3465
RE as Fat (MJ/day)	4.6 ± 2.05	5.22 ± 2.14	13.5	13.82 ± 2.13	17.24 ± 2.05	24.8	**<0.0001**	0.4275	**0.0555**
RE as Protein (MJ/day)	1.73 ± 0.27	1.89 ± 0.28	9.3	3.3 ± 0.28	3.7 ± 0.27	12.1	**<0.0001**	0.3680	0.1896
RE as Fat and Protein (MJ/day)	6.33 ± 2.31	7.11 ± 2.41	12.3	17.12 ± 2.40	20.94 ± 2.30	22.3	**<0.0001**	0.4262	**0.0636**
Heat Production (MJ/day)	36.25 ± 2.52	35.88 ± 2.64	-1.4	47.58 ± 2.64	46.18 ± 2.52	-3.3	**<0.0001**	0.7903	0.7737
Heat Production of Gain (MJ/day)	8.91 ± 1.91	9.75 ± 1.99	9.4	19.13 ± 19.8	22.20 ± 1.91	16.0	**<0.0001**	0.3966	0.5198
Heat Production of Maintenance (MJ/day)	27.33 ± 4.35	26.15 ± 4.54	-4.6	28.44 ± 4.53	23.99 ± 4.35	-16.3	0.8809	0.6213	0.6523

[+] Difference between high versus low RFI = High RFI/Low RFI - 1
Means within rows with different superscripts differ significantly ($P<0.05$)
RE = Retained energy
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

6.3.4 Comparison of selection lines

Heat production and HPM changed as a result of selection for RFI (Table 6.5). After 1 generation of selection for RFI, the proportion of ME-intake that could be explained by variation in heat production was 0.85 for both the low and high RFI steers. Albeit, the proportion of ME-intake that was explained by the HPG was greater in the low RFI steers due to a greater protein deposition. However, HPM explained more of the variation in ME-intake in the high RFI line (0.70) than the low RFI line (0.68). Subsequent to 2.5 generations of selection for RFI, the variation in heat production and HPM explained less of the variation in ME-intake of the high RFI line with no difference in the proportion of ME-intake explained by the HPG. The proportion of HP and HPM differences became even greater following 3.5 generations of selection for RFI due to the greater HPG in these genotypes as a consequence of a much greater fat deposition.

Table 6.5: Heat production, HPG and HPM of high and low RFI animals genetically divergent in RFI after 1, 2.5 and 3.5 generations of selection.

	Low RFI	High RFI	Difference[+]
Heat production proportion of ME-Intake			
Generation 1 steers	0.85	0.85	0.00
Generation 2.5 steers	0.88	0.86	-0.02
Generation 3.5 heifers fed at 105% ME_m	0.85	0.83	-0.02
Generation 3.5 heifers fed at 180% ME_m	0.74	0.69	-0.05
HPG proportion of ME-Intake			
Generation 1 steers	0.17	0.15	-0.02
Generation 2.5 steers	0.13	0.13	-0.00
Generation 3.5 heifers fed at 105% ME_m	0.21	0.23	0.02
Generation 3.5 heifers fed at 180% ME_m	0.30	0.33	0.03
HPM proportion of ME-Intake			
Generation 1 steers	0.68	0.70	-0.02
Generation 2.5 steers	0.74	0.73	-0.01
Generation 3.5 heifers fed at 105% ME_m	0.64	0.61	-0.03
Generation 3.5 heifers fed at 180% ME_m	0.44	0.36	-0.08

[+] Difference between high versus low RFI = High RFI - Low RFI

6.3.5 Modelling residual feed intake

The Davis growth model was used to simulate RFI based on differences in feed intake and hence, growth rate (Table 6.6). This simulation represents the average steer in a group of steers during a 70-day test. The simulated difference in feed intake between a low feed intake steer and a high feed intake steer in actual feed intake was 1.93 kg/day. However, due to the difference in weight gain, the contrast between the expected feed intakes of a low and a high feed intake steer were much smaller (8.6%) than the actual feed intake difference (20.5%). This generated a difference in RFI between high and low feed intake steers of 1.07 kg/day. Consequently, the low feed intake steers had a higher (more favourable) feed conversion ratio (and RFI) than the high feed intake steers.

The model output predicts higher growth in the high feed intake steer rather than the low feed intake steer, as these model predictions are based on differences in feed intake only. The high feed intake steer grew 0.25 kg/day (22.7%) faster than the low feed intake steer. Therefore, the high feed intake steer deposited more fat and lean muscle as empty body fat weight and empty body protein weight than the low feed intake steer. However, the deposition of fat was greater than the deposition of protein in that the high feed intake steer deposited 0.24 kg/day (31.1%) more fat but only 0.02 kg/day (14.1%) more protein than the low feed intake steer.

These differences in the deposition of fat and protein resulted in the high feed intake steer depositing 9.39 MJ/day more energy as fat and 0.35 MJ/day more energy as protein than the low feed intake steer (Table 6.6). In total, the high feed intake steer deposited 9.74 MJ/day (29.8%) more. Even though the high feed intake steer retained

more energy than the low feed intake steer, it still produced 11.49 MJ/day (16.6%) more heat as a result of this much greater energy intake. However, due to the efficiency of deposition of protein versus fat, the HPM of the high feed intake steer was 6.08 MJ/day (12.7%) more than the low feed intake steer. This was due to a 5.41 MJ/day (23.8%) greater HGP of the high feed intake steer.

Table 6.6: Davis growth model predictions of energy intake, feed efficiency, body composition and energy retention responses to a ±10% change in feed intake over a typical 70 day test period.

	Low FI†	Medium FI	High FI‡	Difference+ (%)
Actual Feed Intake (kg/day)	9.40	10.40	11.33	20.5
Expected Feed Intake (kg/day)	9.96	10.40	10.82	8.6
ME-Intake (MJ/day)	103.40	114.40	124.63	20.5
RFI (kg/day)	-0.56	0.00	0.51	
FCR (kg/kg)	8.40	8.32	8.25	-1.8
Start Weight (kg)	300.00	300.00	300.00	0.0
Mid Weight (kg)	339.16	343.74	348.06	2.6
Weight Gain (kg/day)	1.12	1.25	1.37	22.7
Change EBFW (kg/day)	0.77	0.89	1.01	31.1
Change EBPW (kg/day)	0.10	0.11	0.12	14.1
RE as Fat (MJ/day)	30.18	35.01	39.57	31.1
RE as Protein (MJ/day)	2.46	2.64	2.81	14.1
RE as Fat + Protein (MJ/day)	32.64	37.65	42.38	29.8
Heat Production (MJ/day)	70.76	76.75	82.25	16.3
Heat Production of Gain (MJ/day)	22.78	25.57	28.19	23.8
Heat Production of Maintenance (MJ/day)	47.98	51.18	54.06	12.7

FI = Feed intake; FCR = Feed conversion ratio; RE = Retained energy.
+ Difference between high versus low FI = High FI/Low FI - 1
† Low FI = 0.9 x Medium FI
‡ High FI = 1.1 x Medium FI
RE = Retained energy
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

6.4 Discussion

The feed intakes from steers 1 generation divergent in RFI were different based on whether the divergence in RFI is determined from their sire's RFI (Table 6.1; as reported by Richardson *et al.* (2001)) or their own individual RFI (Table 6.2; as

reported herein). There was approximately half of the divergence in RFI when grouped by sire compared to that based on individual RFI (Tables 6.1 and 6.2, respectively). RFI has a heritability of 0.39±0.03 to 0.44±0.07 as calculated in this population (Arthur *et al.*, 1997, Arthur *et al.*, 2001b). However, 41.2% of the high RFI sires had progeny with low RFIs and 25.0% of the low RFI sires had progeny with high RFIs. The genetic (sire) and phenotypic (individual) discrepancies in RFI were large; the genetic divergence in ME-intake between high and low RFI steers was only 4.40 MJ/day (Table 6.1), but the phenotypic divergence in RFI was 7.76 MJ/day (Table 6.2).

The deposition of protein and fat was additionally affected depending upon whether these animals were grouped based on genetic or phenotypic differences. Progeny of sires genetically divergent in RFI deposited more protein, as indicated by changes in EMA and EBPW, with respect to fat ($P<0.05$) (Table 6.1). As discussed by Richardson *et al.* (2001), the low RFI animals deposit more protein in general agreement with the early work by Richardson *et al.* (2001). Herd and Bishop (2000) reported a moderate negative genetic correlation between RFI and carcass lean content of British Hereford cattle(r_g=-0.43), although the phenotypic correlation was low (r_p=-0.22). In the Trangie Angus selection lines, early estimates of genetic and phenotypic correlations between eye muscle area and RFI were low (r_g=0.12 and r_p=0.03) and with large standard errors, suggesting no correlation at all (Arthur *et al.*, 2001b). The phenotypic divergence in RFI presented herein (Table 6.2) showed no statistically significant differences in the deposition of protein and is in agreement with Arthur et al. (2001b) and the more recent work herein (as discussed in-depth in Chapter 3). Moreover, the phenotypic divergence in RFI did not result in significant differences in the deposition of fat between high and

low RFI phenotypes observed when the animals were grouped genetically based on their sire EBVs (Chapter 3).

Heat production was greater in the high RFI steers regardless of whether they were grouped genetically or phenotypically (Tables 6.1 and 6.2, respectively). Nevertheless, the difference in heat production was more between RFI lines when grouped based on phenotypic RFI as the ME-intake difference was much larger. A similar pattern for HPM was observed. However, the HPG was lower in the high RFI steers when grouped genetically, but greater when grouped phenotypically based on the differences in body composition between these groupings. Richardson *et al.* (2001) reported that HPM was not significantly affected between the RFI lines. This discrepancy between the results presented herein can be explained by the error in the estimation of residual heat production in the calculation by Richardson *et al.* (2001).

Genetic selection for RFI has resulted in a divergence in ME-intake in successive generations. After 1 generation of selection for RFI, the differences in RFI were -0.15 kg/day for the low RFI steers and 0.16 kg/day for the high RFI (Table 6.1). This resulted in a difference in ME-intake of 5.1% in these growing animals with a mid-weight of ~357 kg. This difference became greater such that subsequent to 2.5 generations of selection for RFI, the difference in mid-parent RFI-EBV between the high and low RFI lines was -0.52 and 0.63 kg/day, respectively. In the feedlot, this divergence in RFI resulted in a 6.7% difference in the ME-intake of long-fed steers approaching mature weights with a mid-weight of ~569 kg. After 3.5 generations of selection for RFI, the divergence in mid-parent RFI-EBV was -0.78 kg/day and 0.64 kg/day for the low and high RFI heifers (Table 6.4). When fed at approximately 90% of

predicted *ad libitum* requirements (180% ME_m), the low RFI heifers attained an *ad libitum* ME-intake at 86.9% of predicted *ad libitum* requirements based on SCA (1990). It is, therefore, not unreasonable to suggest that the differences in predicted *ad libitum* ME-intake after 3.5 generations of selection would be greater than in previous generations.

The results from heifers following 3.5 generations of selection for RFI provide evidence that most, if not all, of the variation in heat production can be accounted for by the amount of energy consumed, and that heat production per unit of metabolisable energy-intake did not differ between the RFI selection lines (Chapter 3). Therefore, most of the differences in ME-intake between the selection lines must be due to the differences in energy retained between these genotypes. After 1 generation of selection for RFI, Richardson and Herd (2004) hypothesised that approximately 5% of the difference in ME-intake between high and low RFI genotypes could be attributed to differences in body composition with the remainder due to differences in heat production. The results herein (Table 6.1) concur with the outcomes from Richardson and Herd (2004) in that there were no statistical differences in fat gain or energy retained as fat between these genotypes; there was only a small yet significant difference in the gain of protein where the low RFI animals deposited more protein with respect to fat ($P<0.05$).

This early hypothesis of Richardson and Herd (2004) that only a small proportion (5%) of the difference between RFI lines can be attributed to differences in body composition did not hold true in the subsequent generations where there were much larger differences in body composition between the high and low RFI genotypes. After 2.5 generations of selection for RFI, there was a small but significant increase (9.9%) in the

amount of protein deposited, and hence, the energy retained as protein in the low RFI animals (Table 6.3). This difference was not seen in the EMA but was observed in the changes in EBPW. There was a much larger difference (30.3%) in the amount of fat gained, and hence, energy retained as fat by the high RFI genotype animals that are approaching maturity. After 3.5 generations of selection for RFI, no differences were observed in body composition or energy retained as fat and protein between the high and low RFI genotype animals fed at maintenance (105% ME_m). When fed at 180% ME_m, there was no difference in the amount of protein deposited between the animals. However, the high RFI genotype animals deposited 200.0% more subcutaneous fat and 27.3% more EBFW and as a consequence, retained more energy.

It was observed herein, therefore, that selection for low RFI has resulted in (at best) a small increase in the deposition of protein, but this is dwarfed by the decrease in the deposition of fat. It would appear that selection for RFI has decreased fat deposition with successive generations in the low RFI animals. The results herein (and in Chapter 3) indicate that it would be prudent to include fat deposition into the prediction equations for RFI. Previously, many authors (Basarab *et al.*, 2003, Schenkel *et al.*, 2004, van der Werf, 2004, Knott *et al.*, 2008, Kelly *et al.*, 2010) have suggested that including fatness (usually subcutaneous) into the genetic (statistical) models to predict RFI may improve the ability to select for animals with improved energetic efficiency.

In Chapter 3, it was concluded that most, if not all, of the differences in ME-intake could be accounted by the differences in energy retained. If selection for RFI results in changes in ME-intake and hence, body composition (and therefore energy retained), it would follow that the energetics of these animals may be changing with each generation

of selection. There was no difference in the efficiency of energy utilisation for protein deposition as there was no difference in protein turnover between high and low RFI animals after 3.5 generations of selection for RFI (Chapter 2). Subsequent to 1 generation of selection for RFI, the low RFI animals produced 4.9% less heat (P<0.05). Taking into account the differences in the efficiency of energy use for the deposition of fat and protein, the HPM (equal to maintenance and energy requirements as well as the heat increment of feeding) was 7.9% higher in the high RFI animals (P<0.05). This is due to the low RFI animals having retained more energy as protein.

These changes in heat production and HPM became smaller with successive generations of selection for RFI, whereas the HPG became greater with successive generations. Whilst heat production and HPM was still significantly lower in the low RFI genotype after 2.5 generations of selection for RFI, there was no difference in the heat production or HPM subsequent to 3.5 generations of selection for RFI. As reviewed (in Chapter 3), the literature consistently shows (where there are data) that animals with a low RFI phenotype do not have reduced maintenance requirements (Gabarrou *et al.*, 1997, Richardson *et al.*, 2001, Basarab *et al.*, 2003, Castro Bulle *et al.*, 2007, Lancaster, 2008, Boddicker *et al.*, 2011a, Boddicker *et al.*, 2011b). Additionally, genetic selection for RFI appears to have no effect on maintenance energy requirements across a range of species, including cattle (Richardson *et al.*, 2001), chickens (Gabarrou *et al.*, 1997) and pigs (Boddicker *et al.*, 2011a, Boddicker *et al.*, 2011b).

Whilst a small difference in the HPM of animals divergent in RFI by 1 and 2.5 generations can be explained by the heat increment of feeding ($HEI = 0.09 \times MEI$; (SCA, 1990, NRC, 2000), it is possible that the remaining differences in HPM can be

explained by differences in the activity between the high and low RFI animals. Both of the experiments utilising animals divergent in RFI by 1 and 2.5 generations of selection for RFI were under feedlot type conditions and animals were able to expend some energy during activity related heat production. The heifers subsequent to 3.5 generations of selection for RFI could not show differences in activity as the experiment was conducted in metabolism crates. Luiting's (1990) review of the genetic variation of energy partitioning in laying hens and causes of variation in RFI concluded that 9-33% of the variation in heat production could be attributed to variation in activity. However, it must be noted that the relative importance of activity towards total heat production is much less for ruminants than that of laying hens. Nevertheless, Amdi *et al.* (2010) showed that sheep exhibiting a high behavioural reactivity response (and hence, higher activity levels) have lower RFI than sheep with low behavioural reactivity responses. Whilst not significant, there was a trend for the sires of steers 1 generation divergent in RFI (herein) to be divergent in activity during their RFI test ($P<0.10$). Moreover, the low RFI bulls had lower activity levels (Richardson *et al.*, 1999). The correlation between average daily pedometer count ($r_p=0.24$) and RFI was significant ($P<0.05$) (Richardson *et al.*, 1999). Thus, it may be hypothesised that activity is associated with variation in RFI (Chapter 7).

Regardless of the role of activity in RFI, selection for low RFI has not exerted selection pressure on the energy metabolism of these genotypes by lowering heat production or HPM. As a proportion of ME-intake, heat production and HPM is reduced in high RFI animals (Table 6.5). This became less in the low RFI animals after successive generations of selection for RFI. The conclusion that can be drawn is that selection for RFI has put selection pressure on traits other than those that influence maintenance

requirements. Based on the results herein (Chapters 2, 3 and 5) and the results of others, selection for low RFI has resulted in a reduction in appetite, at constant weight and weight gain, and as a result a leaner phenotype.

In contrast, the simulation of feed intake indicated that there should be differences in heat production of low and high feed intake steers. Animals that eat more, even with the same energetics as observed in growth and body composition model (Table 6.6), inherently have a higher RFI. The high feed intake simulation "steer" grew faster and hence, deposited more protein and much more fat. In the simulation, the difference in fat deposition generated the differences in RFI as fat is approximately five time more energy dense than lean muscle tissue (ARC, 1980). The high feed intake simulation steer still had greater heat production, HPG and HPM than the low feed intake simulation steer. A portion of this greater heat production would be due to the heat increment of feeding associated with the greater feed intakes, which in turn, leads to faster weigh gain, and hence, higher maintenance requirements of the high feed intake simulation steer. The higher HPG of the high feed intake steer was due to greater deposition of protein and fat than the low feed intake steer. However, if a simulation was performed at constant weight, weight gain and composition of gain, these differences in heat production and HPM would be (by definition) non-existent between the low and high feed intake simulation steers due to the assumptions within the model. Nevertheless, this simulation clearly shows that a substantial difference in RFI can be generated by only altering feed intake. Additionally, by altering feed intake (appetite) at constant weight and weight gain, such as is the case for RFI regressions, a greater difference in RFI and body composition can be expected.

Selection for RFI shows a positive correlation with FCR (Arthur *et al.*, 1997, Arthur *et al.*, 2001b, Arthur *et al.*, 2001c). In the simulated feed intake results, FCR and RFI had a negative correlation (r=-1.0). This is not expected from the literature associated with selection for RFI. However, from the growth models, the proportion of maintenance requirements is lower relative to the energy requirements for maintenance and growth (ME-intake) of animals that eat more. Further, as appropriate to higher weight gains, FCR was lower.

Nevertheless, direct selection for feed conversion ratio as an alternative to RFI has potential drawbacks. One issue is that FCR is highly correlated with average daily gain. This suggests that selection for high growth (or selection on lean growth as discussed in Robinson and Oddy (2004)) is much more cost-effective than measuring individual feed intake. FCR would also tend to select for animals with greater lean mass and less fat deposition as the energy content of protein is far less that of fat. The water content associated with lean tissue versus fat is less, and therefore, it requires less energy to deposit 1kg lean muscle than 1kg fat tissue. Additionally, selection for increased FCR results in increased mature size. Increasing the size and energy requirements of cows is not a goal of most commercial operations.

Although RFI is an alternative to genetic selection for FCR, the results herein and elsewhere suggest that selection for RFI (low RFI = low feed intake) changes the composition of gain through a decrease in fatness. It may be that selection for RFI is no better than selection for FCR due to the correlated responses with other traits.

6.5 Conclusions

Theory suggests that RFI can be used for genetic selection with much more confidence in beef production systems than FCR as it should not be correlated with average daily gain, body weight or mature size. Initially, it was believed that a reduction in RFI would enable a decrease in basal metabolism. However, the results defined here indicate that the emphasis of selection pressure has been on the composition of gain and not the maintenance requirements of the animal. Selection for low RFI in the Trangie beef cattle selection lines has resulted in decreased feed intake and fatness, and the most likely mechanism being a reduction in appetite. This decrease in fatness associated with finishing animals may mean that they attract penalties for insufficient fat cover. In the context of the breeding herd, a reduction in appetite and hence, fatness may also result in a reduction in reproductive performance.

Chapter 7

Phenotypic relationships between body composition, ME-intake and energetics of cows and calves from diverse fat genotypes and implications for residual feed intake

CHAPTER 7: Phenotypic relationships between body composition, ME-intake and energetics of cows and calves from diverse fat genotypes and implications for residual feed intake

7.1 Introduction

The results herein have shown that selection for low residual feed intake causes a decrease in appetite and hence, a decrease in *ad libitum* feed intake (Chapters 2-6). This selection of appetite at constant weight and daily gain has an unintended consequence of altering the body composition of these animals. Consequently, it has been observed that low RFI animals have less fat than high RFI animals (Chapters 2, 3, 5 and 6). There may also be a small increase in the deposition of protein in the low RFI animals, but this is dwarfed by the decrease in the deposition of fat in these same animals. It would appear that the divergence in RFI is associated with a divergence in fat deposition in these low RFI animals with successive generations.

These conclusions are supported by the literature, which suggests that residual feed intake and fatness are correlated (Arthur *et al.*, 2001b, Basarab *et al.*, 2003, Richardson and Herd, 2004, Robinson and Oddy, 2004, Kelly *et al.*, 2010), especially when measured as gains over the RFI test period (Nkrumah *et al.*, 2004, Lancaster *et al.*, 2009, Kelly *et al.*, 2010). The jury is still out as to whether or not these correlations still exist at maturity. It is also unclear whether leanness is associated with RFI. To address this issue, additional data were analysed to answer the following questions. If selection

for low residual feed intake decreases appetite and as a consequence, reduces the deposition of fat, does the opposite hold? Do genetically leaner animals have a lower appetite, and hence, a lower RFI as compared to fatter animals?

7.2 Materials and Methods

7.2.1 Animals

The animals utilised in this experiment were part of the Beef CRC Maternal Efficiency Project (Pitchford *et al.*, 2013). The experiment was designed to assess maternal productivity in two differing genotypic backgrounds for fat and RFI. These genotypes were fed at two levels of nutrition across three parities in two southern Australian sites, Vasse Research Centre near Busselton, WA and Struan Research Centre near Naracoorte, SA. The genetic groups comprised high and low fat animals based on EBVs from the industry and the high and low RFI selection lines from the Trangie Agricultural Centre. However, only the high and low fat cows from the 1st and 2nd parities at the Struan site were utilised here as data were not available on the 3rd parity at the time of analysis.

The Angus heifers (n=240, Table 7.1) were selected for high and low rib fat depth based on mid-parent EBVs from Breedplan (Graser *et al.*, 2005). These heifers were purchased from autumn calving Breedplan recorded Angus herds (n= 13) and were 2006 "B" and 2007 "C" drop heifers (Table 7.1). These were joined the following year at the Struan Research Station. These high and low fat heifers represented the top and bottom 10% of the Angus breed, respectively; the high fat heifers had a mid-parent EBV for rib fat of > +0.8mm, and the low fat heifers were < −0.8mm. These heifers were also purchased to match growth EBVs between fat lines (Table 7.1). Both the high

and low fat heifers were sourced from the same property to avoid confounding the fat mid-parent EBV with the property of origin.

7.2.2 Treatment allocation

In 2007, 75 high fat heifers born in 2006 were allocated to 7 high nutrition groups of 5 animals and 5 low nutrition groups of 8 animals each. Seventy-five low fat heifers were allocated to separate groups in a similar manner. In 2008, a further 4 high nutrition groups and 3 low nutrition groups of high fat and low fat heifers born in 2007 were allocated. Heifers were eligible for inclusion in the experiment whether or not they became pregnant in the first year of mating. It should also be noted that all fat line heifers were yearlings at the time of their first mating.

Table 7.1: Data for fat lines heifers joined at Struan Research Centre by year and genetic group based on their mid parent EBVs.

	Low Fat	High Fat	Total
Number of fat line heifers			
2007 (2006 “B” drop)	75	75	150
2008 (2007 “C” drop)	45	45	90
Total Cows	120	120	240
Mid-parent EBVs of fat line heifers			
Rib Fat (mm)	-1.55 ± 0.06	0.76 ± 0.06	
P8 Fat (mm)	-1.57 ± 0.07	1.01 ± 0.07	
EMA (mm)	2.10 ± 0.14	2.30 ± 0.18	
Birth Weight (kg)	2.27 ± 0.15	1.19 ± 0.13	
200 Day Weight (kg)	22.28 ± 0.6	17.47 ± 0.59	
400 Day Weight (kg)	36.06 ± 0.88	30.87 ± 0.93	
600 Day Weight (kg)	43.67 ± 1.21	37.09 ± 1.28	
Retail Beef Yield (kg)	0.83 ± 0.05	-0.43 ± 0.06	
Mature Cow Weight (kg)	40.39 ± 1.54	28.36 ± 1.53	
Days to Calving (days)	-1.79 ± 0.16	-3.05 ± 0.15	
Milk (kg)	5.64 ± 0.30	5.32 ± 0.37	

Two periods were used to assess weight, body composition, apparent feed intake, energy retention and metabolisable energy requirements of the animals (Table 7.2).

These periods were from 1) weaning to the start of mating and 2) the start of mating to weaning. These periods were used for two reasons. Firstly, they represent the approximate peaks in rib fat depth (Figure 7.1) and cow weights (Figure 7.2) at weaning and the trough in cow rib fat depth and weight at the start of mating. Secondly, they were the time periods when the best data were available from a commercial scanner for body composition traits. The data from these periods clearly demonstrated the repeatable genetic and nutrition effects on all cows whilst allowing for seasonal variation (Figures 7.1 and 7.2).

Table 7.2: Experimental dates for B and C drop heifers for calving, mating and weaning in supplementary feeding and grazing periods.

	2007	2008	2009	2010
		B and C Drop Heifers		
Start of calving		14-Mar	16-Mar	19-Mar
End of calving		26-May	3-Jun	29-May
Start of mating	13-Jun	17-Jun	17-Jun	18-Jun
End of mating	15-Aug	18-Aug	23-Aug	19-Aug
Weaning		26-Nov	17-Nov	29-Nov
		B Drop Heifers		
Supplementary feeding				
Start		17-Jan	2-Dec	13-Jan
End		18-Aug	23-Aug	22-Aug
Grazing				
Start	16-Aug	19-Aug	24-Aug	23-Aug
End		16-Jan	1-Dec	12-Jan
		C Drop Heifers		
Supplementary feeding				
Start		17-Jan	6-Jan	13-Jan
End		5-Aug	25-Aug	22-Aug
Grazing				
Start		6-Aug	24-Aug	23-Aug
End			5-Jan	12-Jan

7.2.3 Animal Measurements

Heifers and cows were joined for 9 weeks to bulls with low birth weight EBVs to minimise calving difficulties (Table 7.2). Mating was conducted in single sire groups

with bulls rotated every two weeks. Non-pregnant heifers and cows were not culled. Heifers and cows were pregnancy tested (via rectal palpation) approximately 2 weeks subsequent to the mating period.

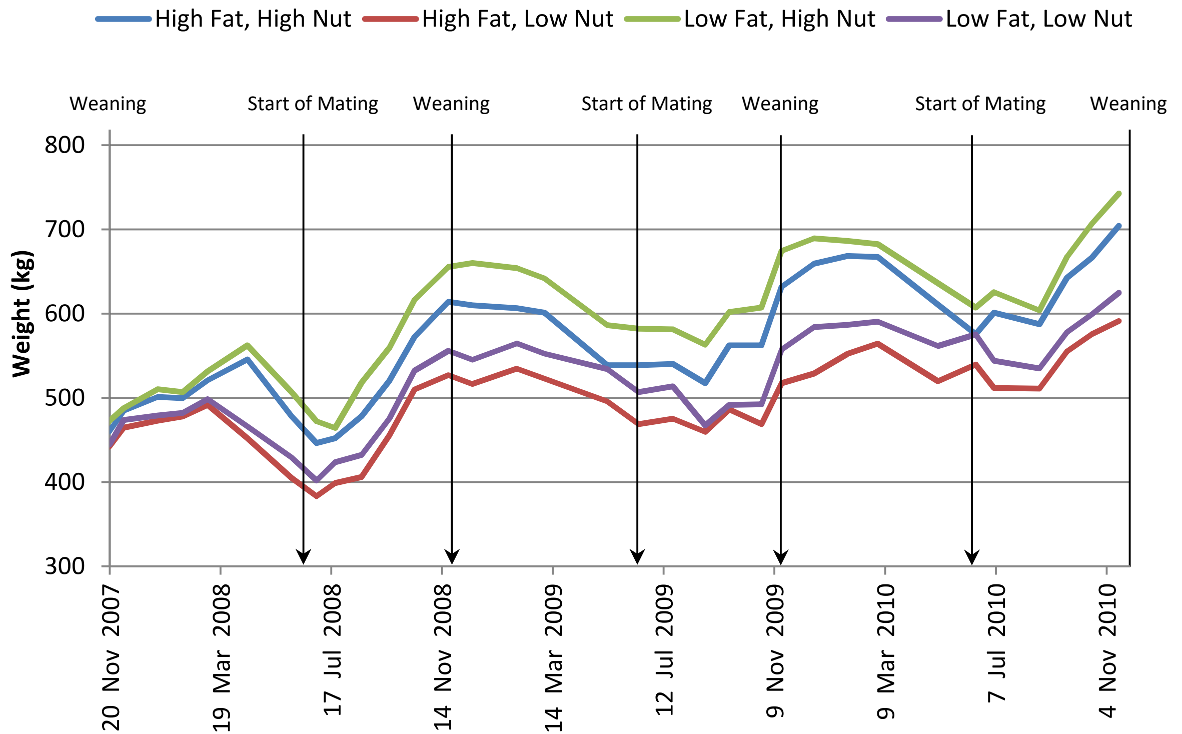

Figure 7.1: Weight of B drop cows at Struan Research Station, representative of seasonal changes in body weight and the critical dates of weaning and start of mating during three calving periods of all cow cohorts.

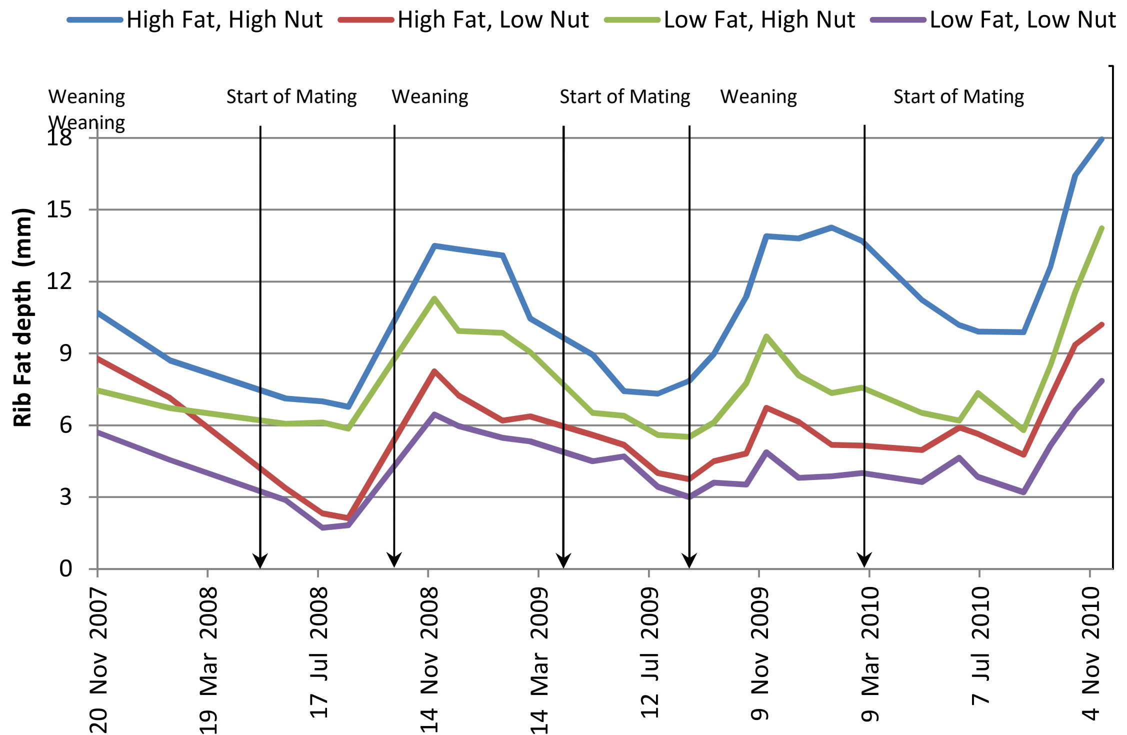

Figure 7.2: Rib fat depths of B drop cows at Struan Research Station, representative of seasonal changes in body composition and the critical dates of weaning and start of mating during three calving periods of all cow cohorts.

Cows were weighed fortnightly and composition (rib and P8 fat scans) was monitored at least bi-monthly. Ultrasound scans for intramuscular fat (IMF %), rib fat, P8 fat and eye muscle area (EMA) were conducted at the start of mating and at calf weaning by an accredited ultrasound scanner. The start of mating scan was chosen to better capture the "trough" in body condition than scanning at pre-calving (Figure 7.1 and Table 7.2). Calves were weighed at birth and fortnightly until weaning. No ultrasound measurements of body composition were taken on the calves.

7.2.4 Feeding

At the end of the spring growing period, calves from every treatment group were weaned (Table 7.2). Cows remained on the TechnoGrazing^{TM} (Kiwitech Int. Ltd., 2001) system until pasture availability had fallen to a level below that required to maintain the

nutritional treatment. This period varied depending on the genotype, year and season (Table 7.2).

After removal from the TechnoGrazing™ system, cows were grouped for supplementary feeding by genotype treatment, nutritional treatment and year of birth. Groups were placed in paddocks with negligible feed on offer and fed a daily silage/straw based ration prepared on site using a feed wagon system. As mated heifers, the ration fed was formulated with allowance for growth (approx 0.1kg/day for low nutrition and 0.3kg/day for high nutrition). From 3 years of age onwards, the ration was formulated to maintain body condition (condition score 2 to 2.5 for low nutrition and 3 to 3.5 for high nutrition (Graham and Clark, 1984)) using the average condition score of the group. Rations were adjusted based on animal performance and physiological status (pregnant/lactating). For low nutrition, the quantity fed was increased for the whole group if any individual animal fell below condition score 2. For high nutrition, the quantity of feed was increased if any individual animal fell below condition score 2.5. The weight of supplement feeding for each group was recorded daily.

During the 9 week mating period, cows remained grouped by genotype treatment (low fat or high fat), nutritional treatment (high or low) and year of birth. During this period, cows were fed a hay based ration in addition to grazing pasture. Pasture availability throughout the mating period at Struan was limited due to low pasture growth rates during winter.

Animals were grazed from the end of mating (August) until weaning (late November) on the TechnoGrazing™ system at Struan Research Centre using small plots for each

replicate of animals. The Dryland TechnoGrazing™ area comprises 6x32 hectares, each of which consists of 8x4 hectare permanent lanes that can then be subdivided into further 90x0.067 hectare cells using temporary electric fencing.

Groups were randomly allocated to a lane on the TechnoGrazing™ area. Overall feed availability was adjusted by allocating an appropriate number of 0.067 ha cells to match feed requirements as estimated by SCA (1990). This was determined by the quantity of feed on offer, feed growth rate and animal performance. The initial allowance was based on meeting the maintenance requirements for the low nutrition group. A 20% difference in intake between the high and low nutrition group was achieved through the difference in numbers of animals (5 vs. 8) between treatments. On-going adjustments to the grazing area were then made to maintain the appropriate condition score of the animals (3-3.5 for high nutrition and 2-2.5 for low nutrition). Animals were generally moved to a fresh allocation of pasture every two days during the spring growing period. Every two weeks during the grazing period, visual estimates of Feed On Offer (FOO) in kg DM/ha (Lodge, 1998) were made on each lane after the cows had been shifted to a fresh pasture allocation. Estimates were made of the FOO both in front of the cows (pre-grazing) and grazing residual behind the cows as a measure of pasture disappearance. Due to the short period of time between each shift, this could be equated to an estimation of apparent pasture intake for the group of animals in their respective lane. However, it should be noted that this technique does not account for losses from trampling, pugging etc.

Apparent pasture intake for each paddock in each grazing period (kgDM/head/day) was calculated as:

$$Apparent\ Pasture\ Intake\ (kgDM/head/day) = \frac{A_j(DM_{i+1,j} - DM_{i,j})}{d_i n_{ij}}$$

Where:

$DM_{i,j}$ is the estimated FOO (kgDM/ha) in paddock j in grazing period i, A_j is the area of paddock j, d_i is the number of days in the grazing period i, and n_{ij} is the number of animals in the herd group in paddock j in the grazing period i (5 high nutrition or 8 low nutrition).

Preliminary analysis of the intake data found that the weekly intake measurements were not normally distributed, but were skewed to the right indicating a greater likelihood of measurement error overestimating intake rather than underestimating intake. Thus, it was decided to log-transform the intake values (kg DM/d or MJ ME/d) before summing them over the period of interest (e.g. between scan dates). On the log-scale, there were very few obvious outliers. The effect of the transformation was to lower the impact of high weekly estimates of the mean and total intake values.

Feed quality assessments were regularly undertaken so that the total MJ of ME fed could be estimated. Pasture samples were collected monthly for quality testing (percentage dry matter (DM%), estimated digestibility, calculated metabolisable energy (ME), percentage crude protein (CP%), percentage neutral detergent fibre (NDF%) and percentage acid detergent fibre (ADF%)) through near infrared spectroscopy (NIR) analysis. Pasture toe cuts were collected from each system from plots prior to grazing (Cayley and Bird, 1991). Sampling time was adjusted to adequately capture the change in growth from spring to summer. For supplementary feeding, the feed quality was

regularly measured. Measurements were also taken when rations were changed or new feed sources purchased.

7.2.5 Modelling body composition

Body composition net of weight associated with the gravid uterus, in the form of empty body fat weight (EBFW) and empty body protein weight (EBPW), of the cow was estimated from the standard reference weight (SRW) and rib fat depth using the model of Williams and Jenkins (1998).

The SRW of the cow was estimated as the weight of the cow minus the weight associated with gestation.

$$SRW\ (kg) = Live\ Weight - \ GU_{Weight}$$

Where:

GU_{Weight} is the weight of the gravid uterus (Uterus + Foetus + Foetal membranes + Uterine fluids)

The weight of the gravid uterus GU_{weight} was calculated as follows (Ferrell *et al.*, 1976a) with on adjustment for calf birth weight (CBW).

$$GU_{Weight}\ (kg) = \ 0.7439e^{0.02t-0.0000143t^2} \times \frac{CBW}{40.7}$$

Where:

t is the day of gestation.

However, this equation assumes a calf birth weight (CBW) at parturition (285 days) of 40.7 kg. Therefore, multiplying by the actual $CBW/40.7$ adjusts for the weight of the gravid uterus due to differences in CBW.

The protein content of the gravid uterus GU_p was calculated as follows (Ferrell *et al.*, 1976a) with the adjustment for CBW.

$$GU_p\ (kg) = 0.002313e^{0.0278t-0.0000176t^2} \times 6.25 \times \frac{CBW}{40.7}$$

Where:

t is the day of gestation.

7.2.6 Modelling energy requirements

Metabolisable energy intake (MEI) in the cow was described as follows

$$MEI = ME_m + ME_g + ME_c + ME_l$$

Where:

MEI is the sum of the metabolisable energy (ME) requirements for maintenance (ME_m), for the growth of the cow (ME_g), for the growth of the gravid uterus (ME_{gu}) and for milk production (ME_l).

ME_m can be estimated by rearrangement of the previous equation such that

$$ME_m = MEI - ME_g - ME_c - ME_l$$

However, the ME requirement for gain (ME_m) was calculated as follows

$$ME_m = \frac{NE_m}{k_m}$$

Where:

The generic equation for the net energy requirements for maintenance (NE_m) (ARC, 1980) is as below and k_m is the efficiency of utilisation of ME for maintenance (k_m =0.7).

$$NE_m(MJ/day) = \left(0.53 \times \left(\frac{Wt}{1.08}\right)^{0.67} + 0.0071 \times Wt\right) \times LS$$

Where Wt is the live weight (kg) of the animal, LS is the lactation status of the cow (Non lactating cows, LS=1; Lactating cows, LS=1.2). During lactation, the maintenance requirements of lactating cows are 20% higher than non-lactating cows (NRC, 2000).

The ME requirement for gain (ME_g) was calculated as follows

$$ME_g = \frac{NE_g}{k_g}$$

The generic equation for the net energy content of gain (NE_g) (ARC, 1980) is as below and k_g is the efficiency of utilisation of ME for retention in live weight gain (k_g =0.4).

$$NE_g(MJ/day) = \frac{4.1 + 0.0332 \times Wt - 0.000009 \times Wt^2}{1 - 0.1475 \times ADG}$$

Where:

Wt is the live weight (kg) and ADG is the average daily gain (kg) of the animal.

Having obtained good estimates of the composition of gain in fat and protein from Williams and Jenkins (1998), the net energy requirements for the retention of fat (RE_{fat}) and protein ($RE_{protein}$) were calculated as follows:

$$RE_{fat}\ (MJ/day) = Change\ EBFW\ \times 39.3$$

$$RE_{protein}\ (MJ/day) = Change\ EBPW\ \times 23.6$$

Where:

RE_{fat} and $RE_{protein}$ are the net energy requirements for the retention of fat and protein, respectively. *Change EBFW* and *Change EBPW* are the growth (kg) in empty body fat weigh and empty body protein weight during the experimental period (i.e. day, week, month etc. during constant tissue growth or loss). In this instance, the experimental

periods were during the “calving” (weaning to start of mating) and “lactating” (start of mating to weaning) periods. 39.3 MJ/kg and 23.6 MJ/kg are the energy densities of retained fat and protein, respectively (ARC, 1980).

The ME requirements for the synthesis of fat (ME_f) and protein (ME_p) were obtained by dividing the RE_{fat} and $RE_{protein}$ by the efficiency of utilisation of ME used for fat (k_f) and protein (k_p) synthesis, respectively. k_f and k_p are 0.70 and 0.20, correspondingly (Geay, 1984).

If the change in weight was negative, then the ME supplied for maintenance from tissue loss ($ME_{tissue\ loss}$) was calculated as

$$ME_{tissue\ loss}\ (MJ) = (Change\ EBFW \times 39.3 + Change\ EBPW \times 23.6) \times k_{tissue\ loss}$$

Where:

$k_{tissue\ loss}$ is the efficiency of ME used for maintenance from tissue loss ($k_{tissue\ loss}$ =0.84).

The ME requirement for gain of the gravid uterus (ME_{gu}) was calculated as follows

$$ME_{gu}(MJ) = \frac{NE_{gu}}{k_{gu}}$$

Where:

NE_{gu} is the net energy content of the gravid uterus (uterus + conceptus, where conceptus = foetus + foetal fluids + foetal membranes) (Ferrell *et al.*, 1976a) and k_{gu} is the efficiency of utilisation of ME for retention in the gravid uterus (k_{gu} =0.14) (Ferrell *et al.*, 1976b)

$$NE_{gu}(MJ) = 0.29189e^{0.0322t-0.0000275t^2} \times \frac{CBW}{40.7}$$

Where:

t is the day of gestation.

The ME requirement for lactation (ME_l) was calculated as follows

$$ME_l = \frac{NE_l}{k_l}$$

Where:

NE_l is the net energy content of lactation (milk) and calculated as

$$NE_l = \left(\frac{NE_m}{k_m} + \frac{NE_g}{k_g}\right)$$

Where:

the NE_m and NE_g are the net energy requirements for maintenance and the net energy content of gain, respectively, of the calf and are calculated as above. k_m and k_g are the efficiency of utilisation of ME used for maintenance and gain, respectively, the value of which is 0.8 for animals fed on milk diets (SCA, 1990). k_l is the efficiency of utilisation of ME used for lactation (k_l=0.62). This equation assumes that all of the nutrient requirements for the calf are met from milk. This results in the overall efficiency of utilisation of ME used for cow lactation, calf maintenance and calf growth equating to 0.5.

Heat production (HP), the heat production of gain (HPG) and the heat production of maintenance (HPM) were calculated as follows

$$HP\ (MJ/day) = MEI - NE_f - NE_p - NE_l - NE_{gu}$$

$$HPG\ (MJ/day) = \left(\frac{RE_{fat}}{k_f} - RE_{fat}\right) + \left(\frac{RE_{protein}}{k_p} - RE_{protein}\right) + \left(\frac{NE_l}{k_l} - NE_l\right) + \left(\frac{NE_{gu}}{k_{gu}} - NE_{gu}\right)$$

$$HPM(MJ/day) = HP - HPG$$

Where:

HPM is the heat production that cannot be explained by the energy retained (as fat, protein, milk and gravid uterus) and the efficiency by which this energy is retained. As such, the HPM is due to the energy requirements for maintenance, activity and any loss from the heat increment of feeding.

7.2.7 Change traits

The GU_{weight}, NE_{gu}, and ME_{gu} between specific time periods were calculated as the difference between t_1 and t_2 where t is the day of gestation.

7.2.8 Statistical analysis

All statistical analyses were conducted using Proc MIXED in SAS 9.1 (SAS, 1989). Tests of significance of fixed effects were calculated utilising type III sums of squares mixed models (Proc MIXED). Two models were fitted, one for the weaning to the start of mating period ("calving" model) and one for the start of mating to weaning period ("lactating cow" model).

Fixed effects fitted in the "calving" model included year of cow birth (2006, 2007), period ("calving" 1, "calving" 2), nutrition (high, low), genotype (low fat, high fat), lactation status (yes, no), pregnancy status (yes, no) and interactions between fixed effects (year of cow birth x feed, feed x nutrition, feed x genotype, feed x pregnancy status, feed x lactation status, nutrition x lactation status, genotype x nutrition). Animal ID was fitted as a random term to account for repeated measures across the two "calving" periods.

Fixed effects fitted in the "lactating cow" model included year of cow birth (2006, 2007), period ("lactating cow" 1, "lactating cow" 2), calving status (yes, no), nutrition (high, low), genotype (low fat, high fat) and interactions between fixed effects (year of cow birth x feed, year of cow birth x calving status, feed x calving status, feed x nutrition, feed x genotype, genotype x nutrition). Lactation length nested within calving status was fitted as a covariate and animal ID was fitted as a random term.

All interactions were tested in the maximal model ("calving" and "lactating cow" models) with non-significant interactions being removed in order of least significance. Means of the "calving" and "lactating cow" models for genotype (low fat, high fat) x nutrition (low, high) interaction were presented as best linear unbiased estimates and standard errors herein. Nutrition by genotype (nutrition x genotype) best linear unbiased estimates and standard errors were calculated. Nutrition and genotype best linear unbiased estimates and standard errors for the analysis with tests of significance were determined (Appendices 7.1 and 7.2).

Residual feed intake (RFI) and residual energy intake (REI) were calculated using Proc GLM in SAS 9.1 (SAS, 1989) for the two time periods, from weaning to the start of mating period ("calving" period) and from the start of mating to the weaning period ("lactating cow" period). The first model estimates the difference between a cow's apparent feed intake (AFI; kgDM/period) and its requirements for the maintenance of cow weight ($MidWt$), cow growth (Cow_{Gain}), growth of the calf ($Calf_{Gain}$) and growth of the gravid uterus (GU_{Gain}).

$$AFI = \mu + \beta_1(MidWt) + \beta_2(Cow_{Gain}) + \beta_3(Calf_{Gain}) + \beta_4(GU_{Gain}) + RFI$$

The second model estimates the difference between a cow's apparent ME-intake (*MEI*; MJ/period) and its requirements for the maintenance of cow weight (*MidWt*), cow growth (Cow_{Gain}), growth of the calf ($Calf_{Gain}$) and growth of the gravid uterus (GU_{Gain}).

$$MEI = \mu + \beta_1(MidWt) + \beta_2(Cow_{Gain}) + \beta_3(Calf_{Gain}) + \beta_4(GU_{Gain}) + REI$$

The residuals from these models (RFI and REI) were regressed against cow genotype (low fat, high fat) with the best linear unbiased estimates and standard errors for cow genotype presented herein.

7.3 Results

The animals used in this experiment were part of the Beef CRC Maternal Efficiency Project. These animals used herein differed genetically in fatness with the "low fat" genotype being -1.57±0.07 mm and the "high fat" genotype being 1.01±0.07 mm different from the Angus Breedplan EBV average in P8 fat depth. These were fed at low and high nutrition, equivalent to a 20% difference in feed intake and subsequently body weight between treatments in a 2x2 factorial design. The data available were from two parities (1st and 2nd).

7.3.1 Maternal efficiency - weaning to mating

Level of nutrition impacted significantly on all weight and body composition traits of cows and calves from weaning to the start of mating ($P<0.05$) (Table 7.3 and 7.4), with the exception of changes in EMA. Cow weight at weaning and their weight at the start of mating were significantly different between the fat genotypes (Table 7.3 and 7.4). This was as expected due to the difference in mid-parent EBVs for mature cow weight

(Table 7.1). The high fat genotype animals had lower mature cow weights and hence, lower start weights (low nutrition = 4.6%; high nutrition = 5.6%) and end weights (low nutrition = 5.8%; high nutrition = 5.6%). However, weight losses by the cows were not different between the fat genotypes. Calf birth weights, weights at the start of mating, and growth rates were not affected by the fat genotype of their dams.

No effect of nutrition was observed on the calving rates of the cows fed high or low nutrition (Table 7.3). However, the high fat genotype had 11.5% more calves than the low fat genotype cows (Table 7.3). There was no genotype by nutrition effect on the calving rate of cows, suggesting that fatness genotype alone accounted for most of the variation in calving rate in this population.

For the change traits, fat genotype only had a significant effect on changes in rib fat depth ($P<0.05$). Overall, fat losses (as seen by changes in rib fat depth) were greater in the high fat genotype (28.3%) as they had more fat at the beginning of the period (Table 7.4). However, there was a trend for the high fat genotype cows fed high nutrition to lose more fat as rib fat than any of the other genotype by nutrition treatments. The fat loss for these cows was 42.2% greater (0.76mm) than the low fat, high nutrition group. Losses in EBFW were greater in the high fat, high nutrition group but this was not significant. From weaning to the start of mating, both genotypes and all nutrition by genotype groups lost protein (as measured by changes in EBPW and EMA). However, there was no significant effect of genotype or nutrition by genotype effects on changes in body protein ($P>0.10$).

Table 7.3: Weight and body composition of high and low fat genotype animals at two levels of nutrition from weaning to start of mating.

	Low Nutrition			High Nutrition			P-Value		
	Low Fat	High Fat	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype	Nutrition x Genotype
Cow Start Weight (kg/period)	514.80 ± 7.03	491.16 ± 7.19	-4.6	569.98 ± 7.60	538.07 ± 7.64	-5.6	**<0.0001**	**<0.0001**	0.5438
Cow End Weight (kg/period)	482.83 ± 6.66	454.79 ± 6.77	-5.8	552.65 ± 7.20	521.47 ± 7.23	-5.6	**<0.0001**	**<0.0001**	0.8115
Cow Weight Gain (kg/period)	-31.91 ± 3.12	-36.48 ± 3.26	14.3	-17.12 ± 3.38	-16.78 ± 3.42	-2.0	**<0.0001**	0.4573	0.3872
Gravid Uterus Growth (kg/period)	37.00 ± 1.17	36.26 ± 1.25	-2.0	39.60 ± 1.23	39.53 ± 1.24	-0.2	**<0.0001**	0.5431	0.6147
Cow Calving Rate (%)	77.0	85.6	11.2	76.9	86.0	11.8	0.1231	**0.0132**	0.9441
Calf Birth Weight (kg/period)	30.95 ± 0.57	29.93 ± 0.54	-3.3	33.19 ± 0.61	32.94 ± 0.59	-0.8	**<0.0001**	0.2705	0.5098
Calf End Weight (kg/period)	73.83 ± 2.89	71.07 ± 2.74	-3.7	87.84 ± 2.93	89.52 ± 2.89	1.9	**<0.0001**	0.7097	0.1287
Calf Weight Gain (kg/period)	47.42 ± 2.44	45.58 ± 2.31	-3.9	59.29 ± 2.47	61.12 ± 2.44	3.1	**<0.0001**	0.9922	0.5908
Change Rib Fat (cm/period)	-1.09 ± 0.22	-1.16 ± 0.23	6.4	-1.80 ± 0.23	-2.56 ± 0.24	42.2	**<0.0001**	**0.0329**	**0.0748**
Change EBFW (kg/period)	-12.25 ± 1.75	-12.48 ± 1.84	1.9	-16.44 ± 1.90	-19.99 ± 1.92	21.6	**0.0002**	0.2271	0.2837
Change Gravid Uterus Protein (kg/period)	5.49 ± 0.18	5.37 ± 0.19	-2.2	5.88 ± 0.19	5.87 ± 0.19	-0.2	**<0.0001**	0.5079	0.5820
Change EMA (cm^2)	-8.46 ± 1.13	-6.08 ± 1.19	-28.1	-7.11 ± 1.22	-6.84 ± 1.24	-3.8	0.7685	0.1889	0.2896
Change EBPW (kg/period)	-3.41 ± 0.39	-3.85 ± 0.40	12.9	-1.17 ± 0.42	-1.57 ± 0.42	34.2	**<0.0001**	0.2307	0.955

EMA = Eye muscle area (*longissimus dorsi* area) expressed as cubic centimetres
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

Table 7.4: Weight and body composition of cows fed at high and low nutrition as well as high and low fat genotype cows from weaning to start of mating.

	Nutrition			Genotype			P-Value	
	Low Nutrition	High Nutrition	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype
Cow Start Weight (kg/period)	502.98 ± 5.3926	554.03 ± 5.7554	10.1	542.39 ± 5.5152	514.62 ± 5.6481	-5.1	**<0.0001**	**<0.0001**
Cow End Weight (kg/period)	468.81 ± 4.9995	537.06 ± 5.3531	14.6	517.74 ± 5.1388	488.13 ± 5.2261	-5.7	**<0.0001**	**<0.0001**
Cow Weight Gain (kg/period)	-34.19 ± 2.5442	-16.95 ± 2.6932	-50.4	-24.51 ± 2.5686	-26.63 ± 2.6779	8.6	**<0.0001**	0.4573
Gravid Uterus Growth (kg/period)	36.63 ± 1.1229	39.57 ± 1.1342	8.0	38.3005 ± 1.1045	37.896 ± 1.1535	-1.1	**<0.0001**	0.5431
Cow Calving Rate (%)	81.3	81.5	0.2	77	85.8	11.4	0.1231	**0.0132**
Calf Birth Weight (kg/period)	30.45 ± 0.397	33.07 ± 0.4281	8.6	32.07 ± 0.4212	31.44 ± 0.4038	-2.0	**<0.0001**	0.2705
Calf End Weight (kg/period)	72.45 ± 2.6377	88.68 ± 2.7079	22.4	80.84 ± 2.7154	80.29 ± 2.6299	-0.7	**<0.0001**	0.7097
Calf Weight Gain (kg/period)	46.51 ± 2.2296	60.21 ± 2.2886	29.5	53.36 ± 2.295	53.35 ± 2.2229	0.0	**<0.0001**	0.9922
Change Rib Fat (cm/period)	-1.13 ± 0.1891	-2.18 ± 0.1791	92.9	-1.45 ± 0.1802	-1.86 ± 0.1887	28.3	**<0.0001**	**0.0329**
Change EBFW (kg/period)	-12.37 ± 1.4531	-18.22 ± 1.5349	47.3	-14.35 ± 1.462	-16.24 ± 1.5315	13.2	**0.0002**	0.2271
Change Gravid Uterus Protein (kg/period)	5.43 ± 0.1699	5.88 ± 0.1716	8.3	5.68 ± 0.1671	5.62 ± 0.1746	-1.1	**<0.0001**	0.5079
Change EMA (cm^2)	-7.27 ± 0.9357	-6.98 ± 0.9884	-4.0	-7.78 ± 0.9414	-6.46 ± 0.9862	-17.0	0.7685	0.1889
Change EBPW (kg/period)	-3.63 ± 0.3145	-1.37 ± 0.333	-62.3	-2.29 ± 0.3176	-2.71 ± 0.3311	18.3	**<0.0001**	0.2307

EMA = Eye muscle area (*longissimus dorsi* area) expressed as cubic centimetres
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

During the period from weaning to the start of mating, there were large nutrition effects on the apparent feed intake of all groups of cows and hence, the apparent ME-intake (Table 7.5 and Figure 7.3). The cows fed at high nutrition had 23.9% higher apparent feed intake and 30.2% higher apparent ME-intake than cows on low nutrition (Table 7.6). This difference was also observed for the predicted ME-Intake requirements (Table 7.6 and Figure 7.3), which were 7.0% greater in cows fed at high nutrition. However, unlike apparent feed intake and due to the variability of ME density of feed during this period of the year, there was no significant difference between genotypes in apparent ME-intake. However, the high nutrition high fat cows did have a higher apparent ME-intake (948 MJ; 3.6%), indicating that when feed is available the apparent feed intake in this group may be modulated by the quality of the feed. Conversely, the estimated ME-Intake requirements of the low fat genotype animals were greater (5.6%) as they are larger cows and appear to have more lean tissue to maintain (Table 7.5 and Figure 7.3).

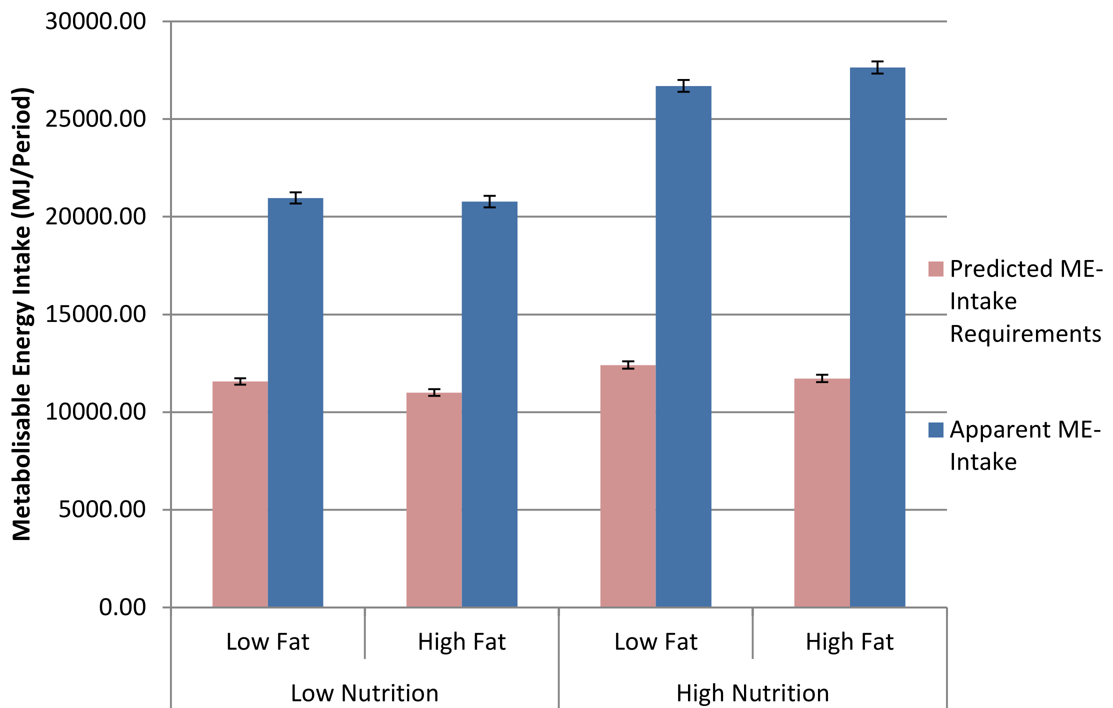

Figure 7.3: Predicted ME requirements and actual ME-Intake of high and low fat genotype animals at two levels of nutrition from weaning to start of mating.

Table 7.5: Apparent feed intake, energy retention, metabolisable energy requirements and efficiency of high and low fat genotype animals at two levels of nutrition from weaning to start of mating.

	Low Nutrition			High Nutrition			P-Value		
	Low Fat	High Fat	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype	Nutrition x Genotype
Apparent feed intake (kgDM/period)	2348.25 ± 29.69	2418.61 ± 31.21	3.0	2909.88 ± 32.22	2995.65 ± 32.61	3.0	**<0.0001**	**0.0035**	0.7699
Total ME-Intake (MJ/period)	20963 ± 280.51	20770 ± 294.86	-0.9	26697 ± 304.39	27645 ± 308.09	3.6	**<0.0001**	0.1316	**0.0226**
RE as Gravid Uterus (MJ/period)	179.37 ± 5.77	175.72 ± 6.17	-2.0	192.11 ± 6.07	191.84 ± 6.11	-0.1	**<0.0001**	0.5524	0.6038
RE as Fat[1] (MJ/period)	-481.39 ± 68.86	-490.36 ± 72.38	1.9	-646.05 ± 74.72	-785.76 ± 75.63	21.6	**0.0002**	0.2267	0.2851
RE as Protein[1] (MJ/period)	-80.43 ± 9.1	-90.83 ± 9.51	12.9	-27.8 ± 9.87	-37.13 ± 9.97	33.6	**<0.0001**	0.2348	0.9486
RE as Fat and Protein[1] (MJ/period)	-561.98 ± 71.76	-581.63 ± 75.43	3.5	-673.87 ± 77.87	-823.4 ± 78.82	22.2	**0.0058**	0.1871	0.3081
$ME_{fat}^{\ddagger}$ (MJ/period)	-546.26 ± 84.46	-546.92 ± 88.78	0.1	-727.1 ± 91.95	-907.02 ± 92.76	24.7	**0.0004**	0.2313	0.2322
$ME_{protein}^{\ddagger}$ (MJ/period)	-12.54 ± 22.6	-69.97 ± 23.75	458.0	127.89 ± 24.52	76.27 ± 24.82	-40.4	**<0.0001**	**0.0073**	0.8847
$ME_{fat + protein}^{\ddagger}$ (MJ/period)	-558.8 ± 90.12	-616.9 ± 94.73	10.4	-599.21 ± 97.79	-830.75 ± 98.98	38.6	0.1123	**0.0725**	0.2786
$ME_{gravid\ uterus}^{\ddagger}$ (MJ/period)	789.56 ± 30.81	721.53 ± 32.33	-8.6	865.8 ± 33.43	861.44 ± 33.82	-0.5	**0.0001**	0.1917	0.2482
$ME_{lactation}^{\ddagger}$ (MJ/period)	1541.94 ± 19.03	1507.87 ± 19.79	-2.2	1685.67 ± 20.62	1717.25 ± 20.82	1.9	**<0.0001**	0.9435	**0.0618**
$ME_{maintenance}^{\ddagger}$ (MJ/period)	9778.03 ± 101.06	9361.14 ± 104.18	-4..3	10449 ± 109.43	9947.44 ± 110.21	-4.8	**<0.0001**	**<0.0001**	0.7293
ME-Intake Requirements (MJ/period)	11566 ± 168.77	10999 ± 175.94	-4.9	12412 ± 182.96	11723 ± 184.81	-5.6	**<0.0001**	**<0.0001**	0.6863
$ME_{maintenance}^{\ddagger}$ Overestimate (MJ/period)	9405.06 ± 302.23	9789.89 ± 317.69	4.1	14287 ± 327.96	15944 ± 331.95	11.6	**<0.0001**	**0.0003**	**0.0169**
Heat production (MJ/period)	20462 ± 280.38	20331 ± 294.72	-0.6	26207 ± 304.25	27289 ± 307.95	4.1	**<0.0001**	**0.0609**	**0.0146**
Heat production of gain (MJ/period)	1266 ± 37.93	1156 ± 39.87	-0.9	1457 ± 41.20	1384 ±41.66	-5.0	**<0.0001**	**0.0071**	0.5855
Heat production of maintenance (MJ/period)	19195 ± 285.78	19165 ± 300.4	-0.2	24750 ± 310.10	25905 ± 313.88	4.7	**<0.0001**	**0.0282**	**0.0201**
ECR1 (MJ/Kg Calf Gain)	482.28 ± 17.38	486.26 ± 17.06	0.8	499.95 ± 19.17	507.78 ± 18.12	1.6	0.2725	0.7400	0.9140
ECR2 (MJ/Kg Calf Gain)	5058.23 ± 459.19	5063.10 ± 459.18	0.1	6274.83 ± 496.52	5710.13 ± 495.81	-9.0	**0.0501**	0.5563	0.7560
ECR3 (MJ/Kg Cow and Calf Gain)	-642.65 ± 78.22	-802.67 ± 92.71	24.9	1251.16 ± 153.25	161.31 ± 19.52	-87.1	**0.0227**	0.3123	0.4629

ME-Intake = Metabolisable energy intake; RE = Retained energy where ([1]) is energy retained in the cow

‡$ME_{subscript}$ designates the metabolisable energy requirements for the subscripted portion of ME-Intake

ECR1 = Energy conversion ratio 1, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth (Means only from cows carrying a calf at the start of mating)

ECR2 = Energy conversion ratio 2, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth

ECR3 = Energy conversion ratio 3, as measured by the ME-Intake of the cow/calf unit per kilogram of cow and calf growth from log transformed data hence s.e. are approximate)

Table 7.6: Apparent feed intake, energy retention, metabolisable energy requirements and ECR of cows fed at high and low nutrition as well high and low fat genotype cows from weaning to start of mating.

	Nutrition			Genotype			P-Value	
	Low Nutrition	High Nutrition	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype
Apparent feed intake (kgDM/period)	2383.43 ± 24.6253	2952.76 ± 26.0114	23.9	2629.06 ± 24.7749	2707.13 ± 25.9536	3.0	**<0.0001**	**0.0035**
Total ME-Intake (MJ/period)	20866 ± 232.63	27171 ± 245.73	30.2	23830 ± 234.05	24208 ± 245.17	1.6	**<0.0001**	0.1316
RE as Gravid Uterus (MJ/period)	177.55 ± 5.5429	191.98 ± 5.5978	8.1	185.74 ± 5.4516	183.78 ± 5.6938	-1.1	**<0.0001**	0.5524
RE as Fat[1] (MJ/period)	-485.88 ± 57.1083	-715.9 ± 60.323	47.3	-563.72 ± 57.4569	-638.06 ± 60.1883	13.2	**0.0002**	0.2267
RE as Protein[1] (MJ/period)	-85.63 ± 7.42	-32.47 ± 7.8547	-62.1	-54.12 ± 7.4913	-63.98 ± 7.81	18.2	**<0.0001**	0.2348
RE as Fat and Protein[1] (MJ/period)	-571.81 ± 59.5144	-784.63 ± 62.8647	37.2	-617.62 ± 59.8778	-702.51 ± 62.7243	13.7	**0.0058**	0.1871
ME_{fat}‡ (MJ/period)	-546.59 ± 70.0419	-817.06 ± 73.9848	49.5	-636.68 ± 70.4696	-726.97 ± 73.8195	14.2	**0.0004**	0.2313
$ME_{protein}$‡ (MJ/period)	-41.26 ± 18.7407	102.08 ± 19.7956	-347.4	57.67 ± 18.8551	3.15 ± 19.7514	-94.5	**<0.0001**	**0.0073**
$ME_{fat + protein}$‡ (MJ/period)	-587.85 ± 74.7358	-714.98 ± 78.9429	21.6	-579.01 ± 75.1921	-723.82 ± 78.7666	25.0	0.1123	**0.0725**
$ME_{gravid\ uterus}$‡ (MJ/period)	755.54 ± 25.4159	863.62 ± 26.8649	14.3	827.68 ± 25.5985	791.48 ± 26.7762	-4.4	**0.0001**	0.1917
$ME_{lactation}$‡ (MJ/period)	1524.91 ± 15.3289	1701.46 ± 16.2519	11.6	1613.81 ± 15.5134	1612.56 ± 16.1206	-0.1	**<0.0001**	0.9435
$ME_{maintenance}$‡ (MJ/period)	9569.58 ± 79.3175	10198 ± 84.3901	6.6	10113 ± 80.7266	9654.29 ± 83.2318	-4.5	**<0.0001**	**<0.0001**
ME-Intake Requirements (MJ/period)	11282 ± 136.76	12067 ± 144.89	7.0	11989 ± 138.24	11361 ± 143.89	-5.2	**<0.0001**	**<0.0001**
$ME_{maintenance}$‡ Overestimate (MJ/period)	9597.47 ± 250.65	15116 ± 264.76	57.5	11846 ± 252.18	12867 ± 264.17	8.6	**<0.0001**	**0.0003**
Heat production (MJ/period)	20391 ± 232.52	26748 ± 245.61	31.2	23334 ± 233.94	23805 ± 245.07	2.0	**<0.0001**	**0.0609**
Heat production of gain (MJ/period)	1211 ± 31.45	1420 ± 33.22	17.3	1361 ± 31.65	1269 ± 33.15	-6.7	**<0.0001**	**00071**
Heat production of maintenance (MJ/period)	19180 ± 237	25327 ± 250.34	32.0	21973 ± 238.45	22535 ± 249.78	2.6	**<0.0001**	**0.0282**
ECR1 (MJ/Kg Calf Gain)	484.37 ± 12.4033	502.7 ± 13.4345	3.8	489.99 ± 13.1729	497.08 ± 12.6723	1.4	0.2725	0.7400
ECR2 (MJ/Kg Calf Gain)	5060.66 ± 327.42	5992.48 ± 353.38	18.4	5666.53 ± 341.12	5386.61 ± 341.5	-4.9	**0.0501**	0.5563
ECR3 (MJ/Kg Cow and Calf Gain)	-711.26 ± -0.8025	693.17 ± 0.7985	-197.5	299.78 ± 0.3468	-321.84 ± -0.3616	-207.4	**0.0227**	0.3123

ME-Intake = Metabolisable energy intake; RE = Retained energy ([1]) energy retained in the cow

‡$ME_{subscript}$ designates the metabolisable energy requirements for the subscripted portion of ME-Intake

ECR1 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth (Means only from cows carrying a calf at the start of mating)

ECR2 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth

ECR3 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of cow and calf growth (data was log transformed hence s.e. are approximate)

During the period from weaning to the start of mating, energy retained or lost was not different between fat genotype animals, but there were large nutrition effects (Table 7.5). The energy retained in the gravid uterus was greater at higher levels of nutrition (8.1%, $P<0.05$) (Table 7.6) and is indicative of the higher birth weights of calves from cows fed at high nutrition (Table 7.3). There was little energy retained by the majority of cows during this period; there was only energy lost to supply energy deficits for maintenance. Energy lost as fat was greater at high levels of nutrition (47.3%; Table 7.6), possibly as these animals could afford to lose fat more as they were heavier during this period (Table 7.3). Both nutritional groups lost protein, but this energy lost as protein were 62.1% greater in cows fed at low nutrition (Table 7.6).

Both levels of nutrition were not supplying sufficient energy for the period between weaning and start of mating (Table 7.5 and Figure 7.4). All nutrition by genotype animal groups used this energy for maintenance (Figure 7.4). Whilst the difference between genotypes was not significant, it must be noted that the high fat animals fed at a high level of nutrition supplied 24.7% more energy, from fat, toward their maintenance energy deficit ($P>0.10$, Table 7.5). The energy cost of the deposition of protein relative to the energy supplied for maintenance was over 5 times greater; hence, energy requirements for protein deposition ($ME_{protein}$) in the high nutrition group were positive even though the majority of animals in this group supplied more energy for maintenance ($ME_{maintenance}$). There was no difference between the nutrition levels in the metabolisable energy used for maintenance from fat and protein combined ($ME_{fat + protein}$). However, the high fat animals used 13.7% more energy from body tissues for maintenance than the low fat animals ($P>0.1$; Table 7.6).

Metabolisable energy requirements for the growth of the gravid uterus were higher in the high nutrition groups relative to the low nutrition groups (Table 7.5; P<0.05), as would be expected from heavier weights of calves born in the high nutrition groups (Table 7.3). There was no discernable difference between the genotypes. There was also no difference in the metabolisable energy requirements for lactation ($ME_{lactation}$) between the animal fat genotypes (Table 7.5), as indicated by the calf weights at the start of mating (Table 7.3). Nevertheless, both animal genotypes had higher energy requirements for lactation when on high nutrition (6.6%; P<0.05; Table 7.6 and 7.10), although calf weights at the start of mating were heavier in both genotypes on high nutrition. Maintenance requirements of the cows were greater in cows fed at high nutrition (P<0.05) (Table 7.6 and Figure 7.4) due to these cows being heavier (Table 7.3). Additionally, the low fat cows also required 4.5% more metabolisable energy for maintenance (P<0.05) (Table 7.6 and Figure 7.4) as these cows were heavier (Table 7.3). This would also be expected from a leaner genotype.

Predicted ME-intake requirements for cow maintenance, lactation, growth of the gravid uterus and growth of the cow were 7.0% higher in animals fed high nutrition relative to those fed low nutrition (P<0.05) (Table 7.6 and Figure 7.4). This is also true of the low fat genotype animals, which had 5.2% greater predicted ME-intake requirements than the high fat cows (P<0.05) (Table 7.6 and Figure 7.4). Due to the over-estimates of apparent feed intake associated with the supplementary feeding period and to some extent, the grazing period, some over-estimates were quite considerable for all nutrition by genotype groups (Table 7.6). However, this interaction was not significant (P>0.10) (Table 7.5; P>0.10). It is, therefore, not surprising that these over-estimates were greater in cows fed at high levels of nutrition (P>0.10) (Table 7.6). Furthermore, the over-

estimate in ME-Intake from predicted requirements was 8.6% greater in high fat cows ($P<0.05$) (Table 7.6) However, this was even more so at high levels of nutrition, where the over-estimate was 11.6% greater (Table 7.5). It must be noted that there is no allowance for the metabolisable energy requirements for activity or hence, the energy use associated with activity. However, activity requirements would be small with respect to these over-estimates (Table 7.5 and 7.6).

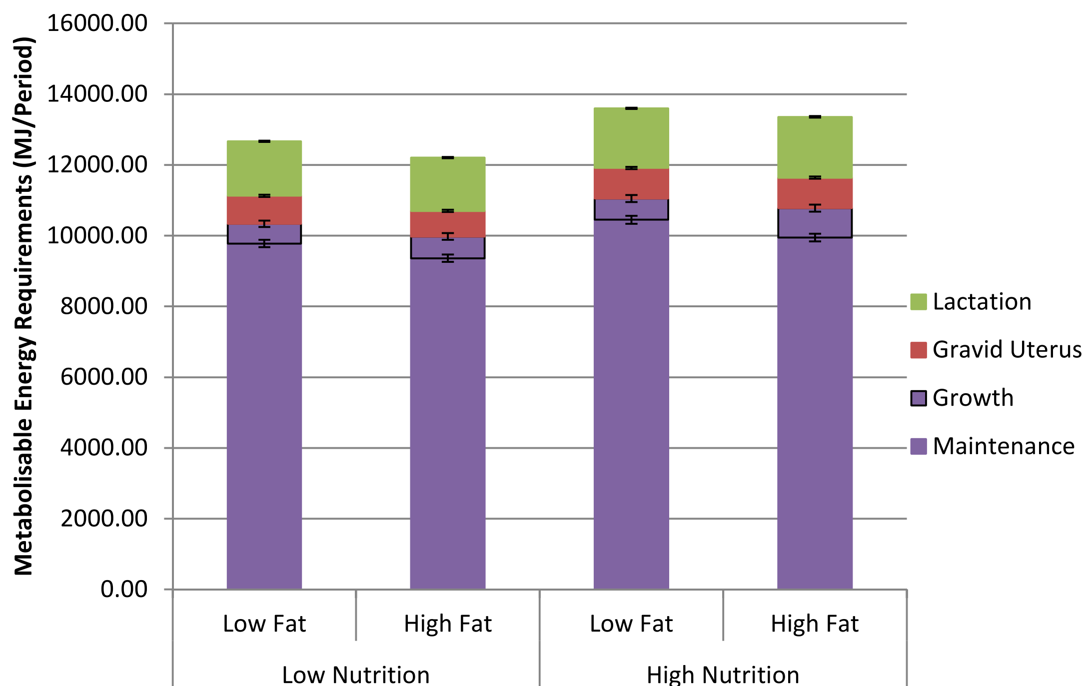

Figure 7.4: Metabolisable energy requirements for cow maintenance, growth, growth of the gravid uterus and lactation of high and low fat genotype animals at two levels of nutrition from weaning to start of mating.

Heat production and HPM was greater in cows fed at high levels of nutrition (31.2% and 32.0% respectively, $P<0.05$) (Table 7.5 and Figure 7.5). However, this highlights the fact that ME-intake was over-estimated within all groups as there should be no real difference between nutrition groups with respect to HPM, except for that which is associated with the heat increment (HEI) of the higher ME-intakes (where $HEI =$

$MEI \times 0.09$). Whilst the high fat genotype cows had a greater heat production and HPM ($P<0.05$), it must be noted that most of this was due to those cows within this genotype that were fed at high levels of nutrition ($P<0.05$) (Table 7.6 and Figure 7.5). The HPG was significantly greater in the low fat genotype cows from the start of weaning to the start of mating at both nutrition treatments (Table 7.5 and Figure 7.5) and overall (6.7%) (Table 7.6). This is consistent with greater ME requirements for protein gain in this genotype and the difference between the k_p and $k_{tissue\ loss}$.

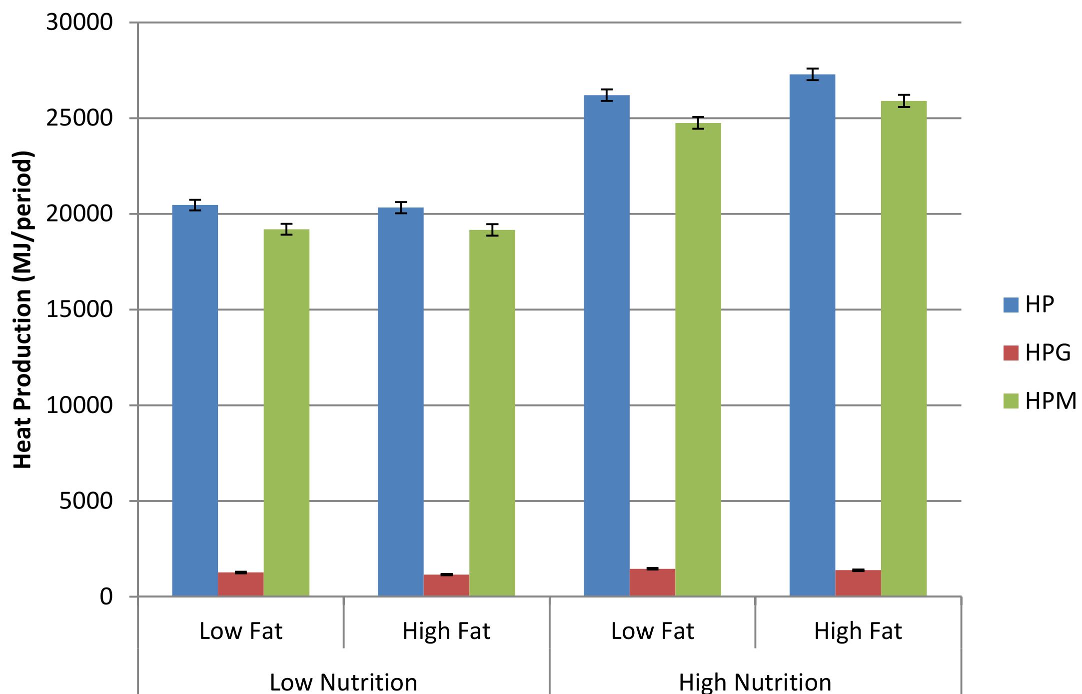

Figure 7.5: Estimated heat production (HP), heat production of gain (HPG) and heat production of maintenance (HPM) of high and low fat genotype animals at two levels of nutrition from weaning to start of mating.

Cows that consumed more feed had heavier calves at the start of mating, which indicates that there is no observable differences in the efficiency conversion ratio 1 (ECR1) from those cows with a calf at the start of mating ($P>0.10$) (Table 7.5). However, when taking into consideration the efficiency of the whole system, regardless

of calving status, while there were large differences in calving rate, there was no significant difference between high and low fat genotype cows in the ECR2 either (P<0.05) (Table 7.6). Nevertheless, cows fed high nutrition tended to require 18.4% more apparent ME-intake for calf gain even though calves from these cows grew faster (P<0.10) (Table 7.5). Weight losses of the cows were greater than calf weight gains and associated with insufficient apparent ME-intakes during the period of weaning to start of mating. This meant that cows fed at low nutrition had a negative ECR3 (P<0.05) (Table 7.5). This nutrition effect on ECR3 indicates that high nutrition is required for a net salvage value of cows and calves. Whilst there was no significant effect of fat genotype on the ECR3, interestingly, on high nutrition, the high fat cows had 87.1% less ME-intake for cow and calf weight gain than the low fat cows (Table 7.5)

The fat genotype of the cows had a significant effect on the residual feed intake (P=0.0006) and residual energy intake (P=0.007) when regressed on these genotypes during the period of weaning to start of mating. The RFI of low fat cows equated to -74.74 kgDM overall (-0.36 kgDM/day) while that of the high fat cows was +74.74 kgDM overall (+0.36 kgDM/day). This equated to a REI of -641.80 MJ overall (-3.11 MJ/day) and 641.80 MJ overall (+3.11 MJ/day) for the low and fat genotype cows, respectively.

7.3.2 Maternal efficiency - mating to weaning

The level of nutrition impacted significantly on all weight and body composition traits of cows and calves from the start of mating to weaning (i.e. whilst gestating and lactating), (P<0.05) (Table 7.7 and 7.8). However, nutrition did not have an effect on the weaning rate of calves (Table 7.7). Due to higher calving rates of the high fat

genotype cows, these cows also weaned 10% more calves (P<0.10) (Table 7.8). Cows and calves fed at high levels of nutrition had heavier weights at the start of mating (14.5% and 20.6% respectively; P<0.05) and consequently, were much heavier at weaning (Table 7.8). This was also true of cows with low fat genotypes which were 6.3% heavier at the start of mating and 5.4% heavier at weaning (Table 7.8). However, there was no effect of fat genotype on the weight of calves at weaning or on the weight gain of the cows or calves during this period. Additionally, there was no difference between weights at the start of mating and weaning of calves from cows of differing fat genotypes (P>0.10). Growth of the gravid uterus was 8.6% greater in cows fed high nutrition (P<0.05) and was also greater by 2.9% in the low fat genotype cows than high fat genotype cows (P<0.05) (Table 7.8). Nevertheless, this greater gravid uterus weight of low fat cows was not significant at parturition nor was the birth weight of calves born from these cows different (P>0.10) (Table 7.8).

Changes in the body composition of cows from the start of mating to weaning were significantly affected by the level of nutrition (Table 7.8). The high nutrition cows deposited more fat in subcutaneous depots and the whole body, and deposited more protein in the gravid uterus (a reflection on the greater growth thereof), eye muscle area and whole body protein weight. Cow genotype also impacted significantly on the growth of these tissues (P<0.05), with the exception of eye muscle area, which was not significantly affected by cow genotype (P>0.10) (Table 7.8). The low fat genotype cows deposited less fat in subcutaneous depots (28.6%; P<0.05) and in the whole body (14.1%; P<0.05), and 11.1% more protein as empty body protein weight (P<0.05) (Table 7.8). Reflective of the difference in gravid uterus growth during this period of

the low fat genotype cows, more protein was deposited in the gravid uterus of these cows as well (Table 7.8).

Table 7.7: Weight and body composition of high and low fat genotype animals at two levels of nutrition from start of mating to weaning.

	Low Nutrition			High Nutrition			P-Value		
	Low Fat	High Fat	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype	Nutrition x Genotype
Cow Start Weight (kg/period)	483.54 ± 7.19	453.38 ± 7.29	-6.2	553.53 ± 7.70	519.41 ± 7.84	-6.2	**<0.0001**	**<0.0001**	0.7788
Cow End Weight (kg/period)	602.90 ± 7.79	573.57 ± 7.94	-4.9	697.37 ± 8.31	656.04 ± 8.52	-5.9	**<0.0001**	**<0.0001**	0.4213
Cow Weight Gain (kg/period)	110.20 ± 3.70	110.36 ± 3.78	0.2	134.39 ± 3.94	125.85 ± 4.03	-6.4	**<0.0001**	0.2219	0.2017
Gravid Uterus Growth (kg/period)	7.94 ± 0.20	7.55 ± 0.19	-4.91	8.45 ± 0.20	8.38 ± 0.20	-0.8	**<0.0001**	**0.0409**	0.1496
Cow Weaning Rate (%)	75.7	80.3	6.2	72.6	82.6	13.8	0.9161	**0.0600**	0.4949
Calf Start Weight (kg/period)	80.40 ± 2.10	77.69 ± 2.15	-3.4	93.60 ± 2.33	97.05 ± 2.20	3.7	**<0.0001**	0.8552	0.1251
Calf Weaning Weight (kg/period)	244.83 ± 3.93	239.60 ± 4.03	-2.1	285.38 ± 4.35	283.69 ± 4.15	-0.6	**<0.0001**	0.3662	0.6444
Calf Weight Gain (kg/period)	76.09 ± 3.65	74.74 ± 3.52	-1.8	102.93 ± 3.78	97.67 ± 3.70	-5.1	**<0.0001**	0.1537	0.5272
Change Rib Fat (cm/period)	2.29 ± 0.26	2.88 ± 0.27	25.8	4.06 ± 0.28	5.30 ± 0.29	30.5	**<0.0001**	**0.0002**	0.1811
Change EBFW (kg/period)	29.89 ± 2.16	34.41 ± 2.22	15.1	48.42 ± 2.30	54.98 ± 2.36	13.6	**<0.0001**	**0.0062**	0.6101
Change Gravid Uterus Protein (kg/period)	0.53 ± 0.01	0.50 ± 0.01	-5.7	0.55 ± 0.01	0.55 ± 0.01	0.0	**<0.0001**	**0.0436**	0.1181
Change EMA (cm^2)	9.04 ± 1.05	8.84 ± 1.08	-2.2	14.06 ± 1.12	13.12 ± 1.15	-6.7	**<0.0001**	0.5548	0.7072
Change EBPW (kg/period)	13.04 ± 0.47	11.98 ± 0.48	-8.1	15.32 ± 0.50	13.20 ± 0.51	-13.8	**<0.0001**	**0.0003**	0.2246

EMA = Eye muscle area (*longissimus dorsi* area) expressed as cubic centimetres
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

Table 7.8: Weight and body composition of cows fed at high and low nutrition as well as high and low fat genotype cows from start of mating to weaning.

	Nutrition			Genotype			P-Value	
	Low Nutrition	High Nutrition	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype
Cow Start Weight (kg/period)	468.46 ± 5.4267	536.47 ± 5.7953	14.5	518.83 ± 5.5371	486.4 ± 5.6977	-6.3	**<0.0001**	**<0.0001**
Cow End Weight (kg/period)	588.24 ± 6.0188	676.7 ± 6.3962	15.0	650.14 ± 6.0971	614.81 ± 6.3335	-5.4	**<0.0001**	**<0.0001**
Cow Weight Gain (kg/period)	110.28 ± 2.9386	130.12 ± 3.1065	18.0	122.29 ± 2.9739	118.11 ± 3.0787	-3.4	**<0.0001**	0.2219
Gravid Uterus Growth (kg/period)	7.75 ± 0.1776	8.42 ± 0.179	8.6	8.2 ± 0.1784	7.96 ± 0.1783	-2.9	**<0.0001**	**0.0409**
Cow Weaning Rate (%)	78.0	78.0	0.0	74.1	81.5	10.0	0.9161	**0.06**
Calf Start Weight (kg/period)	79.04 ± 1.6339	95.33 ± 1.7301	20.6	87 ± 1.687	87.37 ± 1.6798	0.4	**<0.0001**	0.8552
Calf Weaning Weight (kg/period)	242.22 ± 3.0151	284.54 ± 3.2086	17.5	265.11 ± 3.1127	261.65 ± 3.1169	-1.3	**<0.0001**	0.3662
Calf Weight Gain (kg/period)	75.41 ± 3.1197	100.3 ± 3.2249	33.0	89.51 ± 3.2145	86.2 ± 3.1318	-3.7	**<0.0001**	0.1537
Change Rib Fat (cm/period)	2.59 ± 0.2093	4.68 ± 0.2211	80.7	3.18 ± 0.2117	4.09 ± 0.2193	28.6	**<0.0001**	**0.0002**
Change EBFW (kg/period)	32.15 ± 1.7225	51.7 ± 1.8112	60.8	39.16 ± 1.7321	44.69 ± 1.8054	14.1	**<0.0001**	**0.0062**
Change Gravid Uterus Protein (kg/period)	0.51 ± 0.01136	0.55 ± 0.01145	7.8	0.54 ± 0.01142	0.53 ± 0.01141	-1.9	**<0.0001**	**0.0436**
Change EMA (cm^2)	8.94 ± 0.8421	13.59 ± 0.888	52.0	11.55 ± 0.8499	10.98 ± 0.8825	-4.9	**<0.0001**	0.5548
Change EBPW (kg/period)	12.61 ± 0.3727	14.26 ± 0.3938	13.1	14.1704 ± 0.3769	12.5937 ± 0.3905	-11.1	**<0.0001**	**0.0003**

EMA = Eye muscle area (*longissimus dorsi* area) expressed as cubic centimetres
EBFW = Empty body fat weight expressed as kilograms of dry matter
EBPW = Empty body protein weight expressed as kilograms of dry matter

Apparent feed intake, apparent ME-intake and the predicted ME-intake requirements were higher in cows fed at high nutrition than at low nutrition (, regardless of the fat genotype of these animals ($P<0.05$) Table 7.10 and Figure 7.6). Nevertheless, apparent feed intake was 2.4% higher ($P<0.05$) and ME-intake requirements tended to be higher in high fat genotype cows than in low fat genotype cows (1.6%) ($P<0.10$). This appears to be primarily due to the difference between high and low fat cows fed at high nutrition (Table 7.10 and Figure 7.6). The high fat genotype cows at high nutrition had a considerably greater apparent feed intake ($P<0.05$), and hence, greater ME-intake than the low fat genotype cows at this level of intake. Regardless, there appears to be inaccuracies in the measurement of apparent feed intake, as in all nutrition by genotype groups, this was grossly over-estimated from that predicted from the nutrition equations (Figure 7.6). Once again, it must be noted that there is no allowance for the metabolisable energy requirements of activity, and hence, energy use associated with activity, but these requirements should be small with respect to the over-estimates (Table 7.9).

From mating to weaning, the level of nutrition significantly affected retained energy in the body as fat, protein and gravid uterus (Table 7.10), in that animals fed high nutrition deposited more energy in these depots ($P<0.05$). However, the fat genotype of the cow also had effects on the retention of energy in these depots (Table 7.10). Energy retained in the gravid uterus was significantly less in high fat cows (2.5%; $P<0.05$), though this was more evident at low nutrition (3.9%) than high nutrition (1.2%) (Table 7.9). The greater fat deposition of the high fat genotype cows resulted in greater energy retained as fat in this genotype (14.3%; $P<0.05$) (Table 7.10), with no significant variation between genotypes at different levels of nutrition (Genotype x Nutrition, $P>0.10$, Table

7.9). Consistent with leaner genotypes, the low fat genotype animals deposited 11.1% more energy as protein (Change EBPW, Table 7.10). Nevertheless, the overall energy retained as fat and protein was greater (9.4%; P<0.05) in the high fat genotype cows than the low fat genotype cows (Table 7.10), due to the volume of tissue deposited (Table 7.10) and the energy density of fat versus protein.

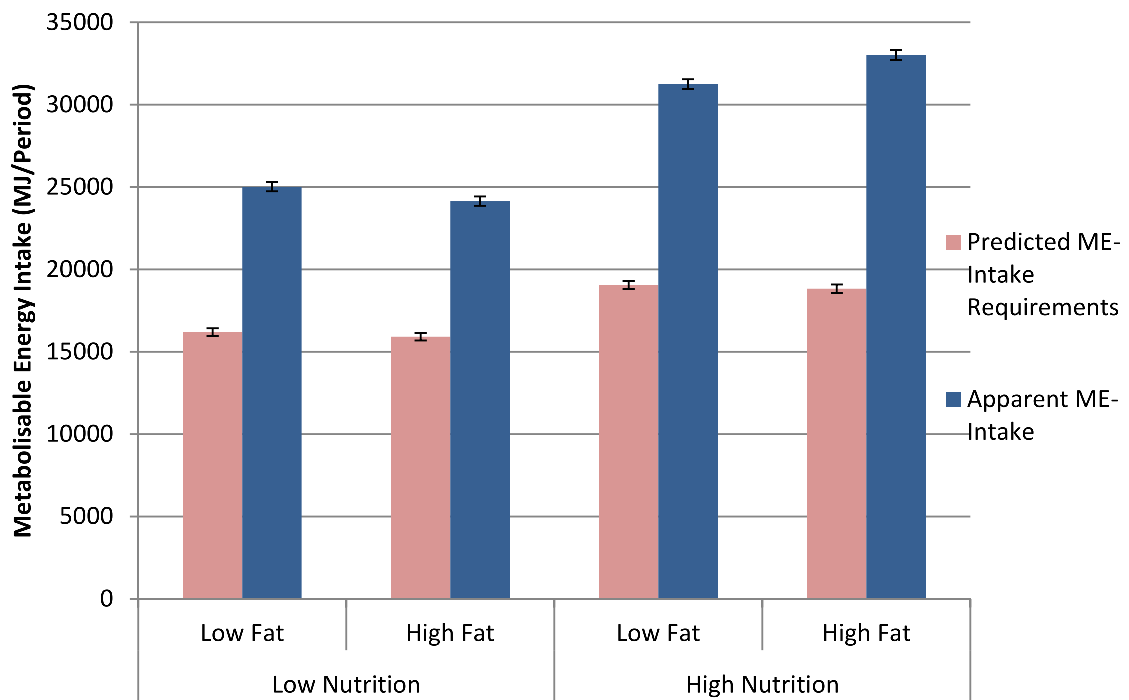

Figure 7.6: Predicted ME requirements and apparent ME-intake of high and low fat genotype animals at two levels of nutrition from start of mating to weaning.

Table 7.9: Apparent feed intake, energy retention, metabolisable energy requirements and efficiency of high and low fat genotype animals at two levels of nutrition from start of mating to weaning.

	Low Nutrition			High Nutrition			P-Value		
	Low Fat	High Fat	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype	Nutrition x Genotype
Apparent feed intake (kgDM/period)	2392.22 ± 28.92	2271.65 ± 29.63	-5.0	2901.63 ± 30.69	3151.23 ± 31.53	8.6	**<0.0001**	**0.0154**	**<0.0001**
Total ME-Intake (MJ/period)	25020.00 ± 278.34	24147.00 ± 285.14	-3.5	31257.00 ± 295.66	33014.00 ± 303.36	5.6	**<0.0001**	**0.0878**	**<0.0001**
RE as Gravid Uterus (MJ/period)	17.27 ± 0.38	16.60 ± 0.38	-3.9	18.26 ± 0.39	18.04 ± 0.39	-1.2	**<0.0001**	**0.0457**	0.3156
RE as Fat[1] (MJ/period)	1174.88 ± 85.14	1352.23 ± 87.25	15.1	1902.82 ± 90.41	2160.82 ± 92.79	13.6	**<0.0001**	**0.0062**	0.6095
RE as Protein[1] (MJ/period)	307.85 ± 11.05	282.74 ± 11.30	-8.2	361.43 ± 11.79	311.73 ± 12.04	-13.8	**<0.0001**	**0.0003**	0.2261
RE as Fat and Protein[1] (MJ/period)	1485.78 ± 88.89	1635.69 ± 91.12	10.1	2270.41 ± 94.39	2473.57 ± 96.89	9.0	**<0.0001**	**0.0324**	0.7452
$ME_{fat}^{\ddagger}$ (MJ/period)	1695.45 ± 119.46	1959.70 ± 122.43	15.6	2718.43 ± 126.88	3087.33 ± 130.20	13.6	**<0.0001**	**0.0045**	0.6353
$ME_{protein}^{\ddagger}$ (MJ/period)	1446.02 ± 57.49	1358.09 ± 58.74	-6.1	1801.22 ± 61.31	1566.42 ± 62.61	-13.0	**<0.0001**	**0.0026**	0.1658
$ME_{fat+protein}^{\ddagger}$ (MJ/period)	3163.42 ± 150.27	3332.06 ± 153.92	5.3	4549.92 ± 159.64	4676.41 ± 163.76	2.8	**<0.0001**	0.2905	0.8795
$ME_{gravid\ uterus}^{\ddagger}$ (MJ/period)	45.31 ± 3.52	43.87 ± 3.61	-3.2	54.91 ± 3.73	53.58 ± 3.83	-2.4	**0.0030**	0.6666	0.9839
$ME_{lactation}^{\ddagger}$ (MJ/period)	4404.07 ± 91.85	4282.84 ± 94.78	-2.8	4897.44 ± 97.43	4890.46 ± 102.39	-0.1	**<0.0001**	0.4392	0.4904
$ME_{maintenance}^{\ddagger}$ (MJ/period)	8552.29 ± 76.21	8212.88 ± 76.69	-4.0	9466.36 ± 81.92	9062.90 ± 82.49	-4.3	**<0.0001**	**<0.0001**	0.6726
ME-Intake Requirements (MJ/period)	16193.00 ± 227.88	15917.00 ± 233.93	-1.7	19061.00 ± 241.95	18832.00 ± 251.90	-1.2	**<0.0001**	0.2313	0.9128
$ME_{maintenance}^{\ddagger}$ Overestimate (MJ/period)	8929.30 ± 364.00	8346.96 ± 372.39	-6.5	12121.00 ± 386.93	14070.00 ± 396.55	16.1	**<0.0001**	**0.0453**	**0.0002**
Heat production (MJ/period)	20876.00 ± 303.43	19939.00 ± 310.81	-4.5	25861.00 ± 322.33	27380.00 ± 330.70	5.9	**<0.0001**	0.3011	**<0.0001**
Heat production of gain (MJ/period)	3350 ± 82.17	3313 ± 84.12	-1.1	4248 ± 87.32	4187 ± 89.54	-1.4	**<0.0001**	0.5317	0.8727
Heat production of maintenance (MJ/period)	17526.00 ± 339.67	16626.00 ± 347.82	-5.1	21613.00 ± 360.88	23193.00 ± 370.78	7.3	**<0.0001**	0.2820	**0.0001**
ECR1 (MJ/Kg Calf Gain)	156.07 ± 3.36	153.47 ± 3.44	-1.7	167.1 ± 3.72	182.53 ± 3.55	9.2	**<0.0001**	**0.0507**	**0.0066**
ECR2 (MJ/Kg Calf Gain)	13048 ± 206.17	13122 ± 211.71	0.6	14618 ± 218.77	14536 ± 224.81	-0.6	**<0.0001**	0.9835	0.6809
ECR3 (MJ/Kg Cow and Calf Gain)	169.51 ± 12.27	151.53 ± 12.61	-10.6	148.69 ± 13.12	172.49 ± 13.38	16.0	0.9949	0.3412	**0.0622**

ME-Intake = Metabolisable energy intake; RE = Retained energy ([1]) energy retained in the cow.

$^{\ddagger}ME_{subscript}$ designates the metabolisable energy requirements for the subscripted portion of ME-Intake

ECR1 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth (Means only from cows carrying a calf at the start of mating)

ECR2 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth

ECR3 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of cow and calf growth

Table 7.10: Apparent feed intake, energy retention, metabolisable energy requirements and ECR of cows fed at high and low nutrition as well high and low fat genotype cows from start of mating to weaning.

	Nutrition			Genotype			P-Value	
	Low Nutrition	High Nutrition	Difference (%)	Low Fat	High Fat	Difference (%)	Nutrition	Genotype
Apparent feed intake (kgDM/period)	2332.09 ± 23.1436	3026.43 ± 24.3004	29.8	2646.92 ± 23.2357	2711.59 ± 24.2614	2.4	**<0.0001**	**0.0154**
Total ME-Intake (MJ/period)	24583 ± 220.85	32136 ± 232.33	30.7	281.38 ± 222.18	285.8 ± 231.48	1.6	**<0.0001**	**0.0878**
RE as Gravid Uterus (MJ/period)	16.94 ± 0.3495	18.15 ± 0.3522	7.1	17.767 ± 0.3512	17.32 ± 0.3509	-2.5	**<0.0001**	**0.0457**
RE as Fat[1] (MJ/period)	1263.56 ± 67.6871	2031.64 ± 71.174	60.8	1538.85 ± 68.066	1756.35 ± 70.9441	14.1	**<0.0001**	**0.0062**
RE as Protein[1] (MJ/period)	295.29 ± 8.7954	336.58 ± 9.2926	14.0	334.64 ± 297.23	8.8953 ± 9.2156	-97.3	**<0.0001**	**0.0003**
RE as Fat and Protein[1] (MJ/period)	1560.74 ± 70.7437	2371.99 ± 74.3705	52.0	1878.1 ± 71.1497	2054.63 ± 74.1497	9.4	**<0.0001**	**0.0324**
ME_{fat}‡ (MJ/period)	1827.58 ± 94.9526	2902.88 ± 99.8495	58.8	2206.94 ± 95.4898	2523.51 ± 99.521	14.3	**<0.0001**	**0.0045**
$ME_{protein}$‡ (MJ/period)	1402.06 ± 45.6676	1683.82 ± 48.2744	20.1	1623.62 ± 46.2136	1462.26 ± 47.8462	-9.9	**<0.0001**	**0.0026**
$ME_{fat + protein}$‡ (MJ/period)	3247.74 ± 119.19	4613.16 ± 125.4	42.0	3856.67 ± 119.93	4004.24 ± 124.92	3.8	**<0.0001**	0.2905
$ME_{gravid\ uterus}$‡ (MJ/period)	44.59 ± 2.811	54.24 ± 2.9521	21.6	50.11 ± 2.823	48.72 ± 2.9465	-2.8	**0.003**	0.6666
$ME_{lactation}$‡ (MJ/period)	4343.46 ± 74.585	4893.95 ± 79.3307	12.7	4650.75 ± 71.4619	4586.65 ± 74.5635	-1.4	**<0.0001**	0.4392
$ME_{maintenance}$‡ (MJ/period)	8297.58 ± 63.2423	9271.45 ± 6.5817	11.7	8985.19 ± 64.8785	8583.85 ± 66.0621	-4.5	**<0.0001**	**<0.0001**
ME-Intake Requirements (MJ/period)	15958 ± 189.65	18939 ± 201.35	18.7	17592 ± 183.94	17305 ± 191.03	-98.9	**<0.0001**	0.2313
$ME_{maintenance}$‡ Overestimate (MJ/period)	8731.22 ± 297.39	13096 ± 306.64	50.0	10555 ± 293.58	11272 ± 305.36	6.8	**<0.0001**	**0.0453**
Heat production (MJ/period)	20407 ± 240.65	26621 ± 253.19	30.5	23368 ± 242.13	23660 ± 252.23	1.2	**<0.0001**	0.3011
Heat production of gain (MJ/period)	3329 ± 64.99	4214 ± 68.42	26.6	3796 ± 65.44	3748 ± 68.11	-1.3	**<0.0001**	0.5317
Heat production of maintenance (MJ/period)	17083 ± 269.01	22411 ± 283.1	31.2	19577 ± 270.74	19917 ± 281.94	1.7	**<0.0001**	0.2820
ECR1 (MJ/Kg Calf Gain)	154.76 ± 2.5752	174.81 ± 2.7417	13.0	161.57 ± 2.6593	168 ± 2.6628	4.0	**<0.0001**	**0.0507**
ECR2 (MJ/Kg Calf Gain)	13085 ± 165.28	14577 ± 173.48	11.4	13833 ± 165.89	13829 ± 173.25	0.0	**<0.0001**	0.9835
ECR3 (MJ/Kg Cow and Calf Gain)	160.52 ± 9.8675	160.59 ± 10.4067	0.0	159.1 ± 9.9521	162.01 ± 103451	1.8	0.9949	0.3412

ME-Intake = Metabolisable energy intake; RE = Retained energy ([1]) Energy retained in the cow

‡$ME_{subscript}$ designates the metabolisable energy requirements for the subscripted portion of ME-Intake

ECR1 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth (Means only from cows carrying a calf at the start of mating)

ECR2 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of calf growth

ECR3 = Energy conversion ratio, as measured by the ME-Intake of the cow/calf unit per kilogram of cow and calf growth

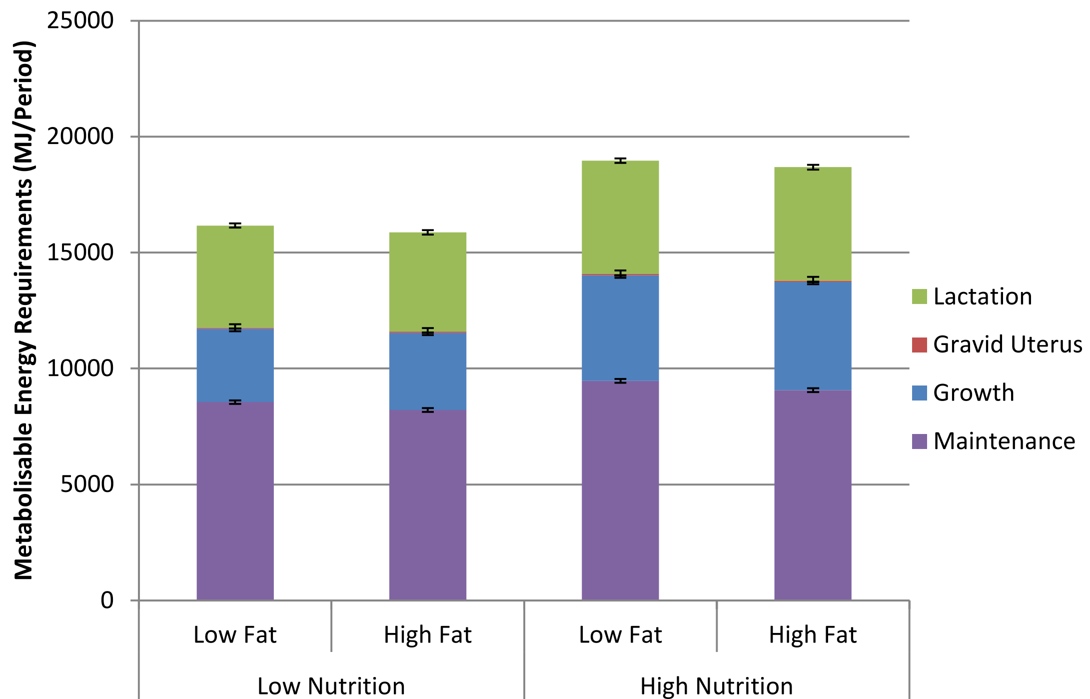

Figure 7.7: Metabolisable energy requirements for cow maintenance, growth, growth of the gravid uterus and lactation of high and low fat genotype animals at two levels of nutrition from start of mating to weaning.

As the energy retained was greater in cows fed at high nutrition from start of mating to weaning, the metabolisable energy requirements for growth was greater in cows fed at high nutrition (P<0.05) (Table 7.10 and Figure 7.7). Metabolisable energy requirements for maintenance were also 11.7% greater due to the heavier weights of cows fed at high nutrition (P<0.05) (Table 7.10). The same was observed for the metabolisable energy requirements during lactation as cows fed at high nutrition weaned heavier calves (Table 7.10). Metabolisable energy requirements for growth of the gravid uterus were negligible (Figure 7.7). High levels of nutrition resulted in heavier birth weights of calves from these cows on high nutrition (Table 7.3). These heavier birth weights resulted in a greater metabolisable energy requirements for the growth of the gravid uterus even though this growth was small from the start of mating to weaning (P<0.05) (Table 7.10).

Due to the differences in energy retained as fat and protein (Table 7.9), the fat genotype of the cow had a significant impact on the metabolisable energy requirement for growth of these tissues (Table 7.10). These requirements for fat deposition were greater in high fat genotype cows than compared to their low fat counterparts (14.3%; P<0.05). However, the low fat cows had a 9.9% greater metabolisable energy requirement for the deposition of protein than the high fat cows (P<0.05). Regardless of these differences, there was no difference between the total metabolisable energy requirements for the deposition of fat and protein between the high and low fat genotype cows (P>0.10) (Table 7.10 and Figure 7.7). This can be explained by the efficiency by which protein and fat are deposited, where protein requires 3.5 times more energy to deposit than fat on a dry matter basis (k_p=0.2 vs. k_f=0.7). This can be seen in the relative differences between energy retained as fat and protein and the metabolisable energy requirement for the deposition of fat and protein, respectively (Table 7.10). Metabolisable energy requirements for lactation were not different between fat genotype cows (P>0.10) (Table 7.10 and Figure 7.7). This is as expected because there was no difference in weaning weights between the cow fat genotypes (Table 7.7). Predicted metabolisable energy requirements for the maintenance of high and low fat cows were greater for low fat cows (4.5%; P<0.05) (Table 7.10 and Figure 7.7), as they had more weight to maintain over this period from the start of mating to weaning (Table 7.10).

The synthesis of these results show that cows fed at high nutrition have increased metabolisable energy requirements for lactation (P<0.05) (Table 7.10). This is because they gave birth to heavier calves, produced more milk by weaning heavier calves, deposited more energy for their own growth, and were heavier. Therefore, they had

more weight to maintain (Table 7.8, Figures 7.6 and 7.7). However, the difference between apparent ME-intake and predicted ME-intake requirements from the nutrition models was considerable between all nutrition and genotype treatments, indicating that apparent ME-intake is over-estimated. This over-estimate in ME-intake was considerably higher in cows fed at high nutrition (50.0%, P<0.05) (Table 7.10 and Figure 7.6). Furthermore, apparent ME-intake was over-estimated to a greater 6.8% extent in high fat cows relative to low fat cows (Table 7.10). However, this is presumably due to a larger over-estimate of high fat genotype cows fed at high nutrition (16.1%; P<0.05) than at low nutrition (-6.5%) (Table 7.9).

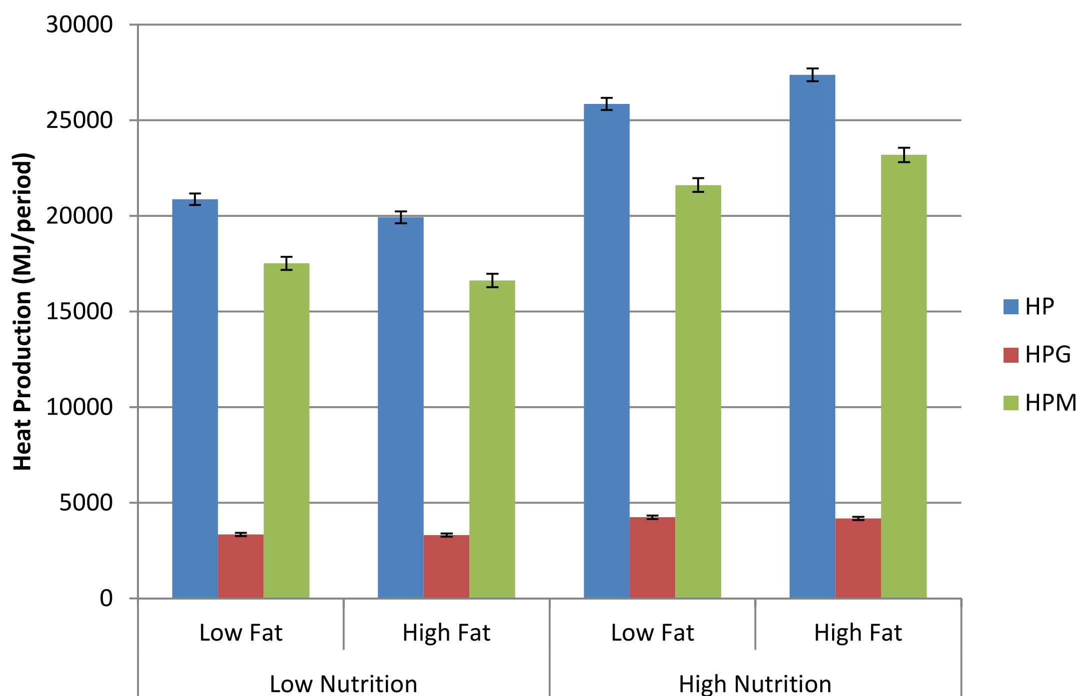

Figure 7.8: Heat production (HP), heat production of gain (HPG) and heat production of maintenance (HPM) of high and low fat genotype animals at two levels of nutrition from start of mating to weaning.

Heat production was greater (P<0.05) in cows on high nutrition (Table 7.10 and Figure 7.8), as was the HPM for these cows. The fat genotype of the cows did not influence the heat production or HPM at low levels of nutrition. Nevertheless, at high levels of

nutrition, the high fat genotype cows had significantly greater heat production and HPM (P<0.05) (Table 7.9 and Figure 7.8). Once again, the accuracy with which the apparent ME-intake is measured is brought into question as there were significant differences in the HPM at high and low nutrition. These differences are more than can be explained by the heat increment of feeding associated with differing nutrition levels and are consistent in this period from start of mating to weaning (Figure 7.8) and in the period from weaning to the start of mating (Figure 7.5). There was no genotype effect on the HPG from weaning to the start of mating (Tables 7.9, 7.10 and Figure 7.8). Cows fed at high nutrition had a significantly greater HPG consistent with greater protein and fat deposition during this period.

The amount of apparent ME-intake required for the growth of the calf was different between nutrition treatments (Table 7.10). At low nutrition, there was a lower efficiency conversion ratio (ERC1) than at high nutrition for cows that calved during the previous period (13.0%; P<0.05). Of those cows that calved, the high fat genotype animals tended to have a lower ECR1 from the start of mating to weaning (4.0% P<0.10). However, these differences in ECR1 were due to the 9.2% decrease in ECR1 of the low fat genotype cows fed at high nutrition (P<0.05) (Table 7.9). Regardless of calving status, there were still large differences between nutrition groups in that cows fed at high nutrition required 11.4% more energy for the growth of their calves ((P<0.05) (ECR2 in Table 7.10). There was no effect of nutrition or genotype treatments on the ECR3 (Table 7.10) even though there were large differences in the weaning rates between the fat genotypes (Table 7.7). However, there was a trend for a lower ECR3 in fat genotype animals fed at high nutrition even though this group had the highest calving rate (P<0.10) (Table 7.9).

The fat genotype of the cows had a significant effect on the RFI ($P<0.0001$) and REI ($P=0.0001$) when regressed on these genotypes during the period of start of mating to weaning. The RFI of low fat genotype cows was -82.58 kgDM overall (-0.53 kgDM/day) and the high fat genotype cows was +82.23 kgDM overall (+0.52 kgDM/day). This equated to a REI of -736.13 MJ overall (-4.68 MJ/day) and 733.03 MJ overall (+4.66 MJ/day) of the low and fat genotype cows, respectively.

7.4 Discussion

The periods studied (from weaning to start of mating and from start of mating to weaning) are representative of the decline in weight and body condition due to seasonal variation and lactation such as may be found in southern Australian breeding operations (Figures 7.1 and 7.2). These periods show some degree of commonality between them in the nutrition and genotype treatment effects. The level of nutrition had a significant effect on the weight of cows and calves, the body composition of cows, the energy requirements of cows and the efficiency of cows in the production system (Tables 7.3, 7.4, 7.5, 7.7, 7.8 and 7.9). The only real deviation from expected of the variable nutrition effects was the absence of an effect on the calving or weaning rates between the high and low nutrition groups. The conclusion from this is that the nutrition treatments herein were not severe enough to produce adverse effects on conception and calving rate at low nutrition. This resulted in there being no difference in the efficiency of ME-intake for calf growth, as calves born from cows on high nutrition weighed more at the start of mating. However, the results presented herein are from a subset of data from a larger experiment which has more power to test potential nutrition effects on calving and weaning rates (Pitchford *et al.*, 2013). A review of nutrition effects on re-

breeding provided conclusive evidence that when nutrition is inadequate (energy and protein), conception rates and hence, calving rates are reduced (Randel, 1990).

In both periods, from weaning to start of mating and from start of mating until weaning, the fat genotype animals differed in RFI and REI, such that the low fat genotype cows consumed considerably less feed and energy than that expected based on their weight, weight gain, growth of the calf and the growth of the gravid uterus. Therefore, the low fat genotype cows had negative RFI and REI, whereas the high fat genotype cows had positive RFI and REI. As discussed herein (Chapters 3, 5 and 6) and in the literature, fat and RFI are correlated and these results are anticipated.

Robinson and Oddy (2004) came to the same conclusion that direct selection for reduced fatness may be more effective at reducing RFI than direct selection of RFI. In the data of Robinson and Oddy (2004), genetic relationships between subcutaneous fat and RFI were r_g=0.72 for P8 fat and r_g=0.48 for rib fat. They also showed that the heritability of RFI is lower (18%) than that of subcutaneous fat (42-45%). Given the high genetic correlation between fatness and RFI, they concluded that selection for reduced RFI would be more effective if selection pressure were exerted on fatness instead. Moreover, Kennedy *et al.* (1993) and van der Werf (2004) showed that selection for RFI is equivalent to selection on the component traits, those being feed intake, weight and weight gain. It follows that if selection for RFI is independent of ADG, the energy content of gain must be less. This is as observed herein and elsewhere. Low RFI or low fat genotype animals deposit less energy in gain through a reduction in the deposition of fat (Arthur *et al.*, 2001b, Basarab *et al.*, 2003, Nkrumah *et al.*, 2004,

Richardson and Herd, 2004, Robinson and Oddy, 2004, Castro Bulle *et al.*, 2007, Lancaster *et al.*, 2009, Kelly *et al.*, 2010).

It would appear that RFI and fatness are physiologically and genetically correlated and to some extent may be the same trait. If this is the case, what is the common underlying cause of variation in RFI and fatness? The earlier results herein suggest that variation in RFI and fatness are caused by variation in actual feed intake, and hence, appetite (Chapter 3). This was observed again herein in that high fat genotype cows had higher apparent feed intakes from weaning to start of mating (Table 7.6) and from start of mating to weaning (Table 7.10). This is further evidence that appetite is correlated with fatness, and hence, RFI. The results also consistently show that genetic selection for RFI is accompanied with corresponding changes in actual feed intake in young animals as well as maturing and mature animals (Chapters 3, 5, 6 and 7).

The results from the fat genotype cows are supported by those in the literature, which suggest that the feed intake during a post-weaning RFI test at *ad libitum* (Herd *et al.*, 1998, Archer *et al.*, 2002, Herd and Pitchford, 2011, Herd *et al.*, 2011) and restricted feed intakes of mature cows (Herd and Pitchford, 2011, Herd *et al.*, 2011) are highly correlated (r_p=>0.50; P<0.05). It must be noted that apparent feed intake herein has not been adjusted for the losses associated with the supplementary feeding or for the losses associated with pugging and trampling during grazing as the adjustments for these were unavailable. Therefore, there is a considerable over-estimation of apparent feed intake herein, but the trends seen by the different genotypes are consistent across years, nutrition treatments and sites.

There is other evidence that selection for fatness and RFI are one and the same. Weights of the low fat genotype cows were greater than the high fat genotype cows at all measurement periods herein. Whilst every effort was made to source cows such that both groups of fat genotype cows were not different in mature cow weight, there was still a difference in mature cow weight EBV (12.0kg; Table 7.1), and this is reflected in the heavier weights of low fat genotype cows across the periods. Across both sites of this experiment (Vasse and Struan), the difference in mature cow weight of low and high fat genotype cows was 13.6kg with the low fat genotype cows being significantly heavier (Pitchford *et al.*, 2013). Interestingly, the trial also included animals that were divergent in RFI from the same source as those herein (Chapters 2, 3, 4, 5 and 6). EBV differences in mature cow weights were reflected in a difference in mature cow weight between the high and low RFI cows of 16.6kg, with the low RFI cows being heavier than the high RFI cows (Pitchford *et al.*, 2013).

These differences in the weights of cows divergent in RFI is the same observation herein and for the whole dataset of cows divergent in fatness (Pitchford *et al.*, 2013). Furthermore, results indicated that cows 2.5-3.5 generations divergent in RFI were also different in cow weight (Pitchford *et al.*, 2013), with low RFI genotype cows being significantly heavier. Herd *et al.* (1998) showed that low RFI cows were 7.1% heavier than high RFI cows as three year olds nursing their second calf ($P<0.05$). Results from 1.8 generations of selection for RFI showed no difference in weights of heifer calves post-weaning between RFI genotypes but the low RFI heifers tended to mature into heavier cows (Donoghue *et al.*, 2011). Furthermore, Arthur *et al.* (2005) reported that at all time periods across the four years that cows were weighed, the low RFI cows divergent for 1.5 generations in RFI were heavier, although this was not significant at

P<0.05. This would suggest that there is a negative genetic correlation between RFI and mature cow weight, which is supported by the significant negative phenotypic correlations (P<0.05) reported in the reviews of Herd and Pitchford (2011), Herd *et al.* (2011) and Herd *et al.* (1998). The fact that there appears to be no difference in the weights of calves divergent in RFI at post-weaning (Arthur *et al.*, 2001b, Arthur *et al.*, 2001c, Schenkel *et al.*, 2004), but there is at maturity (Arthur *et al.*, 2005, Donoghue *et al.*, 2011, Pitchford *et al.*, 2013), leads credence to the hypothesis that RFI changes maturity pattern (type). This hypothesis is supported from the results herein (Chapter 5), which show that low RFI steers have significantly lower ossification scores (a measure of biological maturity) at slaughter than the high RFI genotype steers of the same age.

The results herein show no difference in the weight gain of cows between the high and low fat genotypes between the start of mating and weaning and the weight losses from weaning to start of mating. As RFI is measured independent of weight and weight gain at post-weaning, it can be assumed that no differences would be observed in the weight gains between the high and low RFI cows. Results inferred from Arthur *et al.* (2005) would suggest that this is true. Herd *et al.* (2011) reported a low negative phenotypic correlation between post-weaning RFI and mature cow ADG during RFI testing of mature non-pregnant, non-lactating mature cows (r_p=-0.02; P>0.05). Archer *et al.* (2002) had similar findings where post-weaning RFI was not correlated with average daily gain during the RFI test of mature cows (r_p=0.06; P>0.05). Likewise, Redden *et al.* (2011) found that the correlation between post-weaning RFI and RFI of yearling ewe lambs was r_p=0.05 (P>0.05).

The results demonstrate that high and low fat genotype cows differed significantly in calving and weaning rates, being lower in cows from the low fat genotype. These differences, whilst larger than those associated with RFI in the literature, do show a degree of consistency with this literature. Arthur *et al.* (2005) found that subsequent to 1.5 generations of selection for RFI, there were no differences in the pregnancy, calving, or weaning rates between high and low RFI cows. However, these authors did find a trend towards the low RFI cows calving 5 days later, presumably due to later dates of conception. Donoghue *et al.* (2011) verified these results for pregnancy and calving rates. These authors found that after 1.8 generations of selection for RFI, low RFI cows calved 8 days later than high RFI cows ($P<0.05$). Herd and Pitchford (2011) suggested that if these cows were run under commercial conditions of restricted mating, this difference in 8 days could represent a difference in 8% in pregnancy rates, which is not too dissimilar to those shown herein with the differing fat genotype cows. Both Arthur *et al.* (2005) and Donoghue *et al.* (2011) suggested that this may related due to differences in the onset of puberty. However, this hypothesis is not strong when considering that the onset of puberty in Donoghue *et al.* (2011) was 324.6 days in the high and low RFI heifers, and that for a heifer to calve at 24 months of age, she will be mated some 130 days after the onset of puberty.

It is far more plausible that differences in the day of calving between RFI genotype animals is due to the fatness differences associated with the low and high RFI animals. Data from Donoghue *et al.* (2011) support this hypothesis in that fat heifers were found to cycle earlier ($P<0.05$) and hence, high RFI heifers tended to cycle earlier that low RFI heifers. There is further evidence that differences in pregnancy (and hence, calving and weaning rates) are similar in the fat and RFI genotype cows. Basarab *et al.* (2011)

showed that whilst not significant, low RFI heifers attained puberty 6 days later than high RFI heifers. Furthermore, calving rates of low and high RFI heifers were 72.6% and 84.2%, respectively, with weaning rates of 68.4% and 74.7%, respectively. The death rate of calves from the high RFI heifers was higher, which can be assumed to be due to the four-fold higher rate of twinning in these animals (Basarab *et al.*, 2007). However, calf losses due to abortions were four-fold greater in low RFI heifers. These results are mirrored in the larger data set of Pitchford *et al.* (2013) using heifers 2.5 to 3.5 generations divergent in RFI, where the abortion rate of low RFI genotype heifers was five-fold greater than high RFI heifers ($P<0.02$) (J. Speijers, pers. comm.). Consequently, the low RFI heifers had lower calving and weaning rates.

No differences were observed between high and low fat lines in the efficiency of ME-intake for calf gain and the efficiency of ME-intake for cow and calf gain per cow exposed from weaning to the start of mating and from the start of mating to weaning. However, of those cows that weaned a calf, the efficiency of ME-intake for calf gain was less in the low fat genotype cows, though not overall due to differences in calving and weaning rates between these fat genotype cows (Table 7.9). Arthur *et al.* (2005) reported no differences in efficiency, as measured by weight of calf weaned per cow exposed between cows 1.5 generations divergent in RFI. The results from Arthur *et al.* (2005) are similar to Basarab *et al.* (2007), who found no differences between high and low RFI cows in any of the measures of calf production efficiency. Arthur *et al.* (2005) additionally found that low RFI cows weaned 7kg less per cow exposed than high RFI cows ($P>0.05$). Nevertheless, Basarab *et al.* (2011) found the calving rate was 15.9% greater ($P=0.052$) and weaning rate was 9.2% greater ($P=0.335$) in high RFI heifers. They showed that, whilst not significant at $P<0.05$, the high RFI heifers weaned 12.7kg

more for every heifer exposed than the low RFI heifers. In their data, this equated to weaning 1206kg more in calf weight. This may be cause for concern for selection for Low-RFI animals and warrants further investigation.

7.5 Conclusion

Cows genetically differing in fatness appear to behave similarly to animals differing in RFI. Low fat genotype cows consume considerably less feed and energy than expected based on their weight, weight gain, growth of the calf and the growth of the gravid uterus. Thus, the low fat genotype cows had a lower RFI during both periods of measurement than the high fat genotype cows. Additionally, high fat genotype cows were fatter than low fat genotype cows, as similar to from the differences between high and low RFI genotypes. Low fat genotype cows had higher mature weights (as these genotypes appear to have a later maturity pattern) with no differences in the weight gains of cows and calves or the weaning weights of calves from these cows, similar to low RFI cows. High fat genotype cows had a greater appetite and ate more, as do high RFI cows. Both of these types of cows are possibly fatter as they have greater appetites and eat more (Chapter 8). Whilst not conclusive, high fat genotype cows and high RFI cows tend to both have higher calving rates, weaning rates and weaning weights per cow exposed.

These differences between high and low fat genotypes cows are consistent with cows divergent in RFI. The conclusion is that given the high phenotypic and genotypic correlations between fatness and RFI, selection for feed efficiency may be most easily and cheaply achieved by selecting for fatness. It also highlights that caution should be taken when selecting for low RFI, given the negative effects of high mature cow weight,

a tendency for lower calving and weaning rates (or at least, later conception dates during joining) and the combined effects on lower weaning weights per cow exposed.

Chapter 8

General Discussion

CHAPTER 8: General Discussion

8.1 Introduction

This work was based on the hypotheses of Richardson and Herd (2004) regarding the biological basis of RFI in the Trangie Angus selection lines. These authors proposed that many physiological mechanisms could contribute to the variation in RFI using the data available after one generation of selection in cattle. They hypothesised that the majority of the variation (85%) could be attributed to physiological mechanisms associated with maintenance energy requirements, with 10% due to differences in digestibility and 5% due to differences in body composition. Of the 85% difference in maintenance energy requirements, the heat increment of feeding contributed 9% and physical activity contributed 10%. Protein turnover, tissue metabolism and stress were suggested to contribute 37% of the variation in maintenance energy requirements in steers differing in RFI. The remaining 27% of the variation in RFI could not be accounted for, although the authors suggested that mechanisms such as ion transport may explain this. As yet, there appears to be little evidence to support these hypotheses of Richardson and Herd (2004). The results from experiments herein, and those of others, show that most of the variation in RFI in cattle after 3.5 generations of selection for RFI is due to variation in energy retained as fat and protein and in food intake with the remainder possibly due to differences in activity.

8.2 Biological mechanisms associated with variation in RFI

8.2.1 Appetite

The papers of Kennedy *et al.* (1993) and van der Werf (2004) show that selection for RFI is equivalent to selection on the component traits, those being feed intake, weight and weight gain. The results from herein and from the literature indicate that as expected, selection for divergence in RFI has been associated with divergence in feed intake and not in growth. For example, in heifers divergently selected for RFI over 3.5 generations when restricted to 90% of *ad libitum* requirements (180% ME_m) had a 5% difference in actual feed intake (Chapter 3). Of this feed intake, the low RFI heifers attained an *ad libitum* ME-intake of 86.9% of the predicted *ad libitum* requirements based on SCA (1990). In steers divergently selected for RFI for one generation and 2.5 generations, differences in the *ad libitum* feed intake were 5.1% and 6.7%, respectively (Chapter 6).

This divergence in feed intake was not associated with a difference in the efficiency of high and low RFI animals at near maintenance requirements (Chapters 2, 3 and 6). Divergence in actual feed intake is repeatable at post-weaning and during other stages approaching maturity in *ad libitum* feed cattle (Chapter 3). Since this difference in feed intake is not associated with differences in efficiency, then it can only be due to a divergence in the appetite of these animals at constant weight and weight gain. This is consistent with other research where it has been concluded that residual feed intake is closely related to daily feed intake through phenotypic (Kennedy *et al.*, 1993, Arthur *et al.*, 2001b, Basarab *et al.*, 2003) and genetic correlations (Kennedy *et al.*, 1993, Arthur *et al.*, 2001b).

Between breeds of cattle, there appears to be differences in appetite. Jenkins and Ferrell (2002) reported between breeds, there is considerable variation in the *ad libitum* feed intakes of mature animals in weight stasis between breeds. Their estimates ranged from $0.493WT^{0.73}$ to $0.429WT^{0.73}$ between various breeds of cattle, suggestive of breed, metabolic rate or genetic variation in appetite. The ranking for breeds based on appetite was similar to the ranking for fatness with the exception of one breed, the Charolais which had high appetite and yet appeared not to deposit fat (Jenkins and Ferrell, 2002).

8.2.1.1 Fatness

If selection for divergence in RFI has resulted in divergence in appetite at constant weight and weight gain, there are two possible explanations for the unaccounted feed intake (chapter 3). There may be 1) a difference in the maintenance requirements and/or 2) the energy content of gain must change. No difference in the energy costs were associated with protein turnover between the high and low RFI heifers (Chapter 2). Additionally, no difference was observed in heat production (HP) as measured by CO_2 entry rate (Chapter 3) or by the heat production and heat production of maintenance (HPM) as calculated in these heifers from energetic partitioning (Chapter 6). Thus, one can only conclude that the energy content of gain must have changed as the maintenance requirements are not different.

As maintenance requirements have not changed, the change in the energy content of gain could be due to the differences in protein deposition per unit of energy intake, or rumen efficiency. If rumen efficiency was changed available protein in the small intestine of low RFI animals may be higher and high RFI animals may indeed be protein deficient.

The relationship between energy intake and energy retained has been considered in depth herein (Chapters 3, 5, 6 and 7). It was observed that high RFI heifers 3.5 generations divergent had a ~200% increase in the deposition of fat in their subcutaneous adipose tissues (Chapters 3 and 6). Modelling this response indicated that these high RFI heifers deposit ~26% more fat over the whole body than the low RFI heifers. The conclusion is that the energy intake retained as fat explained most, if not all, of the differences in RFI between these genotypes. These results have been supported by research in pigs. Boddicker *et al.* (2011b) estimated that 87% of the difference in *ad libitum* feed intake between low RFI and control line pig genotypes, which were 5 generations divergent, was reflected in differences in carcass composition. Whilst these conclusions are not implicitly stated in the works of others, there is general agreement that high and low RFI phenotypes have different body composition, primarily due to differences in fatness (Arthur *et al.*, 2001b, Basarab *et al.*, 2003, Nkrumah *et al.*, 2004, Richardson and Herd, 2004, Robinson and Oddy, 2004, Castro Bulle *et al.*, 2007, Lancaster *et al.*, 2009, Kelly *et al.*, 2010).

This relationship between RFI and fatness was demonstrated in cattle RFI selection lines (Chapters 3, 5 and 6). Interestingly, the converse was also true such that cows genetically differing in fatness appeared to be similar to animals genetically differing in RFI (Chapter 7). High fat genotype cows consumed considerably more feed and energy than would be expected based on their weight, weight gain, growth of the calf and growth of the gravid uterus. As a result, high fat genotype cows had a higher RFI than the low genotype fat cows. Additionally, the high fat genotype cows were fatter than the low fat genotype cows, as expected from the differences between high and low RFI

genotypes and expected based on the difference in rib fat EBVs. There was also no real difference in the feed intake of the high and low fat genotype cows on low nutrition, although the high fat genotype cows ate more when on high nutrition. In fact, there was no difference in cows genetically divergent in fatness that could be found from animals divergent in RFI. However, it was not possible to show herein if the differences in the retention of energy as fat can completely explain the higher ME-intake in the fat genotype cows as there was a large error associated with the measurement of ME-intake.

8.2.1.2 Activity related heat production

Most, but not all, of the differences in appetite between high and low RFI steers (1 generation and 2.5 generations divergent) could be accounted for by energy retained as fat and protein and the costs associated with the retention of fat and protein (Chapter 6). In the high and low RFI heifers that were fed in metabolism crates in an animal house, there were no differences in protein turnover (Chapter 2). This indicates that the energetic expenditure associated with the turnover of protein is not different between high and low RFI animals. Moreover, there was no difference in whole body heat production of high and low RFI heifers fed at maintenance (105% ME_m; Chapter 4). However, whole body heat production was slightly higher, though not significant ($P>0.05$), in the high RFI heifers fed at about 90% *ad libitum* requirements (180% ME_m) than the low RFI heifers due to differences in the heat increment of a greater feed intake. Once the energy retained as fat and protein was accounted for, there were no differences in these heifers in heat production (HP) and the heat production of maintenance (HPM) at two feeding levels above maintenance, and therefore, there were no differences in maintenance requirements, heat increment of feeding or activity

(Chapter 6). However, the feedlot steers from one generation and 2.5 generations divergent in RFI were different in both HP and HPM, with HP and HPM being greater in the high RFI steers (Chapter 6).

If there are no significant differences in the HP and HPM of high and low RFI heifers fed at near maintenance and 90% *ad libitum* requirements in metabolism crates but there was in the high and low RFI steers fed *ad libitum* in a feedlot, then these differences may be due to activity related energetic expenditure. This could be associated with differences in feeding behaviour, heat increment of greater ME-intakes and rumination of greater ME-intakes. Activity related heat production has been shown to be positively correlated with RFI. Luiting (1990) was the first of many researchers to show that at least some of the differences in RFI could be explained by differences in activity. In her review, Luiting (1990) showed that 9-33% of the variation in energetic expenditure associated with RFI could be explained by variation in the activity of layer hens. However, Luiting and Urff (1991b) later concluded that up to 80% of the genetic differences in RFI in White Leghorn layers could be attributable to physical activity. Others have also shown that activity accounts for a large amount of the variation in RFI within laying hens (Braastad and Katle, 1989, Katle, 1991).

Similar results to those of layer hens have been observed in other monogastric species. Selection lines in mice for both RFI and heat production have shown differences in activity associated with the divergence in RFI and heat production (Bünger *et al.*, 1998, Mousel *et al.*, 2001, Fenton, 2004). The most notable of these are the observations of Fenton (2004) in mice lines divergent in high and low RFI for 11 generations. The observations of Fenton (2004) showed that high RFI mice were 86% more active than

their low RFI counterparts. Most of these differences were associated with nocturnal activity rather than diurnal activity. This is not the only such finding in mice lines. In mice lines divergent in food intake that were corrected for weight (similar to RFI but without adjustment for weight gain), Bünger *et al.* (1998) showed that 36% of the differences in food intake was due to activity. Similarly, in mice selected for high and low heat loss, 35% of the difference in heat loss between selection lines was due to activity (Mousel *et al.*, 2001). These results in laying hens and mice are supported by the results in pigs (Henken *et al.*, 1991). Henken *et al.* (1991) observed that 6-10% of ME was utilised for physical activity of growing pigs, and that there were large differences between breeds of pigs in physical activity and therefore, efficiency.

In ruminant species, predominantly cattle, activity related energetic expenditure has also been shown to be influenced by RFI although the effect may not be as large as in monogastric species. Richardson *et al.* (1999) reported that high RFI bulls in the feedlot undergoing a RFI test had higher pedometer counts ($P<0.10$), and a correlation with RFI of $r_p=0.24$. These results were supported by data from steers that were progeny of some of these sires (Richardson, 2003), leading to the proposal that a genetic relationship with RFI and activity exists in this population as well. Nkrumah *et al.* (2006) showed that activity related to feeding behaviour was significantly different between high and low RFI steers in the feedlot. These differences in feeding behaviour were associated with a 55% increase in feeding duration ($P=0.006$) and a 97% increase in the number of daily bunk attendances ($P=0.01$) (Nkrumah *et al.*, 2006). Similar results to those reported by Richardson *et al.* (1999) and Nkrumah *et al.* (2006) have been observed in feedlot cattle by Fenton (2004), Basarab *et al.* (2011) and Durunna *et al.* (2011). The conclusion is

that activity and/or feeding behaviour may explain some of the variation observed in RFI in feedlot cattle.

Differences in heat production of maintenance, which may be explained by maintenance requirements, heat increment of feeding and activity between high and low RFI steers in a feedlot are not large. Therefore, assuming no differences in these steers in maintenance requirements as found in heifers in metabolism crates, it is possible that differences in HPM can be explained by differences in the heat increment of feeding (due to differences in ME-intake) and activity (due to differences in feeding patterns). Based on modelling (Appendix 8.1) and the observations of differences in feeding patterns observed by Nkrumah *et al.* (2006), the conclusion is that all of the remaining difference in HPM can be accounted for by differences in ME-intake, feeding duration and the number of bunk attendances (Table 8.1).

Table 8.1: ME-intake and activity related heat production of high and low RFI steers divergent in RFI for one and 2.5 generations.

	Divergence in RFI from Trangie selection lines					
	One generation			2.5 generations		
	Difference	HP of difference (MJ/day)	Percent of HPM	Difference	HP of difference (MJ/day)	Percent of HPM
ME-Intake (MJ/day) [a]	4.4	0.60	12.8	8.6	1.20	25.3
Feeding duration (min/day) [b]	26.2	0.45	9.7	26.2	0.72	15.3
Bunk attendance (events/day) [c]	17.5	2.44	52.3	17.5	3.89	82.1
Total		3.49	74.8		5.81	122.8
Residual HP (MJ/day) [d]	4.66			4.73		

[a, b, and c] From Appendix 8.1.
HP = Heat production
HPM = Heat production of maintenance = Maintenance requirements + Heat increment of feeding + Activity
[d] Heat production of maintenance derived from Tables 6.1 and 6.3 herein.

The difference in ME-intake between high and low RFI steers selected for one and 2.5 generations was 5.1% and 6.7%, respectively (Chapter 6; Tables 6.1 and 6.3). This difference in ME-intake can explain 0.60 MJ/day (12.8%) and 1.20 MJ/day (25.3%) of the difference in HPM of steers one generation and 2.5 generations divergent in RFI through the heat increment of feeding and rumination (Table 8.1) (Appendix 8.1). Assuming a difference in feeding duration of 54.8% between high and low RFI steers as observed by Nkrumah *et al.* (2006), a further 0.45 MJ/day (9.7%) and 0.72 MJ/day (15.3%) of the differences in HPM of steers selected for one generation and 2.5 generations, respectively, may be accounted for. The number of bunk attendances observed by Nkrumah *et al.* (2006) in high and low RFI steers was 96.9% different and could potentially explain a further 2.44 MJ/day (52.3%) and 3.89 MJ/day (82.1%) of the difference in HPM of steers selected for one generation and 2.5 generations, respectively. Thus, the HP related to greater ME-intakes and feeding pattern differences of high RFI steers can explain most of the differences in HPM (74.8%) of steers one generation divergent in RFI and all of the differences in HPM (122.8%) of steers 2.5 generations divergent in RFI.

8.2.2 Biological mechanisms associated with variation in RFI

It would appear from the results presented in this thesis that selection for RFI in the Trangie Angus cattle is equivalent to selection for feed intake and the biological mechanism associated with this difference in feed intake is appetite leading to differences in the composition of gain. This difference was apparent at *ad libitum*, as no difference was observed in feed efficiency on diets near maintenance. When fed at close to maintenance there was no difference in the composition of gain, therefore, it must be appetite leading to the divergence in fat deposition. Selection for a divergence in RFI

(and hence, feed intake at constant weight and weight gain) has resulted in a divergence in appetite between the cattle selection lines utilised herein.

Differences in appetite at constant weight and weight gain between divergent RFI lines were associated with 1) a 2.4% difference in the heat increment of feeding and rumination of divergent ME-intake, 2) a 10.3% difference in the energetic expenditure of activity related feeding behaviour and 3) an 87.3% difference in body composition (Figure 8.1) and (Appendix 8.2).

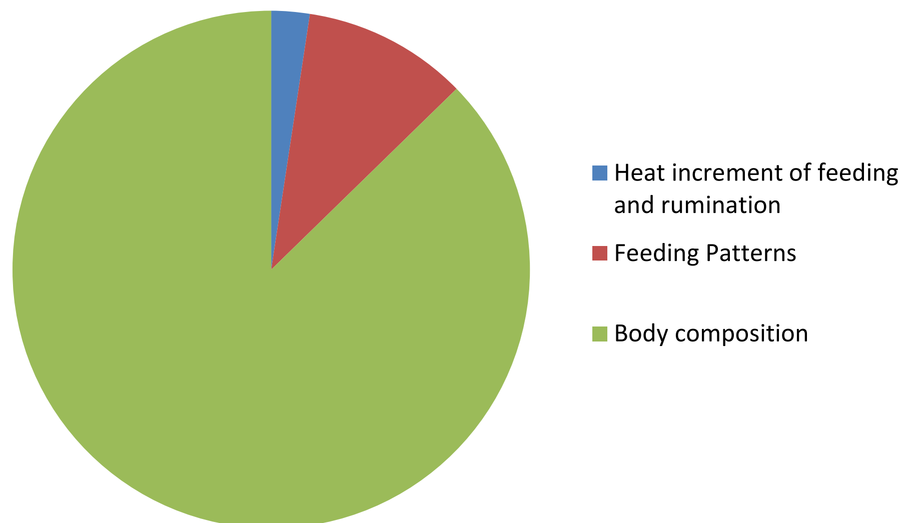

Figure 8.1: Contribution of biological mechanisms to variation in RFI as determined from the experiments on divergently selected steers and heifers herein (Chapters 2, 3 and 6; Appendix 8.2).

The divergence in RFI within the Trangie Angus selection lines has resulted in a divergence in the appetite of these animals at constant weight and weight gain. For animals fed in metabolism crates, this divergence in appetite completely explains the differences in body composition, namely the deposition of fat, with no difference in HP or HPM once body composition was accounted for. However, for animals in the feedlot,

body composition explained most, but not quite all, of the difference between animals divergent in appetite. Significant differences remained in the HPM between these animals. However, these differences can be explained completely by differences in activity associated with feeding behaviour and the heat increment of feeding and rumination of the greater ME-intakes. These relationships from animals divergent in RFI do not deviate from expected based on the differences in appetite or from growth and nutritional models. However, basal metabolism, the trait that was hoped and expected could be manipulated, appears unchanged through selection for RFI.

8.3 Biological mechanisms not associated with variation in RFI

8.3.1 Protein metabolism

Richardson and Herd (2004) proposed that protein metabolism, tissue metabolism and stress accounted for 37% of the variation in RFI of steers post-weaning after one generation of divergent selection for RFI. This hypothesis was the premise for the experiments conducted herein (Chapter 2). However, measurements in heifers 3.5 generations divergent in RFI failed to show differences in protein metabolism between high and low RFI heifers (Chapter 2). In other experiments, the fractional degradation rate of myofibrillar proteins as estimated by the quantification of urinary 3-methyl-histidine was significantly related with residual feed intake (Castro Bulle *et al.*, 2007, Sainz *et al.*, 2007). However, Richardson and Herd failed to show that selection for RFI was correlated with the degradation of myofibrillar proteins as measured by urinary 3-methyl-histidine excretion (Richardson *et al.*, 2004).

Further evidence suggesting that protein metabolism is not likely to be associated with the variation in RFI was provided from the steers divergent in RFI for 2.5 generations.

Calpastatin activity was greatest in high RFI steers and as a consequence m- and μ-calpain was less available for post-mortem proteolysis in the high RFI line, meaning less protein degradation (Chapter 5). These results are contrary to those reported by McDonagh *et al.* (2001), but are supported by the associated differences in the ageing rate of meat (Table 5.3). In addition, Baker *et al*. (2006) reported no difference in calpastatin activity between high and low RFI steers. Thus, in total, there is little evidence for an association between protein metabolism and RFI.

8.3.2 Heat production

Protein metabolism was only one of the mechanisms suggested by Richardson and Herd (2004) that contribute to variation in RFI. Overall, Richardson and Herd (2004) concluded that up to 85% of the variation in RFI could be explained by differences in HP of which protein metabolism accounted for the largest proportion. Interestingly, they did not observed differences in HP between high and low RFI steers divergent in RFI for one generation (Richardson *et al.*, 2001). Further experiments herein (Chapter 3) also failed to show significant differences in HP between the high and low RFI heifers 3.5 generations divergent in RFI, as determined by CO_2 entry rate. Lancaster (2008) reported similar results, in that at maintenance and during times of restricted feeding, heat production (as measured by heart rate) was similar between RFI phenotypes or was greater in low RFI beef cattle. In growing cattle, similar results have been reported by Castro Bulle *et al.* (2007). However, Nkrumah *et al.* 2006 reported that high RFI animals had increased HP above that which could be explained by differences in ME-intake. However, observations show that this difference in HP could be explained by the feeding patterns and activity associated with variation in RFI.

Others, such as Gabarrou *et al.* (1997), reported in high and low RFI cockerels deprived of feed, there was no difference in HP. This led these authors to conclude that there was no difference in basal metabolic rate. In pigs, Boddicker *et al.* (2011b) showed no difference in feed intake between the selected and control RFI lines when the pigs were fed at weight stasis (i.e., maintenance). There is no evidence that variation in RFI can be explained by differences in ME-intake other than that associated with differences in the heat increment of feeding associated with the greater ME-intakes of high RFI animals.

8.3.3 Maintenance requirements

If the heat production of animals selected for divergence in RFI has not changed, it follows that the maintenance requirements of these animals has not changed. Herd and Pitchford (2011) hypothesised that the variance in RFI is much greater at *ad libitum* than when feed is restricted. A 36-fold difference was observed in the variance of feed intake of the heifers fed 90% *ad libitum* than at near maintenance where there was essentially no variance in feed intake or weight gain and therefore, efficiency *per se* (Chapter 3). Herd *et al.* (2006) reported in Angus cows that the variance in RFI at near-maintenance conditions was not associated with RFI under *ad libitum* conditions as heifers or as mature cows. Others have found similar results in composite beef heifers (Roberts *et al.*, 2007), lactating dairy cows (Veerkamp *et al.*, 1995), layer hens (Bordas *et al.*, 1995) and rainbow trout (Silverstein, 2006) as reviewed by Herd and Pitchford (2011). Their review concluded that due to the annual variation in energy availability, whether due to feed quality or quantity, there may be negligible impact of variation in RFI during periods of restricted energy intake (Figure 8.2).

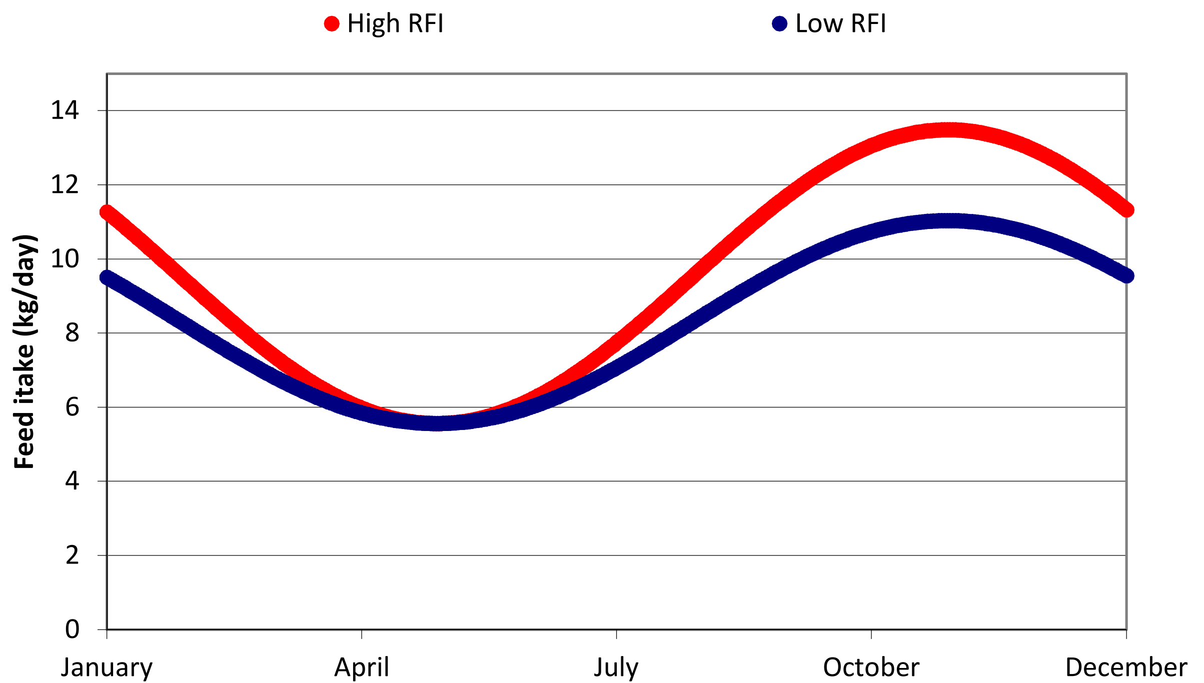

Figure 8.2: Hypothesis of the variance in feed intake of high and low RFI animals associated with energy restriction in a southern Australian production system.

8.4 Value of low maintenance requirements in production systems

Feed consumption of the cow in breeding operations can represent up to 75% of annual feed requirements (Gregory, 1972, Klosterman, 1976, Archer *et al.*, 1999). Of this, maintenance requirements of the cow can represent 60-75% and consequently, about 50% of the total energy requirements of the breeding operation (Ferrell and Jenkins, 1984, Archer *et al.*, 1999). This demonstrates the importance of reducing cow maintenance requirements in breeding operations. As yet, selection for low RFI fails to reduce the maintenance requirements of these animals. However, this is not surprising given that there has only been 3.5 generations of selection for RFI, and where RFI selection does not directly select on maintenance requirements.

This poses four important questions:

1) How much variation in maintenance requirement exists?

2) Has any change been affected in the maintenance requirements of animals?

3) What effect will low maintenance requirements have on the fitness of these animals?

4) What can be measured to alter maintenance requirements?

Most of the primary literature on the variation in maintenance requirements in animals has been available for more than 40 years and was reviewed by Ferrell and Jenkins (1985). Ferrell and Jenkins (1985) showed that there is more variation in the requirements for maintenance than the requirements for growth, gestation and lactation. This variation in maintenance requirements appears to be positively associated with production potential, whether that is potential for growth or lactation and may rather be reflective of feed intake for productive purposes and not maintenance *per se.*

Data from their review suggest that little of the variation in maintenance heat production is attributable to variation in body composition. However, a large proportion of the difference in maintenance heat production appears to be from the heat production of protein synthesis of the liver and gastrointestinal tract, correlated with the mass of these organs. The mass of these organs ($kg/kgBM^{0.75}$) varies between breeds, maturity type, diet, stage of maturity and physiological state. From the feedlot trial (Chapter 5), liver weights at slaughter were not significantly different between high and low RFI genotype steers (unpublished). The regression of liver weight on RFI genotype subsequent to 2.5 generations of selection for RFI was not also significant (P=0.47; High RFI = 6.26±0.08kg and Low RFI = 6.35±0.08kg).

Has any change been affected in the maintenance requirements of animals? Johnson *et al.* (2003) compared the energetics of a circa 1900 steer extrapolated from Kellner and

Goodwin (1909) with that of a circa 1996 steer extrapolated from NRC (1996) (Table 8.2). This comparison really only showed small differences in the energetics of these animals even though the phenotype has changed remarkably. The most notable difference was in the reduction in faecal energy losses but this was associated with grain feeding compared with roughage diets (Johnson *et al.*, 2003). These comparisons would contribute to the idea that maintenance requirements due to basal metabolism (fasted heat production) and the costs associated with increased protein deposition comprise a much greater proportion of gross energy intake in today's animals than those of Kellner's era. The evidence suggests that basal metabolism (as measured by fasted heat production) is unchanged over the last 100 years of animal breeding. Perhaps this is not surprising because 1) there has been no direct selection on fasted heat production and 2) data from the International (human) HapMap Consortium shows genes controlling basic processes (and by extension, maintenance requirements) are three-fold more conserved (i.e. less likely to be involved in recombination/mutational events that are not lethal) than genes controlling adaptive traits (such as feed intake) (Frazer *et al.*, 2007).

Table 8.2: Disposition of dietary energy of circa 1900 vs. circa 1996 steers. Adapted from Johnson *et al.* (2003).

Item	Circa 1900[a] steer, MJ/day	% of GE intake	Circa 1996[b] steer, MJ/day	% of GE intake	Beef system, cow through feedlot, %
Gross energy intake	221.3	100	179.9	100	100
Faecal energy	66.5	30	27.2	15	39.6
Urine energy	7.1	3	7.1	4	4.9
Gaseous energy	14.2	6	4.6	3	5.4
Heat of tissue synthesis	26.4	12	37.2	21	8
Fasted heat production	72.4	33	72.0	40	36.3
Retained in empty body	34.7	16	31.8	18	5.8

[a] Data from respiration calorimetric monitored "well-fed ox" extrapolated from Kellner and Goodwin (1909)

[b] Data from slaughter balance derivation for a 600kg feedlot steer, extrapolated from NRC (1996)

Given that it is possible to reduce maintenance requirements (despite the evidence is for the contrary), what effect will low maintenance requirements have on the robustness of these animals in a production system? Rauw *et al.* (1998) suggested that improvements in production efficiency may have undesirable consequences on fitness traits and can lead to behavioural, physiological and immunological problems. The high energy costs of animals with high production efficiencies may result in less energy for maintenance requirements associated with overcoming challenges in the production environment. It may be that a reduction in the appetite of low RFI animals with continued selection will compromise the ability to maintain physiological functions such as reproduction and health in animals in variable nutritional environment.

What can be measured to alter maintenance requirements? The research (described above) suggests that there may be variation in maintenance requirements. However, given that fasted heat production has not changed in the past 100 years of animal breeding, it is unlikely that substantial gains will be made with regard to the reduction in maintenance requirements, at least using traits currently under selection. Therefore, other than improvements in output traits, improvements in the production system are unlikely to be achieved through a reduction in maintenance requirements. Johnson *et al.* (2003) in the comparison of a circa 1900 vs. circa 1996 steer showed that most of the advances in the efficiency of feed usage have been through improvement in the digestibility of diets (Table 8.2). However, improvements in the heat losses associated with feed energy (i.e. heat lost in faeces, urine and methane) itself may yield the biggest improvement in the efficiency of feed usage without impacting on the carcass. These energy losses are, in effect, a dead loss as energy is wasted that could be stored as protein and fat by the animal.

Wilkes *et al.* (2011) compared the growth and energy balance of Damara and Merino lambs and found that the energy retention of Damara lambs was 24% greater than that of Merino lambs on an *ad libitum* roughage diet (7 MJ ME/kgDM) with no difference in feed intake. This was partially due to a 19% greater loss of energy in the faeces of the Merino lambs with a digestibility of 51.5% and 41.9% for Damara and Merino lambs, respectively. However, when the lambs were fed *ad libitum* high quality feed (11 MJ ME/kgDM), no differences in the digestibility were observed between Damara and Merino lambs. On this diet, the Damara lambs retained 5% more energy due to a 5% increase in feed intake. Whilst the differences in digestibility are much more impressive on *ad libitum* roughage diets in this study, it does suggested that improvements can be made in feed utilisation through improvement in digestibility and reduced faecal loss. Therefore, improving digestive efficiency through use of improved pastures could have one of the largest impacts on the efficiency of grazing animal production and genetic selection per se.

Recent interest in greenhouse gas production and the environmental implications has bought methane production by ruminants into the limelight. However, a decrease in methane emissions from ruminants not only has environmental benefits but also improvement in the efficiency of feed utilisation through the reduction in dietary carbon losses (Johnson and Johnson, 1995, Martin *et al.*, 2010). Blaxter and Clapperton (1965) advised that methane production was related to the digestibility of feed and that as feed intake increases to levels above maintenance, methane production decreases per unit of feed intake. This relationship was found to equate to a 1.6% reduction in the gross energy released as methane per unit of feed intake (Johnson *et al.*, 1993). This suggests

that as appetite and growth rate increase, methane production per unit of growth would be expected to decrease. However, in the context of RFI, this does not hold true as selection for RFI and hence, appetite, at constant weight and growth rate, means that per unit of growth or body weight maintained, methane production in high RFI animals would be more that low RFI animals. Even so, selection for RFI to reduce methane emissions has attracted much interest (Herd *et al.*, 2003a, Hegarty *et al.*, 2005, Nkrumah *et al.*, 2006, Hegarty *et al.*, 2007, Jones *et al.*, 2011, Torok *et al.*, 2011). However, the fundamental hypothesis underpinning this interest is as yet unproven as the relationship between gross energy intake and methane production remained unchanged in these animals. Reducing carbon losses as methane may be more profitably focused on manipulating the microbial profile of the rumen to increase carbon availability for production (Johnson and Johnson, 1995, Martin *et al.*, 2010).

8.5 Practical alternatives to RFI

8.5.1 Growing animals

Growth rate of progeny to be slaughtered can impact significantly on the maintenance requirements of production systems. A faster growing animal effectively reduces the feed intake associated with maintenance energy requirement as fewer days are necessary to meet slaughter requirements. Modelling this reduction in maintenance energy requirement in growing animals would imply that total energy requirements for maintenance and growth can be halved for each doubling of growth rate (ARC, 1980). For example, a 250kg steer growing at 1.25kg/day to a slaughter weight of 500kg would require 16096MJ for maintenance and growth. By comparison, a 250kg steer growing at 0.63kg/day to a slaughter weight of 500kg would require 30567MJ for maintenance and growth. This is effectively half that of the steer growing at 1.25kg/day. For this reason,

crossbreeding cows to fast growing terminal sires can reduce the maintenance energy requirement for the cow and calf per kg beef produced, note this example also highlights the importance of providing high quality feed.

8.5.2 Breeding animals

Selection for low RFI resulted in leaner cows of higher mature weights, reduced calving and weaning rates, and increasing the days to calving in subsequent matings. The cumulative effect of these changes is that the cows would not be suitable in most breeder production systems. If this is true, what alternatives may exist to reduce maintenance feed requirements of the cow as a whole?

Goddard *et al.* (2011) suggested that herd feed conversion efficiency, the amount of beef produced compared to the amount of feed consumed within the production system, can be modulated in several ways. They proposed that this can be achieved by increases in the net reproductive rate, increasing growth rate without the associated increases in cow maintenance requirements (through increased size), and decreasing RFI. However, reducing RFI is unlikely to lead to improvements in whole herd feed conversion. So what are the alternatives for producers?

One means of improving whole herd feed conversion efficiency may be through the use of cows of more moderate size. Ferrell and Jenkins (1984) showed that there were no difference in the maintenance energy requirements (MJ/kgBW$^{0.75}$/day) between crossbred cows of differing mature sizes. This indicates that even though there is no difference in the energy requirements for maintenance for weight adjusted cows, high mature cow weights result in higher maintenance energy requirements. Hence, there are

increases in the overall energetic needs for production of the cow/calf system (Jenkins and Ferrell, 2002).

Additionally, high mature cow weights have some negative correlations that reduce output traits from the production system and hence, further increase the proportion of maintenance requirements of the cow relative to the production system as a whole. Nugent *et al.* (1993) showed that cows of breeds with high mature cow weights had extended calving intervals associated when the nutritional environment was restricted. Thus, when nutrition is restricted, net reproduction decreases through extended calving intervals (Morris *et al.*, 1993, Jenkins and Ferrell, 1994). Cows with lower mature cow weights have reduced maintenance requirements (as a direct response to weight) and an associated improvement in whole herd feed conversion efficiency through increases in net reproductive output. This is due to reduced dystocia and where short mating practices exist in a limiting nutritional environment, a reduction in calving intervals.

Moderating the lactation potential of cows also reduces maintenance requirements. Ferrell and Jenkins (1984) showed that non-lactating, non-pregnant cows with high genetic lactation potential (i.e. dairy and dual purpose types) had increased maintenance requirements (MJ/kgBW$^{0.75}$/day) compared to cows with low lactation potential. This has been confirmed by other authors. Regardless of lactation status (i.e. non-lactating cows, heifers, bulls and steers), animals with high genetic potential for lactation have higher maintenance requirements relative to those with low lactation potential (Gareett, 1971, Chestnutt *et al.*, 1975, Vermorel *et al.*, 1976, Truscott *et al.*, 1983, Ferrell and Jenkins, 1985, Taylor *et al.*, 1986, Montano-Bermudez *et al.*, 1990). Moreover, the effect of high lactation potential on the negative energy balance in a low nutritional

environment affects the timing of postpartum ovulation and increases calving intervals. The net reproductive losses associated with short mating periods are well defined.

Dilution of the maintenance requirements of the whole herd can be achieved through increases in net reproductive rate (Goddard *et al.*, 2011). Positive fat cover (above average for EBV) has been implicated as one of the major factors controlling net reproductive rates. DeRouen *et al.* (1994) reported that body condition score (fatness) at calving in primiparous cows resulted in greater subsequent conception rates and reduced postpartum anoestrus in primiparous cows with greater body condition scores. Spitzer *et al.* (1995) found that higher body condition scores reduced the time in returning to estrus after calving in primiparous cows. In mature cows, Selk *et al.* (1988) showed that higher pre-calving body condition scores results in higher conception rates. However, in mature cows, animals with pre-calving body condition scores of greater than 5 (1-9 point scale; equivalent to 2.5 on 0-6 point scale) show no additional improvement in pregnancy rates (Richards *et al.*, 1986, Selk *et al.*, 1988, Kunkle *et al.*, 1994). In this case, the extra energy associated with the deposition of fat, such that body condition score is greater than 5 (1-9 point scale) would be nutritionally expensive for no additional return. This is less well defined in the primiparous cows where DeRouen *et al.* (1994) reported improvement in pregnancy rates with body condition scores up to 7 (1-9 point scale).

Increases in the reproductive output through twinning will also improve net reproductive rate. Computer simulation has suggested that feed inputs can be reduced by 24% in cows that produce twins (Guerra-Martinez *et al.*, 1990). Furthermore, in the simulations of Herd *et al.* (1993), there was a 25% increase in the efficiency of lean

production associated with twinning as opposed to traditional systems. De Rose and Wilton (1991) demonstrated a 73% increase in the combined weight of twins compared to singletons per cow that calved. Whilst pre-weaning growth rates of twins was less than those of singletons, this study showed no differences in the post-weaning growth rates of twin or singleton calves. Moreover, the profitability of twins after weaning was greater than that of singletons in that feedlot profitability in twin calves was 26% greater than singleton calves due to improved feedlot performance. Additionally, lifetime profitability was 22% greater in twin calves than singleton calves as twins grew more rapidly with respect to their birth weights and cow maintenance requirements were shared between two calves (De Rose and Wilton, 1991). Gregory *et al.* (1990) concluded that cow productivity could be up to 40% greater in cows that produced twins. However, the associated increase in dystocia, stillbirths, calf death within 3 days of calving and negative effects on the re-breeding of cows that bore twins make this a less attractive option for decreasing the feed requirements of the cow (Gregory *et al.*, 1990).

8.6 Conclusions

Direct selection for feed efficiency in beef cattle (FCR) in the past has indicated some potential drawbacks. One issue is that FCR is highly correlated with average daily gain; therefore, selection for high growth alone is much more cost-effective than measuring individual feed intake. Another problem is that this measure of feed efficiency would tend to select for animals with greater muscle mass and less fat deposition. Additionally, selection for increased FCR results in increased mature size and increasing the size and energy requirements of cows would not be a goal of most commercial operations.

Due to these issues with selecting for feed conversion ratio (FCR), it was anticipated that RFI may be an alternative to genetic selection for FCR (Koch *et al.*, 1963). It was thought that RFI could be used for genetic selection with much more confidence in beef production systems as it was supposed to be independent of average daily gain, body weight and mature size. However, all the evidence from the experiments conducted herein show that the only biological mechanisms that appear to be affected through selection for RFI is appetite and activity at constant weight and daily gain. The 2 main implications are not trivial: 1) animals that have a greater appetite and consume more energy at constant weight and daily gain deposit more energy as fat, and 2) animals that deposit more energy as fat do this due to a greater appetite.

The mechanisms controlling appetite in the Trangie Angus selection lines remain unknown. It would appear that in these animals, there are weaker negative feedback mechanisms from fatness to reduce the feed intake in the high RFI animals. This is a very complex system and beyond the scope of this thesis. However, these animals would be a useful resource to study factors controlling appetite in ruminants.

All the evidence concludes that reducing maintenance requirements through selection for RFI may not be possible and may be detrimental to animal fitness. However, if RFI is to be used as a tool for improving feed utilisation, then adjustment for body composition would need to be considered. Given that improving feed utilisation is only reasonable in the growing animal, then feed conversion would be much easier to implement given the high generic and phenotypic correlations between FCR and growth rate. Currently, producers do not have good measures for the variation in feed utilisation for maintenance to target in selection programs. In the absence of such measures,

producers should be encouraged to focus on measurable output traits in their selection programs.

Appendices

Appendix 3.1

Estimation of the relationship between CO_2 entry rate and heat production in beef cattle

The prediction equation of heat production from CO_2 entry rate in sheep has been applied to estimation of heat production herein (Chapter 3) previously in sheep (Corbett *et al.*, 1971). The equation of this estimation is given below and is the mean of 56 intravenous observations herein.

$$HP\ (kcal/hour) = 2.30 \times RCO_2 + 24.02$$ (Corbett *et al.*, 1971)

Therefore, converting this equation to a MJ/day basis

$$HP\ (MJ/day) = 0.0096 \times RCO_2 + 2.41$$

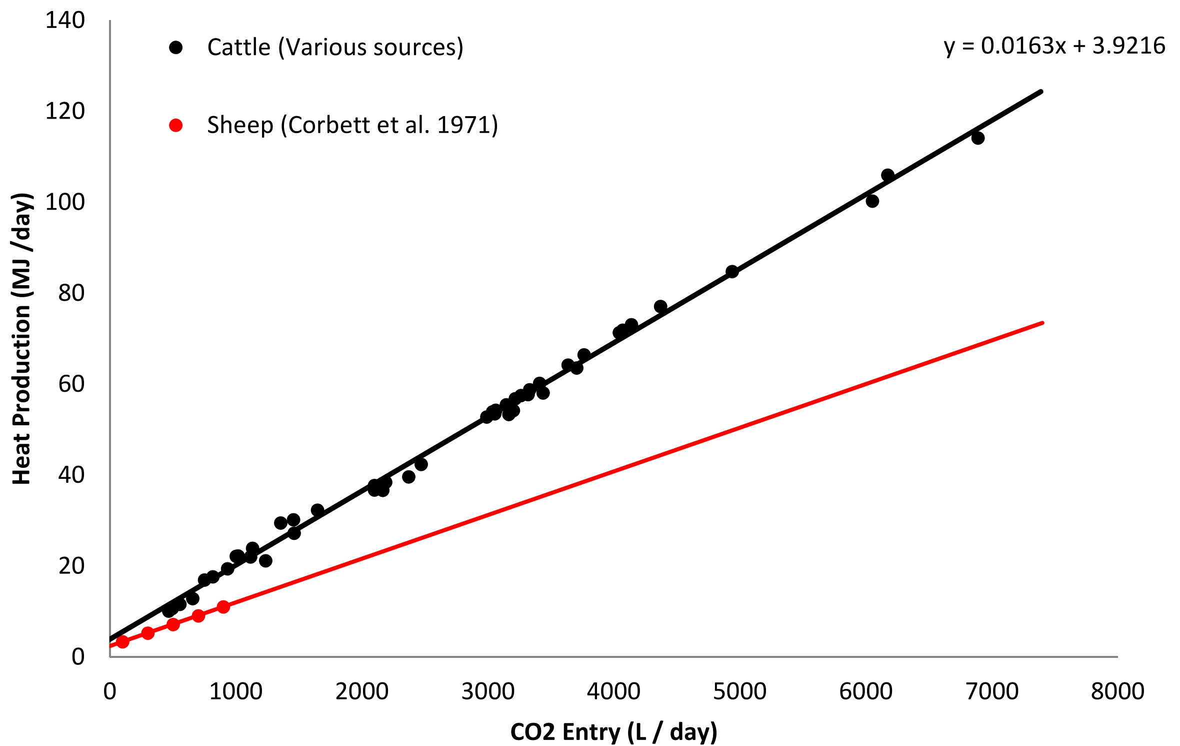

Figure 9.1: Relationship between CO2 entry rate and heat production in beef cattle from literature sources and in sheep from Corbett et al. (1971)

When compared to literature sources (below) (number of observations = 46) of the relationship between CO_2 entry rate and heat production in cattle, it can be observed that the prediction equation by Corbett *et al.* (1971) under-predicts heat production by as much as a third (Figure 9.1). Therefore, heat production can be more accurately calculated in cattle from CO_2 entry rate using the equation (Figure 9.1) below:

$$HP\ (MJ/day) = 0.0163 \times RCO_2 + 3.92$$

The various literature sources (and their various assumptions) used to generate this prediction equation are below.

Blaxter, K & Wainman, F 1966, 'The fasting metabolism of cattle', *British Journal of Nutrition*, vol. 20, no. 01, pp. 103-111.

Brouwer, E 1965, 'Report of sub-committee on constants and factors', *Energy metabolism*, pp. 441-443.
(Assuming RQ = 1.00)

Huntington, GB & Tyrrell, HF 1985, 'Oxygen consumption by portal-drained viscera of cattle: Comparison of analytical methods and relationship to whole body oxygen consumption', *Journal of Dairy Science*, vol. 68, no. 10, pp. 2727-2731.

Junghans, P, Voigt, J, Jentsch, W, Metges, C & Derno, M 2007, 'The 13C bicarbonate dilution technique to determine energy expenditure in young bulls validated by indirect calorimetry', *Livestock Science*, vol. 110, no. 3, pp. 280-287.
(Assuming REC = 0.73)

Lapierre, H, Tyrrell, H, Reynolds, C, Elsasser, T, Gaudreau, P & Brazeau, P 1992, 'Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: whole-body energy and nitrogen metabolism', *Journal of Animal Science*, vol. 70, no. 3, pp. 764-772.

Reynolds, CK, Tyrrell, HF & Reynolds, PJ 1991, 'Effects of Diet Forage-to-Concentrate Ratio and Intake on Energy Metabolism in Growing Beef Heifers: Whole Body Energy and Nitrogen Balance and Visceral Heat Production', *J. Nutr.*, vol. 121, no. 7, July 1, 1991, pp. 994-1003.
(Assuming RQ = 1.00)

Varga, G, Tyrrell, H, Huntington, G, Waldo, D & Glenn, B 1990, *Utilization of nitrogen and energy by Holstein steers fed formaldehyde-and formic acid-treated alfalfa or orchardgrass silage at two intakes*, *Journal of Animal Science*, vol. 68, no. 11, Am Soc Animal Sci, pp. 3780-3791.

Appendix 5.1

Table 9.1: Means, standard deviations and ranges in values for traits for the feedlot steers 2.5 generations divergent for RFI and for their sires and dams.

	Mean	s.d.	Min.	Max.
Steers				
Number	208			
Age at induction (days)	447	17	396	496
Weight at induction (kg)	439	31	365	520
Rib fat at induction (mm)	7.8	2	3	13
EMA at induction (cm^2)	64	6	50	82
ADG Days 1 to 35 (kg/day)	1.38	0.57	-1.09	4
Weight at Day 35 (kg)	487	32	412	592
ADG Days 35 to 113 (kg/day)	1.23	0.28	0.28	2.1
Weight at Day 113 (kg)	583	35	510	682
ADG Days 114 to 251 (kg/day)	0.92	0.2	0.14	1.65
ADG Days 1 to 251 (kg/day)	1.08	0.13	0.66	1.43
Final weight (kg)	710	41	630	832
Feed intake Days 35 to 113 (kg/day)‡	11.3			
Feed intake Days 114 to 251 (kg/day)‡	10.8			
Feed intake Days 1 to 251 (kg/day)‡	11.1			
FCR Days 35 to 113 (kg/kg) ‡	9.2			
FCR Days 114 to 251 (kg/kg) ‡	11.7			
FCR Days 1 to 251 (kg/kg) ‡	10.3			
RFI Days 35 to 113 (kg/day) ‡	2			
RFI Days 114 to 251 (kg/day) ‡	2.2			
RFI Days 1 to 251 (kg/day) ‡	2.9			
Hot carcass weight (kg)	415	27	354	494
Dressing percentage (%)	58.5	1.4	54.1	64.5
EMA on carcass (cm^2)	77	3.3	68	85
Rib fat depth on carcass (mm)	17.9	5.6	6	34
Seam fat (cm^2)	24.2	6.2	9.7	44.2
Ausmeat marble score	3.2	1	2	6
MSA marble score	504	107	350	830
IMF (%)	14.5	3.1	8.3	22.7
Fat colour code	1.2	0.4	0	2
Meat colour code	1.9	0.4	1.3	3
Ossification	141	10	110	160
Ultimate pH	5.5	0.1	5.4	5.7
Calpastatin Activity	2.6	0.8	1.2	3.9
Peak Force day 1 (kg force)	3.3	0.7	2.0	7.1
Compression day 1 (kg force)	1.2	0.2	0.7	2.0
Cook Loss day 1 (%)	11.3	2.6	5.6	20.9
L* day 1	39.7	3.2	30.0	47.7
a* day 1	27.2	2.6	18.0	33.7
b* day 1	13.4	1.9	6.2	17.3
Peak Force day 7 (kg force)	3.0	0.6	2.0	5.3
Compression day 7 (kg force)	1.1	0.2	0.6	2.0
Cook Loss day 7 (%)	11.1	2.2	5.8	18.3
L* day 7	40.7	3.1	30.4	50.5

a* day 7	28.4	2.5	19.3	36.1
b* day 7	14.1	1.9	7.1	18.8
Sires				
Number	26			
Number of progeny	8	5.4	1	21
RFI-EBV (kg/day)	0.02	0.69	-0.92	1.24
Accuracy of RFI-EBV (%)	67	17	44	87
Dams				
Number	208			
RFI-EBV (kg/day)	-0.07	0.36	-1.13	0.87
Accuracy of RFI-EBV (%)	63	8	49	77

‡ Individual animal feed intakes were not able to be measured.
ADG=average daily gain; EMA=area of eye-muscle
L* = Minolta lightness colour value
a* = Minolta red-green colour value
b* = Minolta yellow-blue colour value

Appendix 8.1

Calculation of energetic differences arising from ME-intake and feeding patterns.

Heat production resulting from differences in ME-intake (HP_{DMEI}) due to the heat increment of feeding (*HEI*) and time of rumination is calculated as follows.

$$HP_{DMEI}\ (MJ/day) = HEI + Rumination$$

Where:

$$HEI\ (MJ/day) = 0.09 \times MEI \times DMEI$$

$$Rumination\ (MJ/day) = 5.52 \times DMEI \times 0.002 \times Wt$$

Where:

The time associated with rumination is 5.52 (hours/day) adapted from Gonzalez *et al.* (2009), and 0.002 (MJ/kg/hour) is the energy cost of ruminating per kg live weight (*Wt*) from SCA (1990) and *DMEI* is the difference in ME-intake between high and low RFI steers from Chapter 6, Tables 6.1 and 6.3.

Heat production associated with differences in feeding duration (HP_{FD}) due to activity related HP differences between high and low RFI steers of eating and standing at the bunk is calculated as follows.

$$HP_{FD}\ (MJ/day) = Eating + Standing$$

Where:

$$Eating\ (MJ/day) = 0.0025 \times 0.44 \times Wt$$

$$Standing\ (MJ/day) = 0.0004167 \times 0.44 \times \mathrm{Wt}$$

Where:

The energy cost of eating is 0.0025 MJ/kg/hour derived from SCA (1990), 0.44 is the time difference (proportion of 1 hour; i.e. 26.2 minutes) derived from Nkrumah *et al.*

(2006) which is the difference in feeding duration between high and low RFI steers from Nkrumah et al (2004), and 0.0004167 (MJ/kg/hour) is the energy cost of standing compared to lying down is derived from SCA (1990).

Heat production associated with differences in bunk attendance (HP_{BA}) due to activity related to changing body position (*CBP*; i.e. lying down and standing again) as well as walking to and from bunks can be calculated as follows

$$HP_{BA}\ (MJ/day) = CBP + Walking$$

Where:

$$CBP\ (MJ/day) = 0.00026 \times 17.5 \times Wt$$

$$Walking\ (MJ/day) = 0.0026 \times 17.5 \times 0.05 \times Wt$$

Where:

The energy cost associated with *CBP* is 0.00026 (MJ/kg/event) and is derived from SCA (1990). The number of bunk visits is 17.5 (events) which is derived from the difference in the number of bunk attendances between high and low RFI steers from the trials of Nkrumah *et al.* (2006). The distance between walking to and from the bunk is estimated at 0.05 km/event (50 metres) as shown by trends in Richardson *et al.* (1999).

Assumptions:

The calculations assume that 1) the heat increment of feeding per unit of feed intake above maintenance is not different between high and low RFI animals, 2) rumination times are based on a linear relationship between ME-intake and rumination, 3) rumination times are approximate to those observed by Gonzalez *et al.* (2009), 4) differences in feeding behaviour observed by high and low RFI steers from Nkrumah *et*

al. (2006) and 5) that each bunk attendance (17.5 events) is associated with a CBP and walking event of 0.05km.

Appendix 8.2

Calculation of the effect of different feed intakes between high and low RFI animals.

The calculation of the effect of differences in feed intake, and hence, high and low RFIs in steers and heifers presented in Chapter 6 was calculated as follows. No difference in heat production of maintenance (HPM) was observed in heifers fed in metabolism crates (Table 6.4). This suggests that all of the differences between the ME-intakes of high and low RFI heifers presented in Chapter 6 can be explained by the energy retained as fat and protein and the energy costs associated with the retention of fat and protein (i.e. k_f and k_p). In these heifers, ~60% of ME-intake could be explained by energy retained as fat and protein and the energy costs associated with the retention of fat and protein. However, in feedlot steers (Tables 6.1 and 6.3), there were significant differences in the HPM between high and low RFI steers (Chapter 6). The mean difference in HPM was 8.7% greater in high RFI steers than low RFI steers (Tables 6.1 and 6.3). Therefore, assuming no difference in maintenance requirements between high and low RFI animals (as presented in Chapters 2.3 and 6), the remaining 87.3% (i.e. $0.60/(0.60 + 0.87) = 0.873$) difference must be due to the energy retained as fat and protein and the energy costs associated with them.

As concluded in the discussions in Chapter 6 and 8, this 8.7% greater HPM of the high RFI steers in the feedlot must be due to differences in activity associated with differences in feeding patterns. This 8.7% difference in HPM equates to a total difference of 12.7% (i.e. $0.87/(0.60 + 0.87) = 0.127$) of which, 19% (i.e. 2.4% overall) could be accounted for due to differences in the HP_{DMEI} (mean of steers one and 2.5 generations divergent in RFI). The remaining 81% of the difference in HPM (i.e.

10.3% overall) due to the differences in feeding patterns (HP_{FD} and HP_{BA}; mean of steers one and 2.5 generations divergent in RFI) of high and low RFI steers (presented in Table 8.1 of Chapter 8).

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