Genomics of Feed Efficiency for Livestock

Presented By

Michelle Leanne Fenton

Submitted in total fulfilment of the requirements of the degree of

Doctor of Philosophy

Department of Animal Science

Faculty of Sciences

The University of Adelaide

12 August, 2004

Table of Contents

Abstract

The work presented in this thesis was undertaken to identify regions of the mouse and cattle genomes' containing quantitative trait loci (QTL) and candidate genes for net feed intake (NFI). Prior to mapping QTL in mice, a number of experiments were performed on mice from the eleventh generation of selection for divergent NFI to quantify the effects of selection for NFI on growth, body composition, protein turnover, metabolic rate, activity, and gastric emptying. Eleven generations of selection for and against NFI did not significantly alter body weight or growth rate. Mice from the high NFI selection line contained 32% less body fat, were 85% more active with no significant difference in body weight, growth rate or protein synthesis. There was a significant difference in gastric emptying of solid food between the mice lines with the high NFI mice grinding (25%) and emptying (21%) their gastric contents faster than the low NFI mice. This difference indicates the potential for gut hormones that regulate gastric emptying such as cholecystokinin, gastrin, motilin, secretin, and peptide YY as candidate genes for NFI.

Cattle that were more efficient (lower NFI) had lower maintenance requirements, and consumed less feed per day. The eye muscle area, peak force of the LD muscle and meat, fat and bone weight significantly influenced NFI. However, only 17% of the variation in NFI was accounted for by these traits. Also, eating rate, number of feeding sessions per day and time spent feeding influenced NFI, accounting for 15% of the variation in NFI. Low NFI cattle ate slower, spent less time feeding and had fewer trips to the feeder than those animals with higher NFI suggesting a possible action of the serotonin and/or dopamine systems as candidate genes for NFI.

Multiple interval mapping revealed nine NFI QTL in F_2 mice from the eighth generation of selection for divergent NFI. Interval mapping, a simpler method for QTL mapping was used in Limousin-Jersey backcross cattle and detected four QTL for NFI on BTA 1, 8, 9, and 20. The comparative gene maps between cattle and mice revealed that NFI QTL located on BTA 1 and 20 were comparative with the NFI QTL detected on MMU 16 and 13 respectively. A further two QTL that were almost significant in cattle on BTA 6 and 16 were also comparative with two of the mouse NFI QTL (MMU 1 and MMU 5 respectively). Based on these cattle QTL results and certain assumptions regarding NFI, selection for NFI in stud bulls would appear to be profitable for a producer. Validation of

these QTL in other beef cattle breeds is required before commercialisation of any DNA based test.

There were 19 genes in these four regions that could act as candidates for NFI located in these regions. Overall, the neurotransmitters appear to be the most promising candidate genes making up 11 of the potential 19 candidate genes identified. However, further biochemical and gene expression studies are required in cattle to confirm or contradict this.

Declaration

This thesis contains no material which has been accepted for the award of any degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is given. I give consent for to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Michelle Fenton

Acknowledgements

This project has been both challenging and rewarding thanks to the encouragement and support of many people. Firstly, I would like to thank my supervisors Wayne Pitchford and Cindy Bottema for not only their advice and knowledge, but also their patience and friendship. I greatly appreciate the opportunities that both Cindy and Wayne have provided me with during the course of my studies to travel to conferences and other laboratories, and attend various courses related to my studies.

A project such as this would not have been possible without financial support. The University of Adelaide and Meat and Livestock Australia provided my scholarship whilst MLA funded the majority of the project. There are a number of people that have provided me with valuable data throughout my studies. Paul Arthur and Jason Archer from NSW Agriculture provided me with the Trangie data set and advice for Chapter 6. Special thanks must also go to Paul for his contributions as an external reviewer of my project.

The opportunity to work on the protein synthesis, body composition and activity data in Chapter 4, created by Hutton Oddy, Robert Herd and the NSW Agriculture staff at the Beef Centre in Armidale was greatly appreciated. Whilst I helped set-up, and analyse the results, a large team of people from the Beef Centre conducted the laboratory work. I would also like to acknowledge and thank Erin Symonds for conducting the gastric emptying laboratory work.

A big thank you to my fellow PhD students Toby Hughes for creating the mouse NFI lines, his expert advice and friendship. Also, Adam Kister for the many hours he spent producing the cattle maps for Chapter 8.

To Megan Bray and Elke Stephens thank you for all your help, not only in the mouse house but also in keeping me positive and motivated. Megan, the therapy sessions on the way out to Roseworthy helped maintain my sanity.

To my husband Ben, Mum, Dad, Mark and Graeme who have all ridden the roller coaster with me thank you for your unconditional support, love and encouragement without which I would not have been able to complete this thesis.

Finally its over!

Table 1 Abbreviations

Abbrev.	Definition	Units
ABR	Absolute rate of protein breakdown	g/day
Act	Activity	
ADG	Average daily gain	g body weight/day (mice), kg body weight/day (cattle)
Age_{LD}	Aging rate of *longissimus dorsi*	ln kg/day
Age_{ST}	Aging rate of *semitemdinosis*	ln kg/day
AGR	Absolute rate of protein gain	g/day
AS	Average session length	Seconds/day
ASR	Absolute protein synthesis rate	g protein/day
b	B constant, one of the constants that is used to describe the gastric emptying curve – describes the rise of the curve	
BMR	Basal metabolic rate	ml O_2/g/hr
Bone	Total weight of bones	kg
%cdose	Percentage cumulative dose, total percentage of the given dose that comes out in the breath as $^{13}CO_2$	
DFI	Daily feed intake	g feed/day (mice), kg feed/day (cattle)
EMA	Eye Muscle Area of the *longissimus dorsi*	cm^2
EC_{fs}	Energy cost of fat synthesis	kJ/day
EC_{pd}	Energy cost of protein degradation	kJ/day
EC_{ps}	Energy cost of protein synthesis	kJ/day
ER	Energy retained	
Fat	Total fat weight	kg
Fat%	Body fat percentage	% body weight
FS	Number of feeding sessions	No. sessions / day
FSR	Fractional protein synthesis rate	%/day
FT	Total time spent feeding	Seconds/day
FR	Rate at which feed is consumed	kg feed/day
GE	Gross efficiency	g body weight gain/g feed (mice) kg body weight gain/ kg feed (cattle)
GEC	Gastric emptying coefficient, describes the shape of the emptying curve – indication of delivery to the duodenum	Describes the shape of the emptying curve – indication of delivery to the duodenum
HP	Heat production	kJ/day
HSCW	Hot standard carcass weight trimmed to Ausmeat standards	kg
IMF	Intra-Muscular Fat	%
k	K constant, one of the constants that is used to describe the gastric emptying curve – describes the rate of decline	

Lean	Lean mass	g body weight
M	Oxygen consumption	O_2/hr
m	M constant, one of the constants that is used to describe the gastric emptying curve – % dose recovered at infinity	
Marb	Ausmeat marbling score	
maxPDR	Maximum percentage dose response, height of the gastric emptying curve - indication of post-gastric events most likely due to liver metabolism	
Meat	Total weight of meat	kg
MEI	Metabolisable energy intake	kJ/day
MHP	Maintenance heat production	kJ/day
MS	Average meals size	kg feed/visit to feeder
MR	Maintenance requirements	Kg feed/ kg mid weight
MWT	Mid-weight, average weight of the animal across the test period	g body weight (mice) kg body weight (cattle)
MMWT	Metabolic mid-weight, mid-weight$^{0.73}$	g body weight$^{0.73}$ (mice), kg body weight$^{0.73}$ (cattle)
NFI	Net feed intake, feed intake net of growth rate and body weight	g feed/day (mice), kg feed/day (cattle)
Offal	Weight of visceral organs, calculated as the difference between mid-weight and HSCW	kg
P8	Subcutaneous fat depth at the P8 site on the rump	mm
PF_{LD}	Peak force required to shear the *longissimus dorsi*	ln kg
PF_{ST}	Peak force required to shear the *semitendinosis*	ln kg
QTL	Quantitative trait loci	
RMR	Resting metabolic rate	
RQ	Respiratory quotient	
$t_{1/2}$	Gastric half-emptying time, estimate of gastric emptying	
t_{lag}	Lag time, time taken for the first 5% of gastric emptying – an estimate of the time taken to masticate solid food into small enough particles to enter the stomach	
t_{max}	Maximum time, time taken to reach maximum emptying rate	
V	Velocity	km/hr
WW	Weaning weight	g body weight
Wt6	Six week body weight	g body weight

Chapter 1.

Introduction

1.1 Introduction

One of the main objectives of livestock breeding programs has been to increase the efficiency of production. Increasing production, decreasing costs, or a combination of both, will achieve this. Most livestock industries have focussed on increasing production, through improved growth rates or fertility. However, in the pork and milk production enterprises, feed can account for 70% and 80% of total costs. In pig breeding programs, feed efficiency, or its inverse food conversion ratio, has been incorporated in selection objectives and selection criteria (de Vries and Kanis 1994). A recent study of financial risk in dairy herds concluded that purchased feed expenditure had the largest impact on long-term farm returns (Schmidt et al. 2001). In dairy cattle breeding programs, selection objectives and criteria have generally focussed on outputs, which have not included feed efficiency (Persaud et al. 1991). Measurement of feed intake in progeny testing schemes in dairy cattle is not practical, but the introduction of nucleus breeding schemes in dairy cattle has enabled recording of food intake, such that feed efficiency can be included in the selection criteria.

In beef cattle and sheep breeding programs, outputs (i.e. weight and growth) rather than input (i.e. feed intake) have also dominated selection criteria (Banks 1994; Barwick et al. 1994), due to the difficulty in recording inputs. However, as with dairy cattle, establishment of nucleus breeding herds in beef cattle and sheep present the opportunity to include feed efficiency in selection objectives and corresponding selection criteria. It has been estimated that in a typical beef breeding herd, the cow herd uses 65-85% of the total energy required for beef production (Montano-Bermudez et al. 1990). Thompson and Barlow (1986) modelled the effect of changes in feeding and growth parameters on the efficiency of the cow/calf unit. When the cost of replacement females was included, it was estimated that the dam consumed 89% of the total feed costs of cattle production. This high value is in part due to cattle being large, slow maturing species with low annual reproductive rates, which cannot be easily altered (Pitchford 2002). Thompson and Barlow (1986) however, concluded that one of the most promising avenues for increasing the biological

efficiency of the total production system would be to decrease the maintenance feed costs of breeding cows.

Previous efforts to improve feed efficiency have concentrated on improving gross feed efficiency (weight gain/feed intake). This has resulted in faster growing animals that generally grow to a larger mature size, due to the high correlation between gross efficiency and growth. Associated with the larger mature size has been an increase in mature feed intake. Current research is focussing on the genetic variation in feed intake that is not explained by body size or growth rate, net feed intake. Any improvements in feed intake are made independent of an animals weight or weight gain, avoiding the problems associated with selecting for gross efficiency.

Unfortunately, the cost of measuring feed intake in cattle is prohibitively expensive for phenotypic selection. This makes it an ideal trait for marker– or gene- assisted selection. The overall aim of this project was to determine a way to reduce the cost of measuring feed intake in beef cattle through the location of genetic markers or genes.

Chapter 2.

Review of Literature

2.1 Growth

2.1.1 Definition

'Growth' is a word frequently used to describe many biological phenomena. The most common definition of growth is - an increase in size. In livestock industries, growth can be defined as the process where a newborn animal becomes sexually mature or its carcass is ready for sale. Body growth involves hyperplasia (cell multiplication) and/or hypertrophy (an increase in cell size), including fat deposition, muscle and bone growth. When an embryo develops it produces a large amount of specialised cells (e.g nerve and skeletal muscle cells) that grow by hyperplasia. As the animal matures these specialised cells can no longer replicate, this process is replaced by hypertrophy or the incorporation of satellite cells only. Other tissues such as hair follicles and gastrointestinal epithelia continue to divide throughout life.

Growth is a complex process that is affected by genetics, nutrition, disease, hormones, tissue-specific regulatory factors and the animals' environment. Good nutrition provides the animal with a balanced supply of amino acids, lipids, calories, vitamins, minerals, and water these provide the energy required for growth. Hormones influence growth, directly or indirectly, by altering biochemical reactions. Somatrophin, thyroxine, androgens, estrogens, and glucocorticoids are thought to be the only hormones that have a direct effect on whole body growth (Carlson 1969). Changes in body weight are closely related to the production of degradation of tissue mass, which is associated with protein metabolism.

2.1.2 Muscle Growth

Muscle growth is the net product of protein turnover, the continual process of synthesis and degradation. Protein turnover is energetically expensive, and any change in the relative rates of protein synthesis and breakdown has the potential to affect the efficiency of muscle growth (protein accretion). Tomas et al. (1988) demonstrated that only small changes in protein synthesis and degradation rates are needed to achieve large changes in protein accretion, in chickens. Complex interactions between nutritional, endocrine and genetic systems regulate growth, but the interactions between the systems are not fully understood. Variation in relative rates of protein synthesis and degradation exists in chickens (Maruyama et al. 1978; Tomas et al. 1988) and rats (Chan et al. 1985) with genetically different growth rates. These studies have shown that increased rates of muscle

protein gain are associated with decreased rates of protein degradation. In work done on lambs, Oddy et al. (1995) suggested that selection for weaning weight altered the dynamics of protein metabolism primarily through changes in protein breakdown in muscle. Selection for physiological or metabolic traits that alter nutrient partitioning and the efficiency of nutrient use for growth can alter the genetic 'set' for protein turnover and this appears to be expressed by the rate of muscle protein breakdown (Tomas and Pym 1995). Metabolic 'efficiency' will be improved if the rate of protein accumulation is increased due to a decrease in the rate of breakdown, as opposed to an increase in the rate of synthesis (Tomas et al. 1988).

2.1.3 Lipid and Tissue Growth

Body composition varies with species, breed, age, sex, plane of nutrition, and physical environment (Hafez 1969). Differential growth changes in various parts of the body cause changes in body composition with age, such that after maturity the percentage of adipose tissue in the body increases significantly. Males tend to have less adipose tissue than females and consequently are better able to convert feed into body weight (production of adipose tissue requires more feed than the production of muscle or bone). Lipid metabolism changes with age, and partly controls various productive functions such as lactation, fattening, work, and growth. Caloric balance has a greater affect on body fat than species, age, sex, climate, or composition of the diet (Emery 1969). When caloric intake is greater than the potential for growth of muscle and bone, fat is deposited. Young animals grow rapidly and are more difficult to fatten, but the final distribution and amounts of fat at maturity are similar whether fattening occurs throughout growth or near maturity (Oddy 1999).

2.1.4 The Growth Curves

When an animal has access to an unlimited amount of food, the change in body weight, with age tends to follow the sigmoidal growth curve (Figure 2.1). The animals' growth rate under these conditions increases rapidly to a point of inflection, where the growth rate slows down and approaches an asymptotic weight (mature weight). The S-shaped or sigmoidal curve contains two distinct phases, an accelerating (exponential) phase that begins at conception when growth rate is increasing and a decelerating (asymptotic) phase that occurs sometime after birth. The two phases meet at a point of inflection, which is the

point of maximum growth. The sigmoidal shape is universal among species, breeds and production systems, so it was suggested that a universal growth equation could compare growth characteristics among these systems (Equation 2.1).

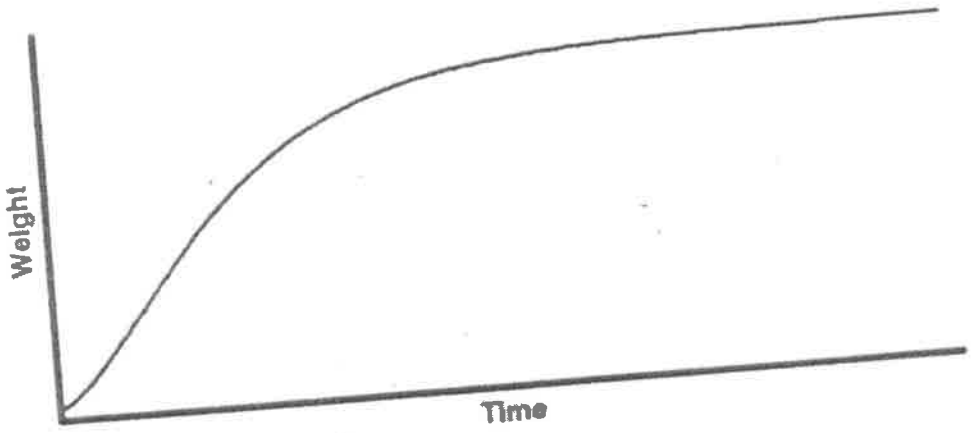


Figure 2.1 Growth Curve

Brody (1945) described the growth curve with the equation:

$$W = A(1 - be^{-kt}) \qquad \text{Equation 2.1}$$

Where W is weight at time t, A is the limiting value of W towards which the curve tends (ie the mature weight or asymptote) and k represents the rate of change per unit time (ie it's the spread of the curve or the maturation rate).

The biggest problem in applying Brody's growth and other growth equations is that they do not take into account feed intake. Since feed is one of the major expenses in all livestock industries, an equation that takes into account feed intake would be of greater benefit. Parks (1982) and Taylor (1982) proposed that growth and feeding of animals from a wide range of species and mature weights could be described by a series of equations that allow feed, growth, and efficiency relationships to be compared.

2.1.5 Genetic Size Scaling

Parks (1982) described the relationship between feed intake, body weight and time with a series of curves. Parks' first equation describes the asymptotic relationship between daily feed intake and time:

$$\frac{dF}{dt} = C(1\text{-}e^{-t/t^*}) \qquad \text{Equation 2.2}$$

where:

F = cumulative feed intake;

t = time;

C = mature daily feed intake;

$1/t^*$ = rate of maturity of daily feed intake with respect to time.

By integrating the above equation cumulative feed intake can be derived

$$F = C(t\text{-}t^*(1\text{-}e^{-t/t^*})) \qquad \text{Equation 2.3}$$

The second equation describes the relationship between body weight and cumulative feed intake, using the same mathematic principals

$$W = A(1\text{-}e^{-BF}) \qquad \text{Equation2.4}$$

where:

W = body weight;

A = asymptotic body weight at maturity;

B = rate of maturity.

Substitution of equation 2.2 into Parks' exponential equation above gives Parks' growth curve equation

$$W = A(1 - e^{-BC(t-t^*(1-e^{-t/t^*}))}) \qquad \text{Equation 2.5}$$

Here body weight is expressed as a function of time and follows the typical sigmoidal shaped growth curve. Also the efficiency of growth can be calculated as the product of A and B, and the maintenance requirement per unit body weight can be calculated by dividing C by A. The obvious advantage of using this equation in livestock production over the previous equations is that it takes feed intake into account. Taylor (1982) proposed that differences in the stage of maturity explain most of the differences observed

in production parameters (e.g. food intake, food efficiency, growth rate and body composition) when comparing animals of different mature weights. Taylor (1980a) suggested two scaling rules that can be applied to overcome the confounding with the stage of maturity:

1. All cumulative input and output variables are to be scaled to mature weight;
2. All age or time variables be scaled by mature weight raised to the power 0.27.

As a corollary of these two rules, rates of inputs and outputs should be scaled by A to the power of 0.73.

Using these scaling rules and corollaries Taylor (1980b) derived a number of standardised functions that describe weight, growth rate, food intake, feed efficiency, milk production and body composition, independent of mature weight. However, Taylor (1980a) stated that although scaling removes the majority of variation in production parameters, there is usually some variation independent of mature weight, which could potentially be important in animal breeding in the future.

2.1.6 Efficiency of Growth

The relationship between energy retention and feed intake is curvilinear (ARC 1980), following the law of diminishing returns. The efficiency of growth becomes the slope of the curve when energy retention is positive (Figure 2.2). Therefore the efficiency of growth changes according to the level of feeding. The efficiency of growth also changes with stage of maturity, in terms of the amount of feed required above maintenance per unit weight gain (Archer 1996). Animals deposit mostly lean tissue (protein) when they are young, which changes as they mature, when they deposit more fat (Searle et al. 1988). Since fat is more energetically expensive to deposit than lean tissue, confounding of the efficiency of growth and stage of maturity occurs. At the same live weight, animals with larger mature weights are more efficient because they are less mature and therefore depositing proportionately less fat than animal with smaller mature size (Taylor 1982).

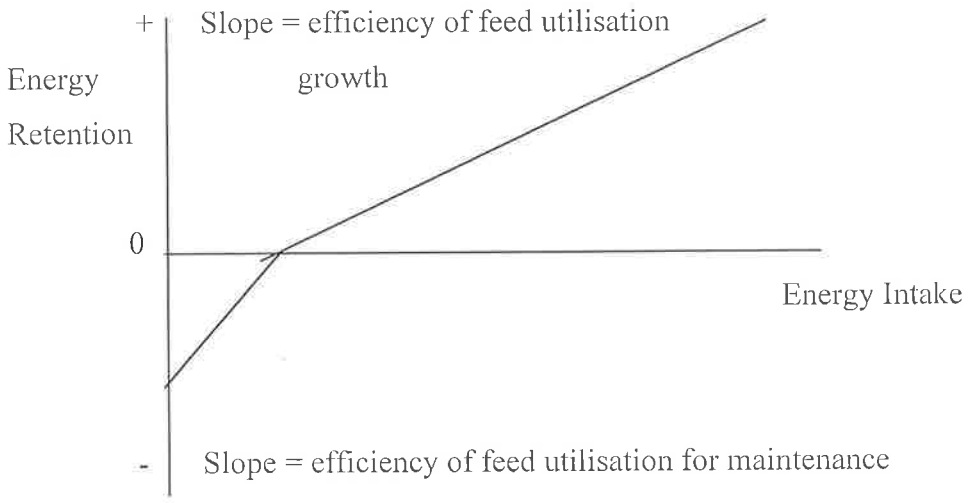


Figure 2.2 Energy retention versus energy intake (from ARC 1980).

2.1.7 Selection for Growth

Growth is the end product of many different physiological processes and is controlled by many genes. It is commonly accepted (Barlow 1978; Koch et al. 1982; Herd et al. 1997) that growth rate to any age is heritable, responds to selection, and is positively correlated with weights at other ages. Yüksel (1979), and Hood and Pym (1982) observed that selection for increased gain resulted in an increase in food intake, an increase in total fatness (depending on the time or weight estimates relative to age of selection), and an increase in gross efficiency. Results from long term selection experiments on mice for increased or decreased growth rate have indicated that there is a small effect on the growth curve (Biondini et al. 1968; Eisen et al. 1969; Timon and Eisen 1969; Brown et al. 1977) however, there is some conflict over whether the chemical composition of the body is altered. Several authors (Biondini et al. 1968; Timon et al. 1970) have reported that increased growth is associated with increased proportions of fat deposition whilst others (Brown et al. 1977) have reported no difference in body fat at maturity after selection for growth. This agrees with work performed by Herd et al. (1997), where fourteen years of selection for daily gain in cattle from birth to yearling resulted in different mature sizes but no differences in the rate of maturation of weight, length, height or girth and no significant differences in dressing percentage or subcutaneous fat depth. The fast growing steers were consistently heavier than the slow growing steers but there was no increase in total fat as a proportion of bodyweight in the fast growing steers.

2.2 Efficiency

2.2.1 Efficiency - Definition

Efficiency can be defined as the ratio of outputs to inputs with outputs and inputs given in a variety of units (biological, physical, financial). In this case biological efficiency would be the ratio of output (body weight or weight of lean product) to input (the total weight of food consumed). In many production systems, selection has been for high growth rate or body weight. These animals have been selected to improve the output of the system, without considering the input of the system. If an increase in growth is met by an equal increase in the amount of feed consumed, then there would be no improvement made in overall production efficiency.

2.2.2 Feed Efficiency

A major aim of all livestock industries is to increase the profitability of the system. Income and expenditure determine profitability. The most common methods of increasing profitability have been to improve the marketability or quality of the product, or to improve the efficiency of the overall system. Currently in Australia, consumer demands dictate the market specifications of beef. Consequently, a lot of work and research has been done on improving the quality of beef carcasses. However, feed costs are approximately one-half of the total cost of production for most livestock production systems, the cost of feed is the single largest cost to the beef producer. Therefore, selecting for feed efficiency is of economic importance.

In a review of feed efficiency Blaxter (1966) suggested that there are at least 5 major determinants of feed efficiency:

1. Voluntary Feed Intake - if animals of the same size are given the same diet *ad libitum* the animal which eats the most is the least efficient.

2. Nature of Feed - when feed is of lower nutritive value, the feed requirements per unit weight increase for both maintenance and weight gain.

3. Maintenance Cost - two animals will be equally as efficient as feed converters if they consume the same multiple of their feed needs for maintenance. The animal which requires less feed for maintenance could be more efficient.

4. Composition of Gain - if two animals gain at the same rate, the one which deposits the least fat, will be the most efficient because fat is almost five times more expensive to deposit than protein.

5. Age - efficiency of feed utilisation declines with age, mostly due to increased proportions of fat.

The importance of food in the beef and sheep industries is often overlooked because it is harder to determine an exact cost and is expensive to measure on an individual animal scale. Ferrell and Jenkins (1985) and Montano-Bermudez et al. (1990) estimated that in a typical beef breeding herd, between 65% and 75% of the total feed energy requirements of the individual breeding cow is used for maintenance. It has also been estimated by Montano-Bermudez et al. (1990) that the cow herd uses 65 - 85% of the total energy required for beef production. Furthermore, Dickerson (1970) estimated that at least 50% of the total feed intake of beef production was used for body maintenance of adult and slaughter animals. By modelling the effect of changes in feeding and growth on the efficiency of the cow/calf unit, Thompson and Barlow (1986) estimated that when the cost of replacement females is included the food costs of the dam can be as much as 89% of the total food costs. Therefore, feed efficiency is becoming a major consideration in many experimental and stud breeding programs.

One method of selecting for feed efficiency is to select for gross feed efficiency, which can result in high growth rates and mature weights, and associated higher feed requirements at maturity (Archer 1996). In beef cattle the maintenance costs of the breeding herd are high relative to productive output. Thus increasing maintenance requirements could lead to the cost of extra feed outweighing the gains that are made by increasing gross feed efficiency.

Net feed intake, (feed intake independent or net of body weight and growth) has been suggested as an alternative measure of feed efficiency (Koch et al. 1963). Arthur et al. (1996) and Archer et al. (1997) have shown that there is genetic variation in net feed intake in Australian beef cattle and that net feed intake is moderately heritable ($h^2 = 0.44 \pm 0.07$). Therefore it has been possible to make gains in feed efficiency by selecting for net feed intake.

2.2.3 Gross Efficiency

Gross feed efficiency, the measure of gain to feed intake appears to be the most widely used measure of efficiency in the literature. Gross feed efficiency in cattle has been shown to be highly correlated with growth rate both phenotypically and genetically (Brelin and Brannang 1982). The estimated genetic and phenotypic correlations between feed conversion ratio and daily weight gain were –0.93 ± 0.56 and –0.55 respectively, using four studies on cattle. A number of studies as cited by Buttazzoni and Mao (1989) have showed there is a high positive phenotypic correlation and very high genetic correlation between gross efficiency of milk production and milk yield. It was suggested by Korver (1988), and Mrode et al. (1990) that there would be little justification in measuring feed intake to improve gross efficiency because improvements can be made in gross efficiency by selecting for production.

However, improvements in gross efficiency in animals that have low reproductive rates and high maintenance requirements relative to production, (such as beef cattle) result in larger animals but do not always improve the efficiency of the overall system (Webster 1980). Animals with higher growth rates and therefore higher gross efficiency appear to be heavier at maturity and consequently feed requirements at maturity are higher. If the gains made in growth efficiency are lower than the increases in feed requirements at maturity then there will be no change in overall efficiency.

It should also be remembered that gross efficiency is measured as a ratio and it may be difficult to improve by direct selection because:

1. Statistical properties of ratios are poor and response to selection can be erratic;
2. Using ratios as selection criteria can result in different responses in component traits and therefore the trait can not be predicted properly;
3. Ratios can produce false indications of economic efficiency (Gunsett 1984).

Moreover ratios can cause problems when used in linear selection indices, particularly when one of the component traits is also in the index.

2.2.4 Net feed intake

Net feed intake can be defined as the variation in feed intake independent of the variation in weight gain, weight maintained and sex. Net feed intake was first suggested by Koch et al. (1963) as an alternative measure of feed efficiency. By examining a range of statistical adjustments that could be used to measure feed efficiency Koch et al. (1963) suggested that feed intake data could be partitioned into two components: 1) maintenance and weight gain; and 2) a residual portion, which adjusts for body weight and weight gain, net feed intake.

Net feed intake may be useful as a measure of efficiency for industries where a large proportion of intake goes into breeding females (e.g. beef cattle) because it is phenotypically independent of the level of production of the animals. To determine net feed intake, a predicted feed intake can be calculated using a linear model that includes terms that describe maintenance requirements and level of production of the animal (growth rate, body weight and sex). Net feed intake (Figure 2.3) then becomes the difference between actual feed intake (actual DFI) and predicted feed intake (predicted DFI) from the residual term.

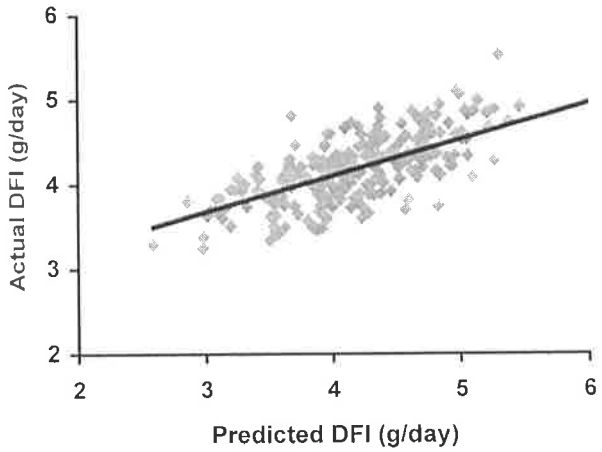


Figure 2.3 Net feed intake in mice (data from Hughes 2003)

The residual amount can then be used to compare efficiency between animals, with negative net feed intake representing an animal that requires less food to reach a given level of production (in this case weight) than would be expected from the average of the population (Archer 1996).

Significant gains have been made in feed efficiency by selecting for net feed intake estimated breeding values in mice. Archer et al. (1997) found that in mice there was a weak phenotypic relationship between post-weaning net feed intake and mature maintenance efficiency but post-weaning efficiency was genetically related to maintenance efficiency. From these results Archer (1996) concluded that selection based on post-weaning efficiency is likely to lead to a favourable correlated response in maintenance efficiency of mature animals.

2.2.5 Response to Selection for Feed Intake

2.2.5.1 Mice

Mice have been widely used in selection experiments on growth, composition and food intake because they provide convenient models for the analysis of physiology and the genetic basis for these traits in commercial species (McCarthy 1982; Stephens 1991). There are three projects that have used mice as a model species to for selection on feed efficiency: University of Edinburgh, UK (Hastings et al. 1997; Bünger et al. 1998); University of Nebraska, USA (Nielsen et al. 1997a, b); and University of Adelaide, Australia (Archer and Pitchford 1996; Hughes 2003). All three groups have clearly demonstrated that net feed efficiency can be improved by selection with moderate realised heritabilities (ranging from 23 to 28).

The Edinburgh group, Hastings (1997) and Bünger et al. (1998) selected for food intake in mice corrected for body weight for 23 generations. Selection for feed intake resulted in a 70-95% difference between the lines in adjusted feed intake, marginal changes in body weight (the high feed intake line was slightly heavier than the low feed intake line) and significant changes in body composition (high intake line had 43% lower fat percentage than the low intake line). Blaxter and Wainman (1966) concluded in a review of feed utilisation that animals with relatively large subcutaneous deposits of fat would be better insulated to heat loss than their leaner counterparts and would require less food to maintain

body heat, which could explain the differences in body composition between the above mice lines. This group also measured activity and found the high efficiency line were 67% less active than the low efficiency line (Hastings et al. 1997; Bünger et al. 1998).

Selection for high or low heat loss in mice for 15 generations by Nielsen et al. (1997a) (The Nebraskan group) resulted in a change in feed intake in the same direction as heat loss with no change in body mass. There was also no change in body fat percentage in the first 13 generations, but from generation 14 onwards the high heat loss line had the lowest fat percentage. Litter size and ovulation rate at second parity was also decreased in the low heat loss animals.

After seven generations of selection for high and low post-weaning net feed intake Hughes (2003) concluded that net feed intake was moderately heritable and genetically correlated with feed intake but genetically independent of growth traits. In addition, the high efficiency line had significantly lower faecal output, larger livers, stomach, caecum and intestines but a smaller heart (Hughes 2003). The Adelaide group also investigated the effect of pre-weaning nutrient supply on post-weaning growth and efficiency (Fenton 1997). The experiment demonstrated that mice from the high efficiency line were less affected by pre-weaning nutrient supply than those from the low efficiency line.

2.2.5.2 Poultry

With the aim of quantifying phenotypic variation in net feed intake in White Leghorn hens, Luiting and Urff (1991a) reported that differences in maintenance requirements caused most of the variation in net feed intake. In a review of literature, Luiting (1990) concluded that the variation in maintenance requirements in poultry were most likely to be explained by variation in feather cover and physical activity, whilst variation in basal metabolic rate, area of nude skin, body temperature and body composition contributed only a small portion. Bordas et al. (1992), Katle and Kolstad (1991), Liuttula (1989), and Luiting et al. (1991) all found an increase in feed efficiency when comparing low and high net feed intake animals, with no change in egg production. In the study by Bordas et al. (1992), selection for net feed intake affected traits related to heat production and dissipation. The rectal temperature, comb temperature, shank length and wattle length in males were greater in the high net feed intake animals. High net feed intake females also had greater shank

length but there was no clear trend for rectal temperature. Since shank length is related to heat dissipation, body size and maintenance requirements, some of the differences observed in intakes between the lines were attributed to these changes. Heat production was also found to be higher in the high net feed intake line by Katle (1991), Katle et al. (1984), and Luiting et al. (1991). Activity related and activity-free heat production was increased in White Leghorn layers with high net feed intake (Luiting et al. 1991).

After it was realised that high net feed intake cocks had higher caloric demand and poor reproductive capabilities, Morisson et al. (1997) examined the effects of selection on reproduction, sperm characteristics and mitochondria of spermatozoa. They found that the percentage of unfertilised eggs was higher in the high intake line, and early embryo mortality was twice as high in the high line. There appeared to be no difference in seminal plasma for pH, uric acid and protein concentrations however, the mean spermatozoa number of an ejaculate was almost 50% lower in the high intake line. Spermatozoa motility was lower and the ratio of dead or abnormal spermatozoa was higher in the high intake line. The two lines had the same mitochondrial activity per mitochondrial inner membrane present in the cell but the high line had 17% less mitochondria.

2.2.5.3 Pigs

There are very few studies reporting information on net feed intake in pigs. Foster et al. (1983) estimated the heritability of net feed intake in three breeds, Landrace, Large White, and Welsh to be 0.30 ± 0.08. Other estimates by Mrode and Kennedy (1993) and von Felde et al. (1996), were 0.30 ± 0.06, 0.38 ± 0.05 respectively. Mrode and Kennedy (1993) also showed a positive genetic relationship with average daily gain and backfat thickness. It has been reported in de Haer et al. (1993) in a study estimating net feed intake in group housed Dutch Landrace and Great Yorkshire pigs that 22% of the variation seen in daily feed intake among pigs was related to variation in metabolic body weight, daily weight gain and the lean percentage in the carcass. A further two studies (Jungst et al. 1981; Webb and King 1983, both cited by Cameron 1998) found no significant response to selection over several generations of selection for feed efficiency.

2.2.5.4 Cattle

Breeding programs for dairy cattle herds have included output traits but generally have not included feed efficiency traits due to the high cost of measurement of feed intake (Persaud et al. 1991). Despite this, the introduction of nucleus breeding schemes in dairy cattle has allowed food intake to be measured and thus feed efficiency can be calculated. Genetic correlation's between feed intake with milk yield 0.59; fat yield 0.76; protein yield 0.64; live weight 0.27; and condition score 0.00 were estimated by Veerkamp and Brotherstone (1997) on Holstein Friesian dairy cattle. The genetic correlations with type traits were quite low except udder depth -0.55; fore udder attachment -0.25; chest width 0.28; body depth 0.34 and rump width 0.24. From this study they concluded that live weight, condition score and food intake can be predicted from the type traits with little loss in accuracy.

As previously mentioned, selection for gross feed efficiency is not an effective measure for improving feed efficiency in beef cattle. As a result, several experiments on net feed efficiency in beef cattle have been carried out. In British beef cattle, Koch et al. (1963) first reported a heritability estimate of net feed intake of 0.28 ± 0.11 for bull and heifer calves. This estimate is relatively consistent with more recent net feed intake studies performed; 0.27 ± 0.23 (Brelin and Brannang, 1982), 0.25 & 0.22 ± 0.11 (Korver 1988; Korver et al. 1991), 0.14 ± 0.12 (Fan et al. 1995) and 0.44 ± 0.07 (Archer et al. 1997). These results indicate that net feed intake is moderately heritable and therefore selection to improve net feed intake should be possible.

To evaluate the variation in net feed efficiency and its relationship with other production traits, Arthur et al. (1996) used 574 weaned bulls and heifers from British beef breeds. Animals were placed in high or low net feed intake groups after feed intake was measured in automated feeders. The results from this experiment indicated that there was significant variation amongst individual animals and sire progeny groups for post-weaning net feed intake. Net feed intake was not correlated with production traits except actual feed intake (0.49), feed conversion ratio (0.47) and fat depth (0.24). Using these animals, Parnell et al. (1995) found the correlation between actual feed intake and metabolic mid-weight was 0.85 in bulls and 0.90 in heifers. The correlation was not significant between actual or net

feed intake with eye muscle area, linear body measurements, age at puberty or oestrus cycle. In a similar study, Perry et al. (1997) estimated net feed intake in 84 sheep and 60 steers from lines selected for divergent weight gain. Animals with low net feed intake (more efficient animals) tended to have lower non-carcass fat and total visceral weights in sheep, and lower non-carcass fat weight in steers. Looking for possible physiological indicators for net feed intake, Richardson et al. (1996) using the same animals as Arthur et al. (1996), found that low net feed intake animals tended to have increased numbers of red blood cells that contained lower haemoglobin content. IGF-1 and IGF-2 concentrations were not significantly different between the two animal groups.

One generation of selection for low net feed intake from the Trangie animals produced steers that grew as fast or faster than high feed intake but ate less feed per unit gain and had more favourable feed conversion ratios. These animals had slightly less subcutaneous fatness and smaller eye muscle area, suggesting a possible association of efficiency with maturity pattern. There was no difference in ability to reach market specification or percentage yield of retail beef from their carcasses (Herd et al. 1998).

2.3 Regulation of Food Intake

2.3.1 Introduction

Food intake is controlled by complex physiological mechanisms that are not fully understood. Nerve pathways, neurotransmitters and hormones all play key roles in the regulation of food intake. Most studies have been performed on rodents or humans, with increasing evidence supporting the concept that the body generates feedback signals to control food intake and regulate body adiposity (Figlewicz et al. 1996). Over the past two decades peptides that are synthesised in the gut or brain which act on neurones to regulate food intake, have been discovered.

Satiety factors, neurotransmitters that signal the brain that the body is full or hungry play an important part in food intake regulation. The central feeding drive system includes a number of anatomical sites where the interactions of a number of neurotransmitters including serotonin, neuropeptide Y and leptin occur. These interactions are believed to be responsible for the generation of the feeding drive (Morley 1990). NPY increases food intake by interacting with a unique NPY receptor. Leptin is the product of the OB gene and is an important signal for the regulation of body weight and body fat. It suppresses food intake in part by inhibiting NPY neurones in the hypothalamus (Wilding et al. 1997). In addition to the short-term satiety signals, there are long-term satiety signals involving absorbed nutrients and hormones released from the gut, such as cholecystokinin. Cholecystokinin (CCK) is a hormone that links the stomach and the brain. CCK is involved in appetite control by producing a satiety signal in response to food intake.

There are also a variety of metabolic signals integrated to control feeding, which are influenced by sensory information (sight, smell, or taste of food), and neural or endocrine information from the brain. Metabolic regulation works at three different time levels: at the meal (initiation, size and frequency), over a period of time after a meal to ensure adequate nutrient intake, and long-term regulation to monitor energy stores and body composition with food intake and energy expenditure to maintain homeostasis.

2.3.2 Metabolic Regulation of Food Intake

Metabolic control systems are just one of the many complex regulatory systems controlling food intake. Metabolic signals are integrated to control feeding and they are further integrated with or influenced by sensory information on the sight, smell, or taste of food, neural and endocrine information from the gastrointestinal tract or higher brain regions. Metabolic regulation of feeding works over three different time scales:

1. Control at the level of the individual meal – its initiation, size, and frequency;
2. Integration over a 24-hour period (in mice and rats, longer for humans) – to ensure a balance of nutrient intake;
3. Long-term regulation – the monitoring of energy stores and body composition, to control feed intake and energy expenditure to maintain homeostasis.

The onset of feeding is associated with a rapid change in the profile of circulating substrates and hormones; glucose, free fatty acids, insulin, glucagon, and norepinephrine (Steffens et al. 1986). The hypothalamus then responds to numerous metabolites (e.g. free fatty acids, ketone bodies, amino acids sugar acids) and hormones (e.g. insulin, glucagon).

Vagal glucose receptors, or glucose-sensitive units, which modulate insulin secretion, and monitor nutrient and intake stores exist in the liver Nagase et al. (1993). After stimulation of hepatic gluco-receptors the information is relayed to the hypothalamus to provide a rapid central response to peripheral changes in carbohydrate status, which may be involved in both the initiation and termination of feeding (York 1990). The satiety effects of certain other hormones such as glucagon, may also be mediated via the hepatic system. Glucose is only one of many substrates that appear to inhibit food intake by suppressing the activity of the hepatic system. Pyruvate, palmitate and glycerol are just some of the other substrates that are also oxidised to yield NADH, which is thought to decrease vagal firing rate and consequently satiety Langhans et al. (1987).

Metabolic signals arise from either the meal consumed or the changing pattern of substrate utilisation and hormone secretion during the inter-meal period. Various signals are required to initiate and terminate feeding. Nerves from the liver convey information to the

central nervous system on the metabolic status of the animal. Brain centres, such as the hypothalamus that are able to monitor both blood and spinal fluid, nutrient related signals, receive neural information from the liver and sensory inputs and initiate and effective feeding response. Central neuropeptides may modulate these integrative processes that provide control over the size and frequency of individual, and long-term energy homeostasis.

2.3.3 Neuropeptide Y

The neurophysical processes controlling appetite and thermogenesis are complex, and are thought to involve many neurotransmitter systems, which may play either key or complementary roles. Neuropeptide Y (NPY) is a 36 amino acid peptide believed to be a key neurotransmitter in the regulation of energy homeostasis. NPY is synthesized in neurones throughout the brains of rodents and humans and is found in large concentrations in the hypothalamus (which is a region crucial to the regulation of appetite). NPY was shown to produce a potent and sustained increase in food intake, combined with a reduction in thermogenesis within 10-15 minutes of injection either into the cerebral ventricles or directly into specific hypothalamic regions (Dryden et al. 1994). Repeated injections of NPY have also been shown to result in obesity due to increased food intake and decreased thermogenesis Stanley et al. (1986).

NPY is thought to inhibit thermogenesis by reducing the firing rate of sympathetic nerves that innervate brown adipose tissue in rodents. Direct injection also increases pancreatic secretion of insulin indicating a shift towards positive energy balance stimulating energy intake and storing excess calories as fat (Wilding et al. 1997). There is also evidence (Dryden et al. 1994; Lambert et al. 1993; Morley et al. 1987; Stanley et al. 1993) to suggest that hypothalamic NPY neurones are activated during conditions of negative energy balance, indicating that one of the important roles of NPY in the hypothalamus is to restore body energy stores (e.g. NPY concentrations are elevated during starvation, food restriction and insulin-deficient diabetes).

It is thought that NPY increases food intake by interacting with a unique NPY receptor that is pharmacologically different from other NPY receptors (Grundemar and Hakanson, 1994). Recently a novel NPY receptor was cloned from the rat hypothalamus whose pharmacological properties matched those expected of NPY feeding receptor and was named the 'Y5' receptor (Gerald et al. 1996). Further studies to clarify the role of the Y5 receptor in the regulation of food intake are currently being done by the above authors.

2.1.1 Leptin

Leptin is a 16-kDa protein, and the product of the *ob* gene. It was first proposed that leptin, a fat-derived hormone might be the 'lipostat' factor postulated to govern energy balance through a negative feed back loop originating in adipose tissue and acting on centres in the brain (Kennedy 1953). This concept was supported by the parabiosis experiments performed by Coleman (1973), which accurately predicted that genetically obese *ob/ob* mice lack this lipostatic factor while the genetically obese diabetic *db/db* mice are unable to respond to it, suggesting a defect in the receptor for that factor. When leptin is injected into *ob/ob* mice, which do not produce functional leptin, food intake is suppressed and body fat content falls without any reduction in lean body mass (Levin and Routh 1996; Halaas et al. 1995). The loss in body fat is due to an increase in energy expenditure combined with hypophagia. The effect of injected leptin has been observed in normal mice but the magnitude of the response was not as great as in the homozygous *ob* mice (Levin and Routh 1996). It is worth noting that rodents have significantly more brown adipose tissue than ruminants.

Leptin reaches the brain via a saturable active transport system that is independent from the insulin transporter (Banks et al. 1996). This system transports leptin across the blood brain barrier, where specialised transport occurs between the circulation and the central nervous system. The leptin receptor is a single chain polypeptide with homology to cytokine receptors. It exists in 2 forms, the short form Ob-Ra has a 34 amino acid intracellular portion and is thought to be the transporter, whilst the long form Ob-Rb has a 304 amino acid intracellular portion and is thought to be involved in intracellular signalling (Mercer et al. 1996; Tartaglia et al. 1995). There are three splice forms of the receptor reported to date and it has been suggested that their functions include leptin transportation and circulation of the leptin transport protein. It appears that the activity of

the leptin receptors depends on the activation of specific tyrosine kinases, which phosphorylate further proteins in a cascade of events leading to alterations in gene activity (Baumann et al. 1996).

The action of leptin in suppressing food intake and stimulating thermogenesis is thought to be mediated in part by inhibiting NPY neurones in the hypothalamus (Stephens et al. 1995). Leptin appears to reduce the levels of NPY mRNA in the hypothalamus, and it may also have a postsynaptic effect, reducing the activity of released NPY. It has been demonstrated that body fat content also correlates with circulating plasma leptin levels (Considine et al. 1996; Cusin et al. 1995; Frederich et al. 1995; Kolaczynski et al. 1996; Larsson et al. 1998). Circulating leptin informs the brain about the abundance of body fat, thereby allowing feeding behaviour, metabolism, and endocrine physiology to be coupled to the nutritional state of the animal (Erickson et al. 1996). In addition, it has been demonstrated that leptin secretion from fat is determined by hormonal status. It is believed that insulin and glucocorticoids increase leptin synthesis and secretion in rats, whereas in fasting, or semi-starved rats, insulin and glucocorticoids reduce synthesis and secretion of leptin. Leptin synthesis and secretion is reduced in fasting, semi-starved and diabetic rats, in parallel with reductions in white fat mass (Wilding et al. 1997).

2.3.4 Serotonin (5-HT)

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter, which is believed to alter food intake in rodents, by altering the quantity of food intake, pattern of eating and appetite for specific nutrients. Other monoamine neurotransmitters include dopamine, noradrenaline and adrenaline. Serotonin suppresses food intake, which is opposite to noradrenaline and adrenaline. Using microinjections of serotonin in various regions of the brain, Shor-Posner et al. (1986) localised its inhibitory actions on feed intake to the paraventricular nucleus (the site where NPY is most active in inducing feeding behaviour). Recent evidence suggests that the hypophagic actions of serotonin may be mediated (at least partly) through the NPY pathway. It is currently believed that NPY and serotonin have opposing effects in the hypothalamus and that serotonin may produce its hypophagic activity directly by inhibiting the firing of NPY neurones in the arcuate nucleus leading to reduced NPY release from the paraventricular nucleus (Wilding et al. 1997)

2.3.5 Cholecystokinin (CCK)

CCK's are a typical satiety signal. Evidence suggests that the physiological role of CCK is as a meal termination signal. CCK is a 33 amino acid hormone that has multiple effects on the gastrointestinal tract and CNS. CCK is synthesised within the gut wall and released into portal circulation in response to nutrients (particularly fatty acids) within the gut. On ingestion of a meal, proteins and fats enter the upper small intestine, stimulating secretion of CCK. When secreted into the blood stream CCK binds to specific receptors on the gall bladder, pancreas, stomach and various nerves to stimulate gall bladder contraction and pancreatic enzyme secretion, delay gastric emptying and regulate satiety (Mutt 1988). As is typical of most gastrointestinal hormones, CCK is produced by endocrine cells of the intestinal mucosa that are concentrated in the duodenum and proximal jejunum. CCK is released into the blood upon ingestion of a meal, with fats, proteins and amino acids being the most potent at stimulating CCK secretion. Rats injected with CCK (peripherally or into the CNS) were shown by Morley (1987) to have reduced food intake. The process by which this occurs has yet to be determined.

CCK is known to act via 2 different receptors: CCK-A receptors and the CCK-B receptor (which is identical to the gastrin receptor). Both types of receptors are found in the gastrointestinal tract and the CNS. When A receptors are blocked, the onset of satiety is delayed, consequently food intake is increased. Dourish et al. (1989) administered B receptor antagonists that blocked the effect of peripheral and centrally administered CCK and also increased food intake.

There are other gastrointestinal hormones such as bombesin, pancreatic glucagon, gastrin and somatostatin that have been proposed as satiety signals (Morley 1990; Figlewicz et al. 1996; Wilding et al. 1997). Like CCK, these peptides appear to decrease single meals size. However, they appear to be less potent than CCK.

2.3.6 Physical Regulation of Food Intake in Ruminants

The most familiar inhibitory signal of food ingestion in humans results from gastric distension; eating stops when the stomach feels 'full' and further eating causes gastric discomfort and nausea. Productivity of all animals (ruminants and monogastrics) depends on their ability to consume and extract usable energy from their available feed. However, pregastric fermentation allows ruminants to extract more energy from roughages, and convert non-protein nitrogen to microbial protein with greater biological value than is possible for monogastric mammals. In ruminants, voluntary dry matter intake may be limited by restricted flow of digesta through the gastrointestinal tract (Allen 1996). An animal's capacity for fill depends on the weight and volume of digesta that causes distention and the flow rate of digesta from the organ in which distention occurs. Distention limits voluntary intake mostly in the reticulorumen when high-fill diets are consumed. Distention in the abomasum has also been shown to limit voluntary intake. Animals with high energy requirements that are feeding on relatively low-energy, high-fill diets are affected the greatest. There are many other factors which influence fill, including particle size, chewing frequency and effectiveness, particle fragility, indigestible NDF fraction (non-dietary-proteins (NDP) take longer to ferment and pass through the reticulorumen and thus have a greater filling affect over time), rate of fermentation of the potentially digestible NDF, and characteristics of reticular contractions.

2.3.7 Measuring Feed Intake

The current methods of measuring feed intake in cattle include testing at pasture, centralised testing stations, and on-farm testing. Testing animals at pasture can be done using controlled-release devices (CRD's) that release chromic oxide or alkane. Faecal samples need to be collected regularly after administering cows with CRD's and recovery rate of chromic oxide/alkane can be calculated. After the concentration of plant alkane/chromic oxide has been measured, dry matter intake can be calculated using the formula of Dove and Mayes (1991).

The current on farm testing methods require either manual feeding and weighing of cattle, or using semi- or fully- automated efficiency testing units (ETU). Feed intake, growth rate, live weight and other data collected on farms would then be sent to a central agency for

analysis (Exton 2001). Manual weighing and feeding reduces the initial cost but has large labour requirements and small numbers of animals can be tested. An automated ETU would be expensive for the producer initially but could be more economical long term than central testing stations. However, a set of guidelines would have to be defined and followed closely, for accurate results.

In North America and South Africa, central test stations are widely used to measure food intake and growth rates of bulls. There are two main problems with adopting this method in Australia; animals coming from different pre-test environments may perform differently when on test. Additionally, central testing stations are expensive and there are often limited facilities available. The most likely use for these facilities would be for bulls that are likely to be used for AI or have good production or marketing traits. The advantage of this system is that it allows almost complete control of the testing procedure and the environment.

To measure feed intake and net feed intake on cattle in Australia currently costs a producer approximately $500 per animal. This is generally not a viable option for the producer and is severely limiting the use of feed intake as part of a selection criteria in cattle. A DNA based test on the other hand may cost approximately $50 per animal, while new DNA chip technologies with multiplexed tests further reduce the cost to around $5 per animal. Mapping the genes associated with feed intake would allow a simple DNA based test at a lower cost to the producer.

2.4 Gene Mapping

2.4.1 Introduction

One definition of a genetic map is the representation of the distribution of a set of loci within the genome. Mapping can be broken down into two basic stages; locating genes to particular chromosomes and then identifying their position along the chromosome. There are three distinct types of genetic maps that can be derived for each chromosome in the genome (apart from the Y chromosome). The three map types - linkage, chromosomal, and physical, are illustrated in Figure 2.4. The maps are distinguished by the methods in which they are derived and the metric used for measuring distances within them.

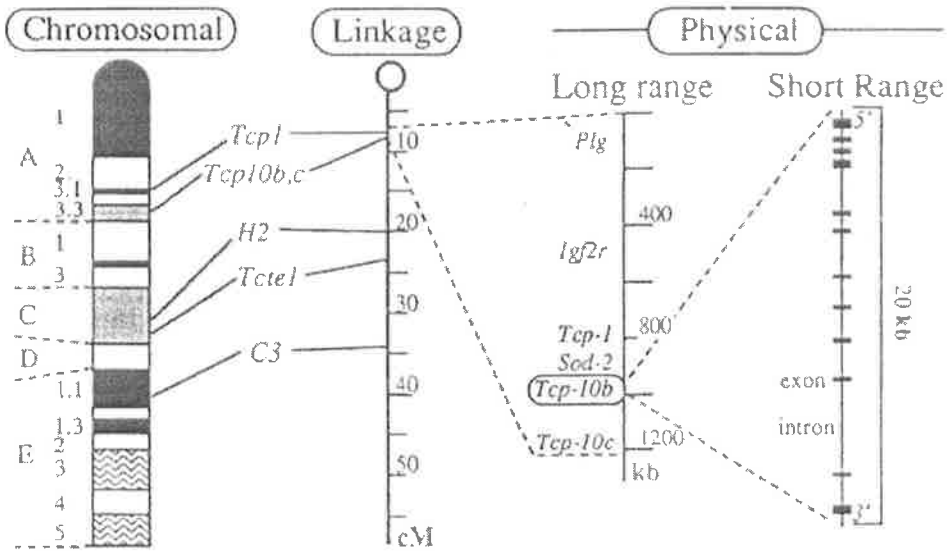


Figure 2.4 Chromosome, Linkage and Physical Maps

2.4.2 Chromosome Maps

The chromosome or cytogenetic map is based on the karyotype of the genome. Chromosomes are defined at the cytogenetic level according to their size and banding pattern (Figure 2.4). Ultimately all chromosome assignments are made by direct cytogenetic analysis or by linkage to a locus that has previously been mapped in this way (Liu 1998). Chromosome maps can be generated in several different ways, each with varying levels of resolution. Indirect mapping involves the use of one or more somatic cell hybrid line that contains portions of the karyotype within the surroundings of another

species' genome. The correlation between the presence or expression of a particular gene with the presence of a chromosome or sub-chromosomal region in these cells allows chromosomal or sub-chromosomal assignment (Liu 1998). *In situ* hybridisation using a locus-specific DNA probe, to visualise the location of the corresponding sequence within a particular chromosomal band directly, is also used. The advantage of this method is that it does not rely on correlations or assumptions and it is the most direct mapping approach that exists. However, it requires a lot of technical work and its resolution is not as high as linkage or physical approaches.

2.4.3 Physical Mapping

Physical maps are based on the direct analysis of DNA. Distances between and within loci are measured in basepairs (bp), kilobasepairs (kb), or megabasepairs (mb). Physical maps have been arbitrarily divided into short range and long range maps. Short-range mapping is commonly done over distances ranging up to 30 kb. This distance is approximately the average size of a gene and the average size of cloned inserts obtained from cosmid-based genome libraries. Due to the small size these cloned regions can be easily mapped with restriction enzymes to high resolution. Sequencing of whole regions of this length is becoming more common.

2.4.4 Genetic Linkage Mapping

The linkage or recombination map was the first mapping method to be developed and is the method used in this thesis. Linkage maps can only be constructed for polymorphic quantitative trait loci (QTL), loci that occur in two or more heritable forms, or alleles. Monomorphic loci (loci with a single allele) cannot be mapped using this approach. The variation observed in most economically important traits comes from the alleles at several loci (QTL) and the interaction of these alleles with the environment (Andersson et al. 1994). Linkage maps are constructed by counting the number of offspring that receive parental or recombinant alleles from a parent that carries two different alleles at two or more loci. From this, counting linkage between loci can be determined. If the loci are linked then their relative order and the relative differences that separate them can be calculated. Genetic linkage analysis of traits and markers is used to identify QTL. Once markers have been linked to a QTL, the marker is used to select for the trait of interest, this is known as Marker Assisted Selection (MAS).

2.4.5 Linkage

If two genes are located on the same chromosome they are said to be linked and do not assort independently. Genes that are in random association are said to be in linkage equilibrium. Linkage disequilibrium occurs when linked alleles are associated with one phase of another allele more often than is expected because sufficient recombination has not occurred, so that some genotypes are not observed. Selection, migration, random effects and mutation can all produce linkage disequilibrium, while recombination reduces linkage disequilibrium. Recombination is the process whereby new combinations of parental alleles may arise in the progeny. Crossover events during zygotene, pachytene or possibly earlier in meiosis, result in recombinant chromatids. Recombinant chromatids are the result of breakage of both maternal and paternal chromatids at the same place but when they repair, one end of the maternal chromatid is connected to the paternal chromatid equivalent. When this occurs there are two parental chromatids and two recombinant chromatids.

2.4.6 Markers

Quantitative traits are traits that are controlled by many genes with small but cumulative individual effects. It has been assumed that these genes have both additive and dominance effects and they interact and show standard segregation patterns of normal genes on chromosomes. It was first shown by Sax (1923) that genetic markers could be used to map individual quantitative trait loci (QTL). It wasn't until after 1980 that the application of these techniques could be implemented due to the lack of segregating markers in the populations of interest (Weller and Ron 1994). To study and locate genes controlling quantitative traits, genetic marker loci that are independent and easily recognisable are required as flags to identify the regions of chromosomes controlling quantitative trait loci. Molecular or DNA markers show variations in the DNA sequence that is not shown in the phenotype. Genetic markers need to be;

1. Polymorphic - exist in two or more allelic forms at the DNA level so that pairs of individuals or lines are likely to carry different alleles at each locus;
2. Abundant - so that comprehensive marker coverage of the genome is achieved
3. Neutral - to the quantitative trait and reproductive fitness;
4. Co-dominant - so all possible genotypes at the marker loci can be identified.

These markers are placed in order on a genetic linkage map as discussed earlier (2.4.4) and are essential for assigning QTL to chromosomes.

2.4.7 Comparative Gene Mapping

There are two main methods used to detect loci responsible for quantitative traits; comparative gene mapping and genome scanning. Comparative gene mapping is the mapping of homologous gene loci in multiple species. Mammals all have a highly conserved genome size and it is thought that they share most of their 30 000 genes. Comparative mapping has shown that even gene order is conserved in mammals it appears that the same genome has been minimally rearranged in most groups during mammalian evolution (CGO 1996).

One of the main benefits of comparative gene mapping is that the knowledge of conserved segments in two different species allows the extrapolation of mapping data from one to the other. The gene of interest is known as a candidate gene. Comparative mapping can often provide a 'first guess' for the regional localisation of a gene that has simply been assigned to a chromosome. One of the most important applications of mouse linkage maps is for comparative mapping. Comparative mapping greatly reduces the need for genome-wide scanning (which is very expensive), because once a marker linked to the relevant gene is identified in one species, it also enables the likely location of the gene in all other species to be found (CGO 1996). A recent example of comparative mapping is the cattle double-muscling gene, myostatin. The mouse myostatin gene was mapped to a region in the murine genome that was homologous to the region of double muscling locus in cattle Grobet et al. (1997).

The use of complementary and interacting human and animal studies generates a powerful method for the search for genes of economic importance in domestic animals and medical importance to humans. Comparisons of the gene maps of humans, mice, and cattle have shown that conservation of synteny is greatest between humans and cattle. However, mapping of some genes by linkage analysis has shown that this conservation of synteny may not represent conservation of gene order (Barendse et al. 1994). If there is no conservation of gene order in conserved syntenies then the size of the region over which information can be interpolated from humans and mouse are limited and therefore more

genes would need to be mapped to determine boundaries of regions of conserved gene order (Barendse et al. 1997).

2.4.8 Genome Scanning

A more strategic method used to detect QTL is to scan the entire genome with evenly spaced markers. Lander and Botstein (1989), developed a method of mapping QTL, that is the most widely used in gene mapping, using a genome-wide scan followed by interval mapping analysis. Genome-wide scanning is relatively expensive however, dense linkage maps, containing several types of highly informative genetic markers are available for many species, with markers at less than 1 cM intervals in mice (Dietrich et al. 1996) and humans (Dib and Faure 1996) and wider intervals in pigs (Rohrer et al. 1996), cattle (Barendse et al. 1997), and sheep (de Gortari et al. 1998). To reduce the cost and amount of work, selective genotyping and DNA pooling can be done. Selective genotyping involves only individuals from the high and low phenotypic tails to be genotyped. Different marker allele frequencies between phenotypic tails indicate the presence of a QTL near the marker. Pooling DNA samples from the phenotypic extremes, followed by PCR and analysis of band intensities takes this approach one step further. These approaches dramatically reduce the amount of time and money spent on genotyping, however the power to detect QTL is also reduced (Darvasi and Soller 1994; Liu 1998).

Marker spacing is also important in determining the power of the experiment to detect QTL. When crosses between inbred lines are used, the power to detect QTL does not improve significantly 40 cM spacing to 20 cM spacing (Lander and Botstein 1989). Markers spaced every 40cM provide 80% of the information of markers spaced every 20 cM. In outbred populations the marker density needs to be increased because a lot of markers are un-informative. Knott et al. (1997) suggest markers be spaced every 10 cm along the genome. The most widely accepted approach is to use markers with an average space of 20 – 40 cM for the initial screening, with additional markers used to bracket the regions of significant effects in the initial screen (Cheverud et al. 1996; Leamy et al. 1998).

2.4.9 The Mouse Map

The mouse is a powerful genetic system for the study of mammalian biology. There are a large number of inbred and congenic mouse strains. These are large numbers of available

markers (over 7000) and this, coupled with their ability to produce large litters rapidly make them a useful model for investigating QTL that are relevant to domestic animals. The first genetic map of the mouse was based on visible mutant phenotypes, however this was a difficult and slow procedure and linkage maps soon took over. Since DNA markers were introduced, the mouse map has rapidly developed which has allowed many QTL to be mapped. The genomic resources for the mouse are currently increasing at an astounding pace. The ability manipulate the mouse genome coupled with the availability of genome sequence make the mouse a valuable and unique resource tool.

2.4.10 The Bovine Gene Map

Bovine gene mapping has turned a complete circle over the past decade, with synteny mapping, being replaced by linkage mapping and searches for economic or quantitative trait loci, and more recently comparative gene mapping from the map-rich genomes of mouse and human becoming popular (Womack and Kata 1995). Even though cattle are large in size, have slow reproductive rates and are expensive animals, they have been relatively widely used for gene mapping. This is mostly due to the high economic value of cattle in many countries. There is also a large amount of genetic diversity between breeds of cattle, with most adapted to their local environment due to natural and artificial selection.

The genome of cattle has the fourth most established map in mammals after humans, mice and rats, in both anonymous DNA polymorphism's and genes (Eggen and Fries 1995). According to Barendse et al. (1997) there are more than 3300 cattle DNA sequences in Genbank. There are approximately 800 markers of which 150 are type I markers or genes but this group is predominantly made up of type II sequence tagged sites (STS) markers (Ferretti et al. 1997). Most of the genes mapped in cattle have been mapped by hybrid cell genetics to chromosomes. However, QTL mapping is of great importance to cattle breeders because most phenotypes of milk and meat production are polygenic in nature and are affected by environmental factors. A saturated microsatellite based linkage map for cattle would provide the foundation for identification of loci contributing to the genetic variance for economic traits (ETL) and the exploitation of MAS for phenotypes of interest (Fries 1993).

2.4.11 Marker and Gene Assisted Selection

The integration of marker information into artificial selection for polygenic traits is known as marker assisted selection (MAS). Artificial selection on an individual's phenotype has been practised in domestic species, consciously and unconsciously for a long time. Lande and Thompson (1990) proposed a method of MAS, which uses linkage disequilibrium created by hybridisation between inbred lines. Selection is made on an index combining phenotypic and marker information. The efficiency of MAS has been investigated by a number of authors; Lande and Thompson (1990) for a single generation, Zhang and Smith 1992, 1993; Gimelfarb and Lande 1994a, b, 1995; Wittaker et al. 1995 over several successive generations using computer simulations. The main conclusion from these studies is that MAS could be more efficient than purely phenotypic selection in quite large populations and for traits showing relatively low heritabilities. MAS is most advantageous when phenotypic selection is difficult such as traits expressed late in life or in only one sex or very expensive to measure. However, the advantage of MAS decreases with the number of generations of selection.

2.5 Summary

Over the past 10 years there have been a number of studies in Australia on the efficiency of feed utilisation in beef cattle. The main objective of these studies has been to examine individual animal variation in feed efficiency and its exploitation for genetic improvement in beef cattle. The results have indicated that there is genetic variation in feed efficiency in Australian beef herds, feed efficiency is moderately heritable and there is potential to reduce the cost of beef production through selection for more efficient cattle. Currently, the limiting factor preventing the inclusion of feed efficiency into a breeding objective is the cost of measuring feed intake. The key to its inclusion is therefore a cost effective way of measuring feed intake. Marker or gene-assisted selection has the potential to reduce this prohibitively expensive cost. The current gap in knowledge is where the genes for feed efficiency are located in the bovine genome. Therefore, the overall aim of this project is to locate the genetic markers or genes that influence feed efficiency.

Chapter 3.

Animal resources and general methods

3.1 Mice

The mice used throughout this thesis originated from a three-way cross between Swiss outbred males and F1 females from a C57BL/6, BALB/c cross (Figure 3.1). The mice were randomly mated for four generations. Archer (1996) found average daily gain (ADG) was lowly heritable, weaning weight (WW), mid-weight (MWT), daily feed intake (DFI), and net feed intake (NFI) were all moderately heritable (Table 3.1).

Table 3.1 Mean (μ), phenotypic standard deviation (σ_P), heritability (h^2) and common environmental effects (c^2) for post-weaning traits from univariate analyses (from Archer 1996).

Trait	μ	σ_P	h^2	c^2
WW	14.2	1.7	0.33 ± 0.06	0.48 ±0.03
ADG	0.37	0.15	0.14 ± 0.05	0.11 ± 0.03
MWT	23.2	2.4	0.35 ± 0.07	0.14 ± 0.03
DFI	4.65	0.43	0.33 ± 0.06	0.09 ± 0.02
NFI	0.00	0.31	0.27 ± 0.06	0.16 ± 0.03
Fat	15.3	1.66	0.22 ± 0.10	0.14 ± 0.04

A summary of the abbreviations can be found in Table 1.

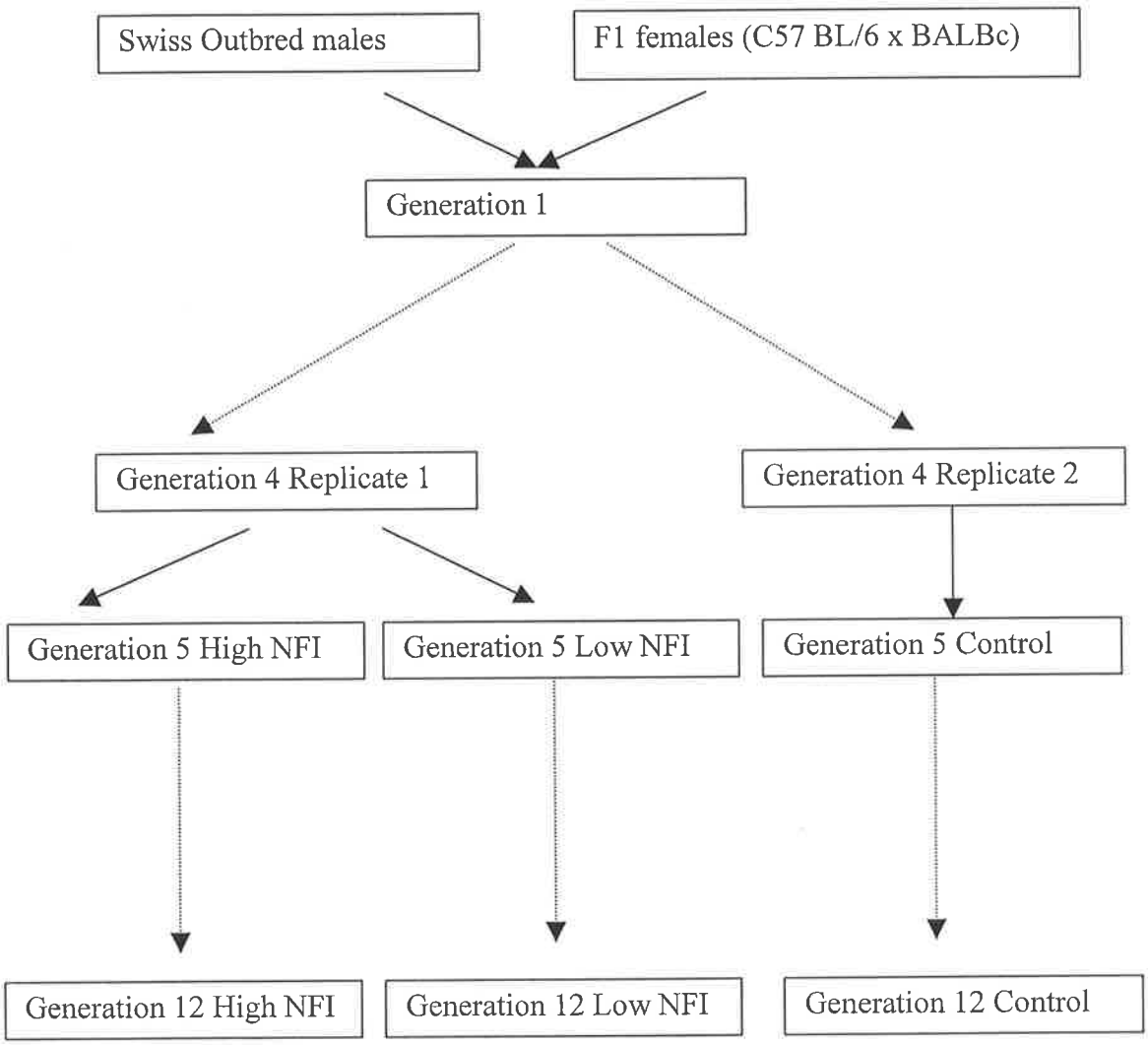
Also from this study Archer (1996) found that NFI was genetically correlated (0.69) with DFI but not with ADG or the two measures of weight (Table 3.2). However, DFI was highly correlated with weight and moderately correlated with ADG (0.36).

Table 3.2 Phenotypic (above the diagonal) and genetic (below the diagonal) correlations between intake and growth traits (from, Archer 1996).

	WW	ADG	MWT	DFI	NFI	Fat
WW		-0.16	0.58	0.29	-0.11	0.04
ADG	-0.15		-0.01	0.11	-0.04	0.20
MWT	0.74	0.49		0.68	-0.02	0.14
DFI	0.42	0.36	0.76		0.69	0.11
NFI	-0.16	-0.06	0.00	0.64		-0.02
Fat	0.01	0.57	0.24	0.09	-0.10	

A summary of the abbreviations can be found in Table 1.

Figure 3.1 The Mating System



Following four generations of random mating, the mice were selected for high and low net feed intake based on estimated breeding values for feed intake calculated using BLUP with DF-REML (Meyer 1993) for seven generations by Hughes (2003) (Figure 3.1). A control line was also maintained by randomly selecting animals from generation four onwards. The response to selection for NFI is plotted against selection differential in Figure 3.2.

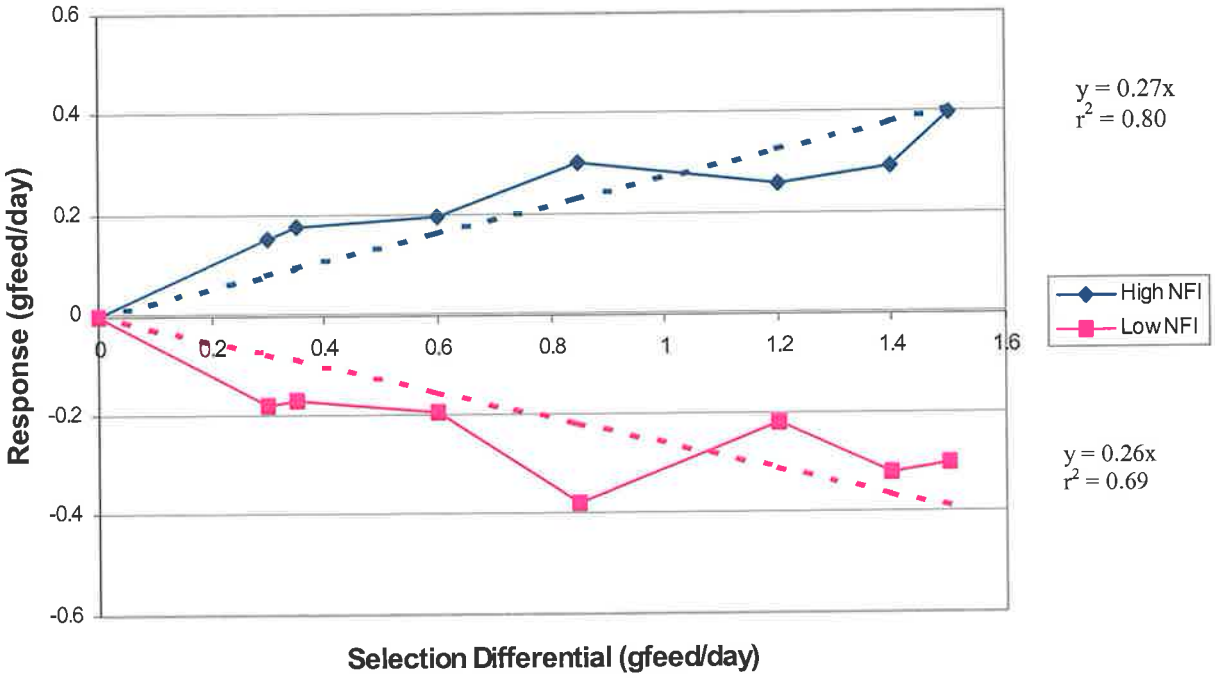


Figure 3.2 Response to selection for net feed intake (including the generations that are used in this study)

Hughes (2003) in studies on previous generations of the selected mice found that there was no difference between males and females in net feed intake. This is because the model used to estimate net feed intake included a term for sex. Males from generation seven of selection for and against net feed intake ate 10% more than females and were 5% and 20% heavier at weaning and mid-way through the test. Average daily gain was significantly higher in the males, which led to a distinctly higher gross efficiency. Surprisingly, males were fatter than females, when tested immediately after a three-week post-weaning feed intake test.

The difference between the high and low net feed intake lines in net feed intake was significant with a 13% difference between the lines in generation 10 (Hughes 2003). There were correlated responses to selection in daily feed intake, and all measures of weight, with the high line eating 20% more and weighing slightly (6%) more than the low intake line. Average daily gain, however differed with the high line gaining 25% less per day than the low intake line.

3.2 Mouse Feed Efficiency Test

The animals were housed in the Surgery Building, Department of Animal Science, University of Adelaide, Waite Campus. The room temperature was set at 24 °C ± 2°C and the lighting regime was 16 hours of light and 8 hours of darkness. At birth, all litter sizes were standardised to five pups with post-weaning feed intake measurements beginning when the animals were 21 days of age. Weaning weight was recorded at this time and the animals were placed in individual boxes that contained a feeding device. The feeding device was a plastic container, with slits on the side to allow the mice to eat, and a wide base to prevent feed spillage. The animals were fed a standard laboratory feed (New Joint Stock Ration, Milling Industries Pty Ltd) *ad libitum*. The weight of the feeder was recorded at day 21, 28, 35 and 42. Body weights were also recorded at these time intervals. The data from the first week (approximately 21 – 28 days of age) were excluded from the analysis to allow the animals to adjust to their new environment.

Post-weaning data consisted of feed intake between days 28 to 35 and 35 to 42 and body weights at days 28, 35 and 42, average daily gain (ADG), daily feed intake (DFI), net feed intake (NFI) and metabolic mid-weight (MMWT - the average weight over the test period raised to the power of 0.73, Table 1).

Net feed intake was calculated as the residual error term of a linear model (PROC GLM SAS 1998) fitted to the daily feed intake data (Equation 3.1). The model included terms for the class variable of sex and management group, co-variates average daily gain and metabolic mid-weight, and the interactions of each class variable with the covariates.

$$DFI = sex + litter\ size + MMWT + ADG + \varepsilon \qquad \text{Equation 3.1}$$

Where DFI is daily feed intake (g feed/day), ADG is average daily gain (g body weight/ day), MMWT is the metabolic mid-weight (g body weight$^{0.73}$) and ε is net feed intake or the residual error term.

3.3 Mouse Body Composition Analysis

Body composition was determined (unless specified) using an EM-SCAN Small Animal Body Composititon Analyser. The body composition analyser measures the change in an electrical field made by the mice inside the machine. If an animal was required for further testing, they were anaesthetised (to prevent movement) using an intra-peritoneal injection of Avertin (0.3 ml/25g). Once the animals were anaesthetised, their body weight and length were recorded and they were placed on a carrier plate and inserted into the analyser. A minimum of five readings were taken and if the coefficient of variation was greater than six percent, then at least two more readings were taken and the highest and lowest readings removed, until the coefficient of variation was below six percent.

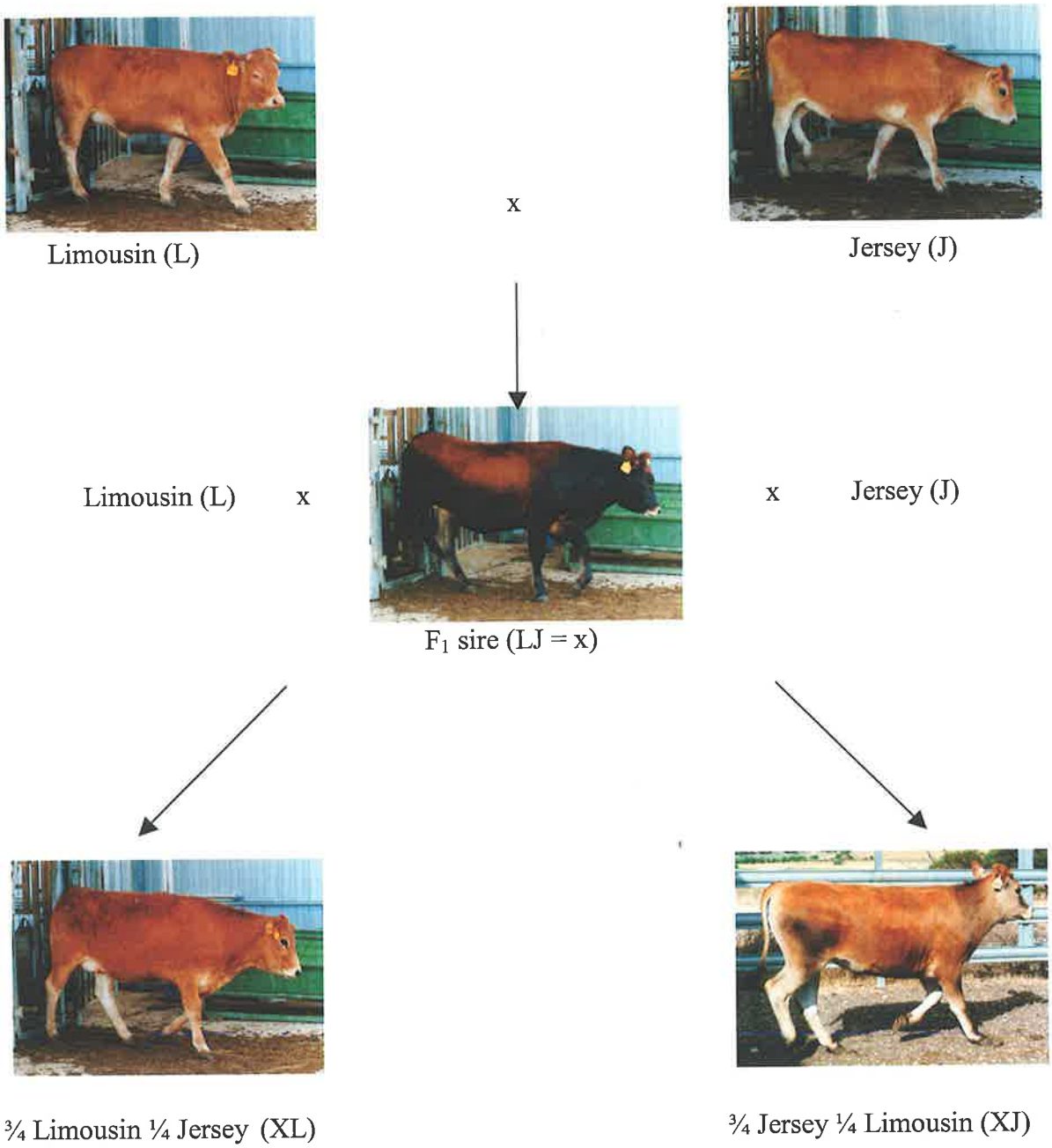
3.4 Cattle

The Davies Cattle Gene Mapping Project began in 1990 with a reciprocal backcross between purebred Limousin and Jersey cattle. The initial aims of the project were: 1) to study the mode of inheritance of important meat quality traits, and 2) to map major genes controlling these traits. To improve the chance of finding the major genes controlling these traits, two cattle breeds with large differences in carcass and meat quality traits were mated. Purebred Limousin and Jersey animals were crossed to produce F_1 progeny. Three F_1 bulls were randomly chosen and mated to the purebred Limousin or Jersey cows to give progeny that were either three-quarter Limousin or three-quarter Jersey (Figure 3.3). The backcross progeny comprised 370 calves: 80 born April 1996, 140 born April 1997, and 150 born April 1998.

The Davies Cattle Gene Mapping Project was unique in that there are progeny from another three Jersey/Limousin crossbred bulls in New Zealand. The three F1 bulls were half-sibling brothers of the crossbred bulls used in Australia. These bulls were also mated to Jersey and Limousin cows to produce ¾ Jersey or ¾ Limousin backcross progeny, as in the Australian project. Approximately 300 traits have been measured in one or both environments, ranging from behaviour, growth, fat and protein metabolism to food intake

and efficiency. Unfortunately, the progeny in New Zealand were not able to have feed intake measured and were finished on grass. However, this allows genotype by environment interactions to be tested in traits that were measured in both environments.

Figure 3.3 Cattle backcross design



3.5 Cattle Feed Efficiency Test

The first cohort (1996 heifers and steers) comprised 80 cattle, and was measured for feed intake at the Tullimba Research Feedlot located in Armidale, New South Wales. In contrast to the following drops of cattle, both the heifers and steers were measured at the same time. The remaining four cohorts were measured at the Struan Research Feedlot located in Naracoorte, South Australia.

Both feedlots utilised Ruddweigh electronic feeders. Cattle in a feeding pen were tagged with electronic ear tags that produce a signal for a unique number. When an animal enters the feeding box, an infra red beam is broken to indicate that recording of feed intake and body weight (Struan only) is to begin. When the animal leaves the feeding box, the infra red beam is reset. The weight of food that the animal has eaten is then calculated as the weight of the food bin after feeding has finished subtracted from the weight of the feed bin before feeding commenced. Also the time taken feeding (ET) and the number of feeding sessions (ES) per day are recorded. This information is then downloaded and processed, to convert the continuous results into daily totals for food intake, body weight, the number of feeding sessions, and the time spent feeding for the day.

Feed intake data was processed by calculating least-squares means for each animal over a test period. Day was included in the model to allow for weather, personnel, time of feeding and any other factors that would affect the intake of all cattle. Average daily gain was calculated as the regression coefficient of weight against day of test. Net feed intake was calculated after modelling daily feed intake for metabolic body weight (MMWT) and average daily gain while on the feed intake test (Table 1). Metabolic mid weight was calculated in the same way as for the mouse experiment, the mid-weight raised to the power 0.73. The initial equation used to calculate NFI included the main effect of cohort and interactions between MMWT and ADG (Equation 3.2). Cohort was defined as the eight combination of year of birth (1996 – 1998) and sex (heifer or steer). However, both interaction terms and cohort were not significant. Thus, a simple model comprising only MMWT and ADG was used (Equation 3.3). It should be noted that this simple model inflated the phenotypic variance and will consequently inflate any estimates of heritability.

$$DFI = Cohort + MMWT + ADG + Cohort.MMWT + Cohort.ADG + \varepsilon \qquad \text{Equation 3.2}$$

$$DFI = Cohort + MMWT + ADG + \varepsilon \quad \text{Equation 3.3}$$

Where DFI is daily feed intake (g feed/day), ADG is average daily gain (g body weight/ day), MMWT is the metabolic mid-weight (g body weight$^{0.73}$) and ε is net feed intake or the residual error term.

Analysis of variance for calculating net feed intake in the Jersey Limousin backcross cattle.

Source	df	SS	F-value	Parameters
Initial model				
Cohort	5	6	0.5	
MMWT	1	294	126.2***	
ADG	1	20	8.5**	
Cohort x MMWT	5	11	0.9	
Cohort x ADG	5	22	1.9	
Residual	301	700		
Final model				-0.295 ± 1.14
MMWT	1	454	131.7***	0.113 ± 0.010***
ADG	1	67	19.4***	0.803 ± 0.182***
Residual	320	1103		
Total	322	1575		

A summary of the abbreviations can be found in Table 1.

The eating behaviour traits of, average session length (AS=ET/ES), eating rate (ER=DFI/ET) and average meal size (MS=DFI/ES) were also calculated. Most traits were normally distributed with the exception of number of eating sessions and time spent eating. The data for these traits were transformed by taking the natural logarithm before being analysed.

3.6 Cattle Behaviour Measurements

The behaviour of the cattle from all cohorts was adapted from those used by Matthews et al. 1997) and described in Fisher et al. (2000). The flight distance of an animal is the distance that the animal will allow a person to approach before it moves. Flight distance was measured in the handling yards, when an animal was at the bottom of the yard, close to a pen containing the rest of the herd. A person would walk slowly towards the stationary animal from a distance of 32 m, so that the animal was aware of their presence (Figure 3.4). When the animal took two paces with its front feet, the distance from the animal to

the person was recorded as the flight distance. Flight distance has been shown to be a highly repeatable (0.51) measure of behaviour (Fisher et al. 2000). Those with longer flight distances were deemed to be 'flighty' or more nervous animals.

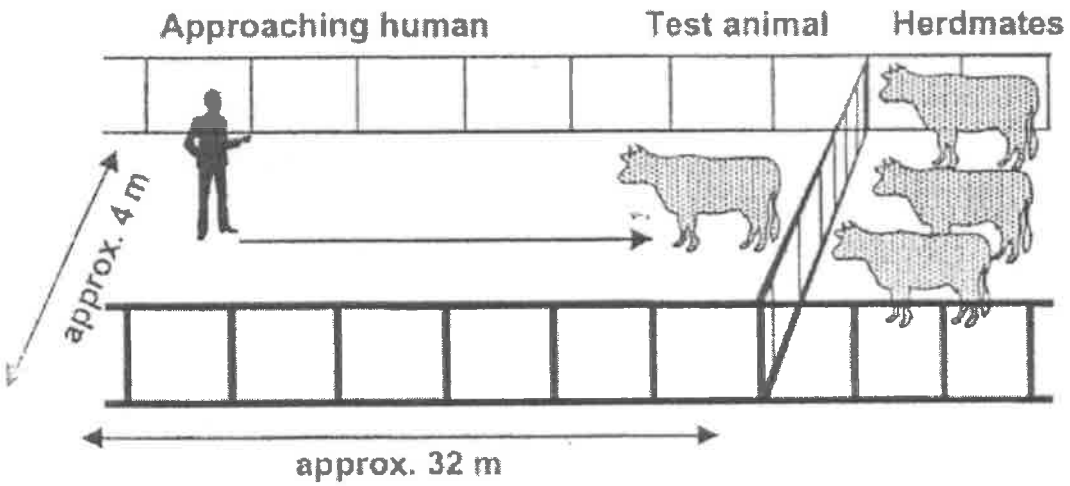


Figure 3.4 Flight distance measurement (adapted from Fisher et al. 2000)

Docility scores were also calculated on all cohorts of cattle. The docility score was calculated using variables from a docility test, similar to that of le Neindre et al. (1995). This test was also conducted in the handling yards using two pens. The first pen contained a group of herdmates, whilst the second pen contained the animal to be measured. Each animal was left alone for 30 seconds and then in the presence of a motionless handler for another 30 seconds. Docility was then measured as the animal handler attempted to contain the animal in a 2 metre by 2metre area in the corner of the pen for 30 seconds. If the handler were able to contain the animal for 30 seconds, then they would try and stroke the animal while contained. The handler was given a maximum of two minutes to achieve this and the test was concluded when the animal was contained for 30 seconds or if two minutes was reached. Animals were considered to be aggressive if they lowered their head, threatened or charged the handler. The docility score was then calculated using a multidimensional analysis (as described by le Neindre et al. 1995) using a number of

variables related to the general activity and performance of the animals recorded during the docility test (aggressiveness, time spent in the corner, number of times the animal attempted to escape, time spend running, and stroking).

3.7 Slaughter Measurements

The 1996- and 1997-drop cattle were slaughtered and processed at Valley Beef Abattoir Grantham Queensland. The 1998-drop cattle were slaughtered and processed at T&R Pastoral Murray Bridge, South Australia. At both abattoirs, a number of samples were collected, the several cuts from the *Longissimus dorsi* and *Semi tendinosis* muscles were taken to measure the ageing rate (Age) and peak force (PF). Warner-Bratzler peak force measurements were recorded at 1, 5, 12, and 26 days ageing after slaughter. For each animal, the natural logarithm of peak force was regressed on days of ageing. The y-intercept represented the initial peak force and the slope representing the ageing rate (ln kg/day).

3.8 Linkage Mapping - CRIMAP

CRI-MAP (Green et al. 1990) was used to detect any errors in the genotyping of both the mouse and cattle experiments and to confirm marker order in the linkage maps. Non-Mendelian inheritance is detected when CRI-MAP analyses pedigree and genotype data file (*.gen file). However, errors in genotyping do not always lead to non-Mendelian inheritance (the perceived error rate is less than the true error rate) so once the linkage map was built and confirmed with the published map, the *chrompic* function of CRI-MAP was used. The *chrompic* function finds the maximum likelihood estimates of the recombination fractions of the specified locus order; these estimates are then used to find the particular phase choice for each sire family and the grand-paternal and grand-maternal phases (Green et al. 1990). *Chrompic* also gives the number of recombination events. Any individuals with double or triple recombination events were carefully examined for genotyping or pedigree errors, as double recombinants are unlikely when markers are spaced at 20cM intervals.

The *all* function was used to build the linkage map. Markers were added one at a time with a LOD score of > 3, starting with the markers with the most informative meioses. The *all* function finds log10 likelihoods for all loci orders that result from placing the inserted loci

in all possible positions. Those orders with an associated log10 likelihood of less than 3 were eliminated (Green et al. 1990). The *flips* (2-6) function was then used to determine if the fixed marker order was correct.

3.8.1 Regression Interval Mapping

Regression interval mapping was also done using QTL Express (Seaton et al. 2002; QTL Express http://qtl.cap.ed.ac.uk ; accessed from 2001 - 2003) for QTL detection in both the mouse and cattle experiment. This program was developed for inbred lines by Haley and Knott (1992) and extended for outbred lines by Haley et al. (1994). The method has been widely used (Knott et al. 1998 and Brockmann et al. 1998). The analysis for QTL Express requires two steps. In the first step, the data on marker positions and actual marker genotypes are used to calculate the probability of an individual inheriting 0, 1 or 2 alleles from each of the two founder lines at positions throughout the genome. These probabilities are combined into "coefficients" that can be used to look at marker information content or marker segregation distortion. The second step of the analysis is where the phenotypic data is regressed onto the coefficients derived from the first step. This approach allows various genetic and environmental models, such as one or two linked QTL, additive, dominance (F_2 design only) and imprinting effects of QTL, effects of environmental factors (fixed effects) or covariates, and interactions of QTL with fixed effects to be fitted. The F_2 program in QTL Express was used for the mouse data while the half-sib program was used for the cattle data.

3.9 Maximum Likelihood - Interval Mapping

3.9.1 Interval Mapping

The aim of interval mapping is to test for the presence of a QTL at many positions between two mapped marker loci (Lander and Botstein 1989). The maximum likelihood approach does this by computing the likelihood of the observed distributions with and without fitting a QTL effect, as opposed to regressing phenotypes on estimated genotype probabilities as in the regression approach.

3.9.2 Composite Interval Mapping

Composite interval mapping as proposed by Jansen (1993) and Zeng (1993) has been applied to the regions found significant by interval mapping. Extra markers, besides the markers flanking the region of interest, are fitted to account for unlinked genetic variation and potentially reduce the residual variance and at the same time increase power. However, the limitations of composite interval mapping are: 1) the analysis can be affected by an uneven distribution of markers in the genome which can lead to incomparable test statistics in marker-rich versus marker-poor regions, 2) it is difficult to estimate the joint contribution to genetic variance of multiple linked QTL, 3) the analysis can not be extended to epistasis, and 4) tightly linked markers fitted as co-factors can reduce the statistical power.

3.9.3 Multiple Interval Mapping

To overcome the limitations of composite interval mapping, Kao and Zeng (1997) and Kao et al. (1999) developed multiple interval mapping (MIM). Multiple interval mapping implements QTL mapping analysis for multiple QTL in multiple intervals for a single trait in a single environment. Model selection is the key component of the analysis and the methods of Zeng et al. (1999) were followed. Selection of the number and position of putative QTL to be included in the MIM model followed a combined forward backward stepwise regression to select a subset of significant markers. Composite interval mapping was then performed utilising the significant markers to scan the genome for candidate positions. Composite and multiple interval mapping were carried out on the mouse data only.

To identify candidate epistatic terms for the initial model, markers and marker pairs were pooled together in a combined forward stepwise regression analysis. This analysis treats the marker marginal effects and pair-wise interaction effects equally when selecting significant epistatic terms (Zeng et al. 1999). Each parameter in the model is then tested for significance using MIM in QTL Cartographer. Any estimates that are not significant are dropped from the model in a stepwise manner. The stepwise selection approach used (as adapted from Zeng et al. 1999) was as follows:

1. Begin with a model that contains m QTL and t epistatic effects.

2. Scan the genome to determine the best position for the $m + 1$ QTL. When this is found, a likelihood ratio test for the marginal effect of this putative QTL is performed. If the test statistic exceeds the critical value, this effect is retained in the model. Determining how to calculate a critical value for this analysis is not yet fully understood. However, a permutation re-sampling was performed (Churchill and Doerge 1994) and a 5% permutation threshold of LOD = 2.05 was obtained for the data set. This permutation threshold is suited for the test of zero *vs.* one QTL and does not address the issue of model selection (Weber et al. 1999). The Bayes information criterion was used based on the work of Zeng et al. (1999), who found that this criterion was the most appropriate when genetic parameters, such as number of QTL, are to be estimated.
3. Search for the $t+1$ epistatic effect among pair-wise interactions that are not yet in the model, and perform the likelihood ratio test on the effect. If the critical value is exceeded, then the effect is retained in the model. This process is repeated until no more significant epistatic effects are found.
4. Re-evaluate the significance of each QTL effect in the current model. If the LOD for a QTL effect falls below the significance threshold conditioned on the other fitted effects, the effect is removed form the model. If the effect has a significant epistatic effect with another QTL, then the effect is retained. This process is also repeated until the test statistic or each effect is above the significance threshold.
5. Optimise the estimates of the QTL positions based on the currently selected model.

Chapter 4.

Studies on the metabolic basis of feed efficiency

4.1 Introduction

The mapping of genes and quantitative trait loci (QTL) both require the same essential criteria: 1) a clear phenotypic measurement, 2) family pedigrees that are segregating for the gene of interest, 3) DNA markers and a genetic linkage map, and 4) information about the physiology and biology of the trait of interest. The first three criteria will be addressed in the following chapters with the final criterion the focus of this chapter. Understanding the biological and physiological mechanisms involved is important when considering genes that could act as candidates for controlling the trait of interest. Combining positional (QTL) information with the candidate gene approach has been successful in identifying a number of the causative mutations in livestock. The discovery that the ryanodine receptor is the site for porcine stress syndrome mutation (Fujii et al. 1991) and that the myostatin locus causes double muscling in beef cattle (Grobet et al. 1997) are two examples of this positional candidate gene approach.

Since the cost of feed is the single largest cost for many livestock systems, interest in feed efficiency has grown significantly in the past decade. Selection for and against net feed intake has been performed in a variety of species including poultry (Luiting 1991; Luiting and Urff 1991a & b; Luiting et al. 1991; Bordas et al. 1992; Bordas and Minivielle 1997), pigs (Mrode and Kenedy 1993; Hermesch 2000) beef cattle (Archer et al. 1999; Herd et al. 2000; Arthur et al. 2001a; Richardson et al. 2001) and mice (Hughes 2003). These studies improve our understanding of feed efficiency and provide an alternative to selection on feed conversion ratio (or gross efficiency).

There are, however, several gaps and inconsistencies between species that exist in the current literature on selection for NFI. McDonagh (2001) examined the genetic associations between NFI, carcass traits and meat tenderness. Interestingly, after a single generation of divergent selection, there was a correlated response in calpastatin and myofibril fragmentation. Steers selected for low NFI had a lower index of myofibril fragmentation and higher levels of calpastatin in their *Longissimus dorsi* (LD-muscle) at slaughter. Calpastatin is a powerful inhibitor of calpain activity: one unit of calpastatin inhibits four units of calpain (Dransfield 1999). Since calpain is the enzyme responsible for protein turnover in muscle, it is possible that these higher levels of calpastatin are accompanied by lower rates of protein breakdown in muscle, which could contribute to the

difference in efficiency of energy utilisation between divergently selected lines of cattle in NFI.

Several studies on protein synthesis have been performed on two lines of mice, one known to contain a major gene for growth and a control line, Bernier and Calvert (1987) and Bernier et al. (1987). The authors found:

1. A linear relationship between heat produced from body protein synthesis and metabolisable energy intake.
2. The increased heat produced from protein synthesis associated with increased metabolisable energy intake was not different between the lines but the energetic efficiency of protein deposition was greater in the high growth line.
3. The relationship between the energy used during protein synthesis and metabolisable energy intake was variable in both lines (assuming no measurement error). The between animal variation suggests that the efficiency of protein deposition can be improved.

Based on these studies, the first hypothesis was developed:
Differences in feed intake observed when divergently selecting for NFI could be associated with differences in protein synthesis, such that the high NFI line would have higher rates of protein synthesis and turnover in both the liver and muscle tissues.

Whilst protein synthesis and metabolism both require significant amounts of the body's energy stores, some of the differences observed between the NFI selection lines in intake could be influenced by the rate of passage of food/chyme through the digestive system. Once a bolus of food is swallowed, there are a large number of events that involve complex digestive, secretory, absorptive, and excretory processes. The digestive system plays an important role in regulating all of these events and processes so that food is efficiently digested and absorbed. For example, the stimulation of the smooth muscles of the oesophagus must be sequentially coordinated to produce peristalsis. There are also a number of endocrine, exocrine, neurotransmitters and feedback mechanisms that signal to the brain and other parts of the body that food/chyme is ready to be moved to the next stage for further breakdown or absorption.

The second hypothesis formed was:
The high NFI mice would have a faster rate of gastric emptying and therefore need to eat more to retain the same amount of nutrients from the feed.

This chapter involves two experiments with the broad aim of detecting candidate genes for NFI by determining differences that exist between the mice NFI selection lines in: 1) body composition, metabolic rate, activity and protein synthesis and 2) gastric emptying. Each experiment was performed on different generations of mice that had been divergently selected for NFI.

4.2 Methods

4.2.1 Mice used for protein turnover, body composition and metabolic study

Mice from the eleventh generation of divergent selection for post-weaning net feed intake (NFI) were used to determine protein synthesis, body composition, activity and metabolic parameters. There were 280 mice which were randomly allocated to one of three experimental groups: group 1 had protein synthesis measured, group 2 had chemical body composition measured and group 3 had basal metabolic rate and activity measurements taken. There were equal numbers of mice from the high and low NFI selection lines and equal numbers of each sex within each of the lines. There were approximately 192 mice measured in group 1 (protein synthesis), 60 mice in group 2 (body composition), and 24 mice in group 3 (basal metabolic rate and activity). Staff at the Beef Improvement Centre, NSW Agriculture, Armidale, conducted the following experimental procedures.

Feed intake and body weights were recorded weekly for 2, 4 or 6 weeks after weaning depending on the age at which they were killed. Feed intake was measured as described in the animal resources and general methods section. The composition of the Ridley Agriproducts mouse feed (Table 4.1) and equation 40 (Table 7) from Noblet and Perez (1993) was used to calculate the metabolisable energy of the feed. A bomb calorimeter was used to determine the gross energy of the feed. The metabolisable energy content was 10 MJ ME/kg DM.

Table 4.1 Chemical composition of mouse feed

Feed Component	Units
Dry Matter	90.7 % of fresh weight
Protein	26.3% DM
Non-Degraded Fibre (NDF)	28.3% DM
Ash	10.1% DM
Gross Energy	16.3 MJ/kg

4.2.2 Protein turnover experimental procedure

4.2.2.1 Animals and feed intake measurements

There were 192 mice (96 from each of the selection line) that had protein synthesis measured. Two days prior to each measurement time, half of the mice were restricted to 60 percent of the previous weeks *ad libitum* intake. Protein synthesis was measured on 48 mice from each selection line at four ages (6, 8, 10 and 12 weeks of age), at two different feed intake levels (*ad libitum* or 60% *ad libitum*). Due to technical problems, only the mice killed at 8 and 10 weeks of age were used for the analysis of protein synthesis. In this serial slaughter experiment, the mice were randomly allocated to a slaughter date.

4.2.2.2 The flooding dose procedure

Mice were injected intraperitoneally with a flooding dose of phenylalanine solution (150mM 1-Phe containing 100μCi 2,6 ^{3}H-1-Phe/ml and approximately 45 % enrichment with d5 1-Phe). Both radio-labelled and tracer doses of phenylalanine were given to the mice so that two different methodologies could be used. The tracer dose was the preferred method for future work on larger animals (cattle and sheep), as the carcasses would not be radioactive. In the flooding dose approach, a large volume of labelled amino acid, in excess of the body's free amino acid pool, was given to dominate and control the enrichment of the free amino acid pool during the measurement period. Mice were weighed and killed by cervical dislocation approximately 10 minutes after injecting. The liver and hind leg muscle were excised immediately and placed in liquid nitrogen and stored at -80°C until analysis. The exact times to freezing were recorded for the calculation of protein synthesis rates.

The tissue samples were prepared for [^{3}H & ^{2}H] phenylalanine analysis following the procedure of Danicke et al. (1999). This procedure was also used to determine the free and bound amino acids on a HP 6890.GC system and a HP 5973 MS engine. The peak area ratios for the fragment ions at m/z 239 and 234 of the phenylalanine derivative were recorded. The calculation of the atom percent excess of phenylalanine in the respective samples was based on the equation of McNurlan et al.(1979).

The rate of fractional synthesis (FSR; %/day) of protein was determined by:

$$FSR = \frac{MPEb}{MPEf \times time} \times 100$$

Equation 4.1

MPEb = mass percent excess for bound amino acids (precipitate)
MPEf = mass percent excess for free amino acids (supernatant)
Time = time after injection in days

The amount of protein in the muscle only was determined using a BCA protein Assay Kit (Pierce, 23225).
The absolute synthesis rate (ASR, g/day) was calculated as:

$$ASR = FSR \times protein$$

Equation 4.2

where FSR is the fractional synthesis rate (%/day) and protein is the amount of protein (grams) determined as described herein (Section 4.2.3).

Measurement of DNA in a tissue can be considered an indicator of the number of cells in that tissue, and the protein to DNA ratio an indicator of cell size, or more precisely, DNA unit size (Cheek et al. 1971; Robinson 1971). DNA and RNA were extracted from the hind leg muscle using Perchloric acid (PCA) while the concentration of RNA was determined using an Orcinol reagent (based on Bioal's test for pentoses) and DNA using a diphenylamine assay.

4.2.3 Body composition experimental procedure

In total, 64 mice had chemical body composition measured (half from the high NFI line, half from the low NFI line). Chemical body composition requires the removal of tissues from the body, so a serial slaughter was performed. Measurements were taken when the mice were approximately six, eight or ten weeks of age. All mice were placed on the post-weaning growth and feed intake test prior to slaughter. Immediately following the growth and intake measurements, the mice were fasted overnight and killed by cervical dislocation. They were then eviscerated and skinned, to give three body components: viscera, skin (including head and feet), and carcass. The three body components were frozen at -20°C overnight, followed by 48 hours of freeze-drying. Total body water was calculated by subtracting the live weight of the animal from the dried weight of the animal. Fat was measured on the dried samples as the weight loss from the thimble following two times 65 minute extractions with chloroform in a Soxtec 1043 Extraction Unit. Nitrogen was determined for the fat-free samples using a Leco Nitrogen Analyser (model FP-200). Protein was calculated as the nitrogen content multiplied by 6.25. Body ash content was determined by the difference (body ash = dry weight – (protein weight + fat weight)). The mice from this experimental group also had dry matter digestibility estimated. This was done by collecting and weighing the faeces of the mice each week.

4.2.4 Basal metabolic rate and activity experimental procedure

The 24 mice in experimental group 3, (12 from each of the selection lines) were measured for activity and metabolic rate. Metabolic rate was determined by indirect calorimetry. Due to restricted capacity to measure metabolic rate, mice were divided into four groups of 6 mice. Two groups were measured in one week and the other two groups in the following week. The apparatus and methodology for the measurement of metabolic rate is described in Geiser et al (1998). Briefly, three mice were placed into individual 1-litre respirometry chambers situated in a temperature-controlled cabinet (33±0.5°). The flow rate (450 ml/min) of dry air drawn through each of the respirometry chambers was measured with a mass flow meter. Oxygen content was measured with a single-channel oxygen analyser. Each of the 3 mice chambers and 1 reference chamber were scanned in sequence for 3 minutes every 12 minutes to determine oxygen content in the outside air and in each of the chambers. The mice were fasted overnight, with some mice measured in the morning and others in the afternoon.

Metabolic heat production was derived from the formula of Miller et al. (1981):

$$MHP(kJ) = 16.17 \times V_{O2} + 5.02 \times V_{CO2}$$

Equation 4.3

where V is volume of respiratory gases in litres at standard temperature and pressure.

Metabolism of carbohydrates, fats, and proteins require different amounts of oxygen to completely oxidise the carbon and hydrogen atoms in each molecule to carbon dioxide and water. The respiratory quotient (CO_2 produced as a proportion of O_2 consumed) gives an indication of what substrate is being metabolised by the animal.

Locomotor activity was monitored continuously for six weeks by passive infrared sensors (Jaycar Electronics, LA-5017) that were placed on top of each mouse cage. Activity events were recorded in 15 minute intervals to a maximum of 255 movements per period then logged to a custom-built eight channel data logger [Electronic Services Unit; University of New England].

4.2.5 Derived values

Metabolisable energy intake (MEI) was calculated by multiplying the daily feed intake by the metabolisable energy content of feed (10 MJ/g). The energy retained (ER) due to the increase in body mass was calculated as the increase in energy stored in body tissues between 6 and 8 weeks of age and 8 and 10 weeks of age. MEI and ER were calculated on all three experimental groups of mice. Energy stored in body tissues was estimated assuming 23.6 MJ of energy per kilogram of protein and 39.3 MJ of energy per kilogram of fat (Pullar 1977) using mice from group two. Heat production (HP) was calculated as the difference between the MEI and ER in body mass. The energy cost of fat synthesis (EC_{fs}) was determined from ER as fat gained multiplied by 0.15 KJ expended/KJ stored (Reeds 1991). The energy cost of fat synthesis was calculated on group 2 mice that had body composition measured.

Whole-body protein synthesis was calculated assuming that absolute synthesis in the muscle accounts for 17.7% of the whole-body synthesis in mice (as reviewed in Garlick 1980):

$$ASR_{wb} = \frac{FSR_m \times [Protein_m] \times CarcassWeight}{0.177}$$

Equation 4.4

Where ASR_{wb} is whole-body absolute synthesis, FSR_m is fractional and synthesis rate of muscle. The cost of whole-body protein synthesis (EC_{ps}) was calculated as whole-body (absolute) protein synthesis multiplied by 23.6 (MJ of energy stored per gram of protein) and 0.15 (KJ expended/KJ stored) (Reeds 1991). Absolute whole-body protein gain per day was crudely estimated from daily growth rate. Absolute whole-body degradation rate was determined as the difference between whole-body protein synthesis and gain. Based on the work of Reeds (1991), the energy cost of whole-body protein degradation (EC_{pd}) was calculated as the absolute degradation rate multiplied by 0.04 (KJ expended/KJ degraded). These measurements were derived from the group 1 mice that had protein synthesis measured.

Resting metabolic rate (RMR) of the mice at 21°C (animals at room temperature) was estimated as twice the metabolic heat production (MHP was measured at 33°C) based on the review of Brody (1945) where heat production in mice at 21°C was twice the heat production at 33°C. The energy cost of activity (EC_a) was estimated based on the work of Taylor and colleagues (1970). In that study, the energy cost of running was related to body size in seven different mammalian species. The authors found that steady-state oxygen consumption increased almost linearly with running speed and could be expressed as a linear equation:

$$M = 2.83 \times V + 3.34$$

Equation 4.5

where M= oxygen consumption in ml O_2/g/hr and V = velocity in km/hr and 3.34= basal metabolic rate. Since activity in the experiment herein was measured in counts/hr and not running velocity (km/hr), several assumptions were made. Firstly, the maximum counts observed in one hour was 1020 in this experiment was the equivalent of running 1 km/hr (the maximum running velocity observed by Taylor and colleagues). Thus, an activity count of 1 would be the equivalent of running 0.001 km/hr. Secondly, it was assumed that the rate (2.83 ml O_2/gbwt/day) at which oxygen consumption increased with velocity in the studies of Taylor was the same for the mice in this experiment. Thus, the equation used for oxygen consumption was

$$M = 2.83 \times Act \times 0.001$$

Equation 4.6

where Act = activity in counts/gbwt/hr. These activity measurements and the resting metabolic rate measurements were determined using the 24 mice in experimental group 3 (Table 4.2).

Table 4.2 Mice groups and numbers of animals used in calculating the energy balance of the NFI selection lines

Trait	Abbrev.	Animals Used	High NFI	Low NFI
Metabolisable Energy Intake	MEI	Group 2	25	24
Energy Retained	ER	Group 2	25	24
Heat Production	HP	Group 2	25	24
Resting Metabolic Rate	RMR	Group 3	11	12
Energy Cost of Activity	EC_a	Group 3	11	12
Energy Cost of Protein Synthesis	EC_{ps}	Group 1	24	24
Energy Cost of Protein Degradation	EC_{pd}	Group 1	24	24
Energy Cost of Fat Synthesis	EC_{fs}	Group 2	25	24

A summary of the abbreviations can be found in Table 1.

4.2.6 Gastric emptying protocol

Forty mice (20 from each of the selection lines) from generation 12 of selection were used for the breath analysis study. Analysing the breath of animals provides a non-invasive method for determining the function of the gastro-intestinal tract (Butler 1996) and more specifically the rate of gastric emptying. A range of techniques have been developed to analyse gastric emptying: 1) marker dilutions (Conover et al. 1987; and Penner and Hollander 1940), 2) ultrasound (Batemann and Whitingham 1982; Bolonid et al. 1985; and Gilja et al. 1997), 3) radioscintigraphy (Griffith et al. 1966; Siegel et al. 1988) and most recently, 4) breath analysis (Bjorkeman et al. 1991; Ghoos et al. 1993; Symonds et al. 2000 & 2001). Breath tests that measure gastric emptying are based on the ingestion of a substrate that is labelled with a stable carbon isotope incorporated in the functional group (Ghoos et al. 1993). The gastric emptying of the test meal becomes the rate-limiting step such that the rate of labelled CO_2 that is excreted in the breath can be used to estimate the rate of gastric emptying (Maes et al. 1994). The potential for differences between the NFI selection lines exists.

The breath testing and analysis were performed by Erin Symonds from the Adelaide Women's and Children's Hospital. A detailed description of the procedure can be found in Symonds et al. (2001). Briefly mice were fasted overnight and then given either 1) a non-nutrient liquid meal, that contained water with a thickening agent (hydorxypropyl methylcellulose and 1 μl/mL [^{13}C]-acetic acid), 2) a nutrient liquid (intralipid and 1 μl/mL [^{13}C]-octanoic acid) or 3) a solid egg yolk meal (0.1 grams) and [^{13}C]-octanoic acid the following morning. All 40 mice were given each of the three types of meals with at least 3 days recovery in between measurement periods. Prior to the administration of the labelled liquid or solid meal, a baseline breath sample was taken. The mice were then placed in individual chambers (Figure 4.1) that contained two three-way valves inserted in the lid to allow a continual airflow. Breath samples were collected at 5-minute intervals for the first 30 minutes, followed by 15-minute intervals for 120 minutes (liquid meal) or 150 minutes (solid meal). Breath samples were obtained after the airflow to the chamber had stopped for 90-120 seconds by a syringe. Stopping the airflow allowed the CO_2 levels to accumulate to greater than 1%. The breath samples were then injected into evacuated 10 mL Exetainer tubes so that $^{13}CO_2$ content of the samples can be determined by isotope ratio mass spectrometry (ABCA 20/20 Europa Scientific). The time taken to excrete half of

the gastric contents ($t_{1/2}$) is used as the indicator of gastric excretion. The $t_{1/2}$ value describes both the gastric half emptying time and the time taken for post-gastric processes of absorption and metabolism of the labelled substrate (Ghoos et al. 1993). The initial delay in gastric emptying due to the stomach grinding the meal into particles that are fine enough to pass through the pylorus is known as the lag phase (t_{lag}). This was calculated for the solid meal only. The mathematical formulae used to derive the $t_{1/2}$ and t_{lag} can be found in Symonds et al. (2000).

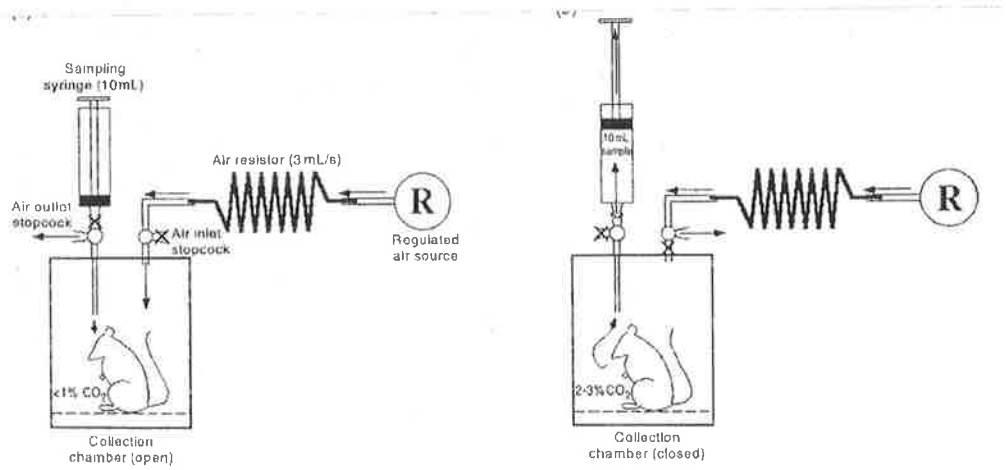


Figure 4.1 Mouse chambers for collection of exhaled breath a) when the system is open to allow a continual airflow through and b) when the system is closed to allow breath to accumulate (taken from Symonds et al. 2001).

4.2.7 Statistical analysis

4.2.7.1 Analysis of protein synthesis, metabolic rate and body composition data

The metabolic rate, body composition and protein synthesis data were all analysed using sex (M, F), line (H, L), slaughter age (8, 10) and level of feed intake (restricted, *ad libitum* - protein synthesis measurements only) as fixed effects and the interactions where significant using the GLM procedure of SAS (SAS 1998). To minimise the impact of a few sires on overall conclusions, a sire model was fitted using the MIXED procedure in SAS (SAS 1998). Dam nested within sire was also fitted as a random effect with the same fixed effects to ensure differences observed were not due to one or two sires but reflected true line differences. To aid interpretation of a number of body composition traits, principal components were formed between visceral, carcass and skin chemical compositions.

4.2.7.2 Spectral analysis of the activity data

The activity data represents a time series of 24 hourly observations (counts/hour). It was first analysed using proc MIXED in SAS (SAS 1998) with sex, line and time fitted as fixed effects and the repeated statement for time within each line, to account for the repeated measures within an animal. Spectral analysis was then used to investigate the cyclical nature of the activity data (circadian rhythms). Spectral analysis partitions the total variation in a time series into sums of squares that are contributed to different cyclical components (Seman et al. 1997). Fourier frequencies were calculated. That is, the cyclical components are identified by frequency and a total of n/2 frequencies are calculated (where n is the number of observations in the time series). For each Fourier frequency, an ordinate, or sum of squares, was calculated by the following equation:

$$I(\omega) = \left[\left\{ \sum_{t=1}^{n} yt \cos(\omega t) \right\}^2 + \left\{ \sum_{t=1}^{n} yt \sin(\omega t) \right\}^2 \right] / n$$

Equation 4.7

where $I(\omega)$ is the ordinate due to each Fourier frequency (ω), n is the number of observations in the time series and t represents time, in hours. Genstat (1989) was used to calculate time series ordinates for each of the NFI lines and sexes within each line. The ordinates are plotted against cycle length on a periodogram. Significant ordinates were identified by an F test [2,2m-2 degrees of freedom] where m is the largest integer that is ≥

(n-1)/2. This tests whether the sine and cosine functions = zero (no significant cycles). To compare the spectra from the high and low selection lines, the procedure of Diggle (1990) was used.

4.2.7.3 Analysis of gastric emptying data

Initially, a simple linear regression using proc GLM in SAS (SAS 1998) was fitted to all variables in the gastric emptying data set (Table 1). The model included line and sex, fitted as fixed effects, and the interaction between line and sex with age was fitted as a covariate. Generally the proportion of feed digested is inversely proportional to feed intake resulting in fewer nutrients per gram of feed available so where the solid meal was given the amount of feed eaten was fitted as covariate to account for any differences in feed intake. Any term that was not significant was removed from the final model. A mixed model was then fitted to any trait data that had a significant line effect using the MIXED procedure in SAS (SAS 1998). Sex and line and the interaction between those two variables were fitted as fixed effects, and sire and dam nested with-in sire were fitted as random effects to determine if the differences observed were due to a sire effect.

4.3 Results

4.3.1 Body composition

At weaning, there was no difference in weight or age between mice from the high or low NFI selection lines at generation 11 of selection. Average daily gain over the test period, metabolic mid-weight and DM digestibility did not differ between the selection lines (Table 4.3). Mice from the high NFI (low efficiency) selection line ate 25% more than mice from the low NFI selection (high efficiency) line. Feed conversion ratio (FCR) was measured between 4 to 6 weeks of age and was 50% higher in the high NFI line. NFI was not different between males and females because sex was included in the model for calculating NFI.

The high NFI mice had 32% less total fat than the low NFI mice with no significant differences in live weight, the amount of protein or ash between the lines during the experimental procedure. The low NFI mice were fatter than the high NFI line from 6 to 12 weeks of age (Figure 4.2), whilst the amount of protein in the whole-body was not different during this time (Figure 4.3). The carcass weight of the high NFI mice was 9% lighter, they had 37% less carcass fat and 7% less carcass protein than the low NFI mice. When expressed as a percentage of the animals' body weight, the differences between the lines in carcass fat and protein remained significant. There were no significant differences ($P < 0.05$) in any of the visceral components except visceral fat was less ($P<0.054$) in the high NFI mice. In line with the other measures of fatness (not presented) the principal components analysis supported these findings with principal component one, which represented overall weight of fat and lean tissue being slightly lower ($P=0.036$) in the high NFI line. This principal component accounted for 49% of the standardised variance of the body composition. Principal component two accounted for 38% of the standardised variance and reflected fat compared to protein, with the high NFI line containing a lower proportion of fat relative to protein.

Table 4.3 Feed intake and body composition means (± standard error) of mice from group 2

Trait	High NFI	Low NFI	% Dev.	Sign.
Number of mice	25	24		
Net Feed Intake (g/day)	0.65 ± 0.13	-0.48 ± 0.09	23	***
Daily Feed intake (g/day)	6.01 ± 0.13	4.95 ± 0.09	21	***
Dry Matter Digestibility (%)	60.1 ± 3.9	62.4 ± 3.6	-4	n.s.
Whole Animal				
Live Weight (g)	18.07 ± 0.47	18.44 ± 2.01	-2	n.s.
Fat (g)	0.75 ± 0.07	1.11 ± 0.47	-32	**
Protein (g)	3.70 ± 0. 11	3.72 ± 0.47	-1	n.s.
Ash (g)	0.88 ± 0.13	0.92 ± 0.10	-4	n.s.
Percentage of Live Weight				
Fat (%)	4.1 ± 1.8	6.0 ± 2.5	-33	**
Protein (%)	20.5 ± 1.0	20.0 ± 0.9	3	n.s.
Ash (%)	4.9 ± 0.40	4.9 ± 0.4	0	n.s.
Component Weights				
Carcass Weight (g)	7.18 ± 0.26	7.82 ± 0.25	-9	**
Carcass Fat (g)	0.229 ± 0.024	0.365 ± 0.040	-37	**
Carcass Protein (g)	1.450 ± 0.056	1.560 ± 0.052	-7	**
Carcass Ash (g)	0.339 ± 0.014	0.355 ± 0.012	-5	n.s.
Skin Weight (g)	5.70 ± 0.13	5.56 ± 0.09	3	n.s.
Skin Fat (g)	0.317 ± 0.032	0.472 ± 0.044	-33	*
Skin Protein (g)	1.457 ± 0.042	1.376 ± 0.038	6	n.s.
Skin Ash (g)	0.346 ± 0.009	0.352 ± 0.007	-2	n.s.
Visceral Weight (g)	4.765 ± 0.120	4.772 ± 0.128	0	n.s.
Visceral Fat (g)	0.209 ± 0.025	0.279 ± 0.026	-24	†
Visceral Protein (g)	0.792 ± 0.105	0.757 ± 0.100	5	n.s.
Visceral Ash (g)	0.192 ± 0.049	0.201 ± 0.046	-5	n.s.

n.s., Fprob > 0.10, † Fprob < 0.10, * Fprob < 0.05, ** Fprob < 0.01, *** Fprob <0.001

% Dev., percentage deviation of the high NFI selection line from the low NFI line.

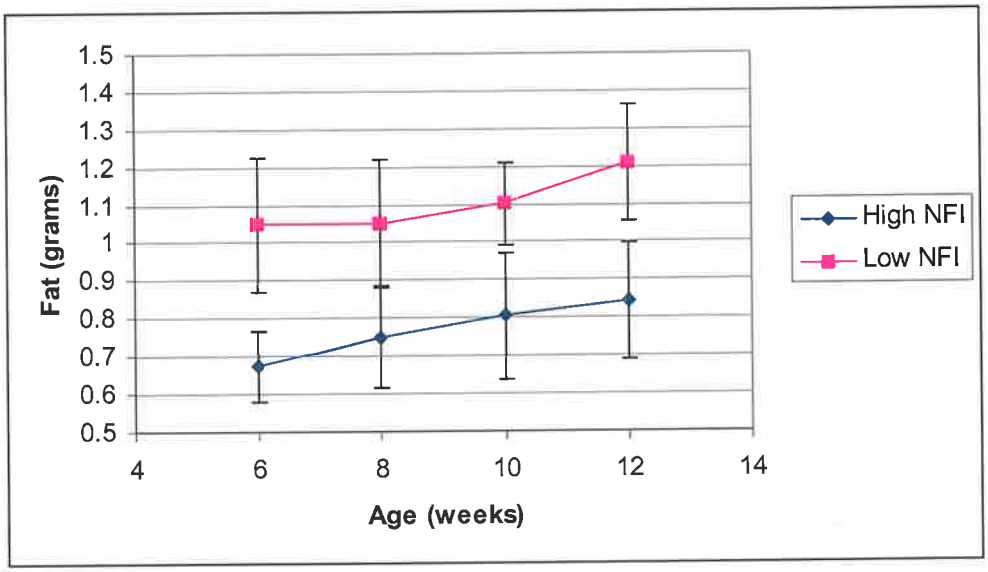


Figure 4.2 **Change in body fat, between the NFI selection lines over time**

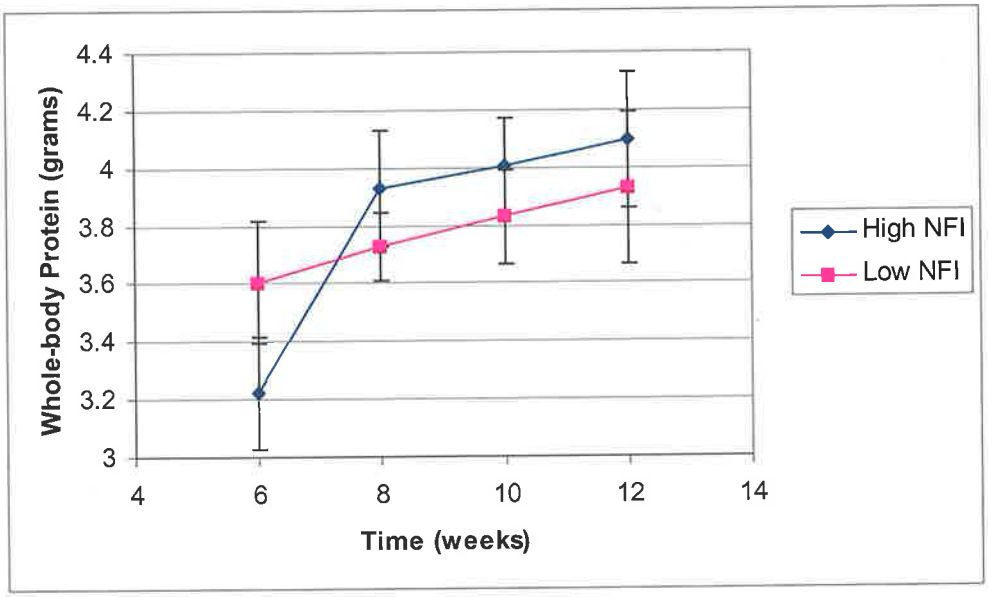


Figure 4.3 **Change in whole-body protein between the selection lines over time**

4.3.2 Protein Synthesis

4.3.2.1 Line Differences in Protein Synthesis

There was no difference between the selection lines in fractional protein synthesis in the liver or muscle or in the rate of absolute synthesis in the muscle (Table 4.4). There was also no difference in the weight of the mice or in muscle protein concentration. However, the low NFI line had significantly heavier leg weights, and therefore, more total leg protein than the high NFI line. The concentration of DNA and RNA in the muscle was 14% and 10% higher in the high NFI line, respectively. However, the total amounts of DNA and RNA were not different between the lines. Because the amount of protein in the muscle was greater in the low NFI line and the DNA and RNA concentrations were not different the protein to DNA ratio (cell unit size) and the protein to RNA ratio were 14% and 10% greater in the low NFI line. There was no difference between the lines in the RNA to DNA ratio or in the ASR to RNA ratio in the muscle. Whole-body absolute synthesis, gain or degradation rates were not different between the selection lines. Although the high NFI line had greater rates of synthesis and degradation, the lines were not significantly different.

4.3.2.2 Intake Differences in Protein Synthesis

The fractional synthesis rate of protein in the liver of *ad libitum* feed mice was 19% greater than mice that had been restricted. However, fractional synthesis in the muscle was not affected by level of nutrition. The rate of absolute synthesis in the muscle was 32% higher in the *ad libitum* fed than restricted mice. This was because the amount of protein in the muscle of the *ad libitum* fed mice was greater. The absolute rates of whole-body protein synthesis, gain and degradation were all greater in the *ad libitum* fed mice. The concentration of RNA was not different between restricted and *ad libitum* fed mice, but the DNA concentration was 9% greater in the restricted mice. The ratio of protein to DNA was 11% higher in *ad libitum* fed mice with no difference in the ratio of protein to RNA. The RNA to DNA ratio was 7% greater in *ad libitum* fed mice with a 20% ASR to RNA ratio. The interaction between line and level of feeding was not significant for any of the measured traits.

Table 4.4 Means (± standard error) for each line and feeding level for mice from group 1 slaughtered at 8 and 10 weeks of age.

	Line		Feeding Level		F Probability	
Intake	**High NFI**	**Low NFI**	***Ad lib.***	**Restrict**	**Line**	**Feed**
Number of mice	48	47	48	47		
Body Weight g	21.5 ± 0.4	21.9 ± 0.5	22.6 ± 0.4	20.8 ± 0.4	n.s.	***
Leg Weight g	0.72 ± 0.02	0.85 ± 0.02	0.81 ± 0.02	0.76 ± 0.02	***	n.s.
FSR_l %/day	74.9 ± 1.9	73.2 ± 1.9	80.7 ± 1.9	67.5 ± 1.9	n.s.	***
FSR_m %/day	7.4 ± 0.6	6.6 ± 0.6	7.3 ± 0.6	6.1 ± 0.9	n.s.	n.s.
Protein mg/g	198 ± 2	199 ± 2	195 ± 2	202 ± 1	n.s.	***
Total Protein mg	142 ±15	169 ± 5	157 ± 5	153 ± 5	***	n.s.
DNA μg/g	531 ± 12	483 ± 16	486 ± 12	530 ± 17	***	**
Total DNA μg	380 ± 10	400 ± 10	380 ± 10	390 ± 10	n.s.	n.s.
RNA μg/g	2009 ± 38	1803 ± 30	1895 ± 39	1918 ± 34	***	n.s.
Total RNA μg	1430 ± 50	1520 ± 50	1500 ± 40	1440 ± 50	n.s	n.s.
Protein/DNA	381 ± 8	429 ± 12	413 ± 10	397 ± 11	***	*
Protein/RNA	100 ± 2	111 ± 2	105 ± 2	107 ± 2	***	n.s.
RNA/DNA	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	3.7 ± 0.1	n.s.	**
ASR mg/day	10.2 ± 1.1	10.9 ± 1.0	11.6 ± 1.0	9.5 ± 1.0	n.s.	**
ASR/RNA	21 ± 2	24 ± 3	24 ± 2	20 ± 3	n.s.	n.s.
ASR/DNA	80 ± 9	93 ± 11	98 ± 9	75 ± 11	n.s.	*
ASR_{wb} mg/day	594 ± 21	580 ± 18	666 ± 19	502 ± 18	n.s.	**
AGR_{wb} mg/day	25 ± 3	23 ± 2	32 ± 4	10 ± 4	n.s.	*
ABR_{wb} mg/day	569 ± 19	557 ± 17	634 ± 18	492 ± 18	n.s.	**

n.s., Fprob > 0.10, † Fprob < 0.10, * Fprob < 0.05, ** Fprob < 0.01, *** Fprob <0.001

Concentrations are in μg or mg per gram of leg muscle.

$_{wb}$ denotes estimates of whole-body rates; FSR_m fractional synthesis rates of leg muscle; FSR_l denotes fractional synthesis rates of liver.

A summary of the abbreviations can be found in Table 1.

4.3.3 Activity and metabolic rate

Basal metabolic rate and metabolic heat production were both 20% higher in the high NFI line when measured as a per weight basis (Table 4.5). The respiratory quotient was not different between the lines or the sexes. The high NFI line was 86% more active than the low line, and females were more active than males.

Further analysis of the activity data revealed that activity was highly repeatable (r=0.82). A spectral analysis was performed separately on each line. Significant cycle lengths for activity are determined by exceeding the *F* critical value (Figure 4.4). Both the high and low NFI selection line exhibited cycle lengths of 12, and 24 hours. There was no difference in the cyclicity between the lines, but the amplitude of the high intake selection line was significantly higher (Figure 4.5).

Table 4.5 Means (± standard error) for Metabolic Traits and Activity

	High NFI	Low NFI	Male	Female	Line	Sex	LxS
No. animals	12	12	12	12			
NFI	0.43 ± 0.12	-0.83 ± 0.10	-0.01 ± 0.13	0.06 ± 0.11	***	n.s.	n.s.
DFI	5.95 ± 0.21	4.74 ± 0.20	5.46 ± 0.20	4.92 ± 0.19	***	***	n.s.
BMR[a]	1.90 ± 0.28	1.58 ± 0.28	1.69 ± 0.39	1.76 ± 0.32	*	n.s.	n.s.
RQ	0.75 ± 0.08	0.74 ± 0.07	0.74 ± 0.07	0.75 ± 0.08	n.s.	n.s.	n.s.
MHP[b]	36.8 ± 6.1	30.7 ± 5.2	32.6 ± 7.0	34.3 ± 5.8	*	n.s.	n.s.
Activity[c]	310 ± 177	167 ± 122	214 ± 126	255 ± 197	***	***	†

n.s.; Fprob > 0.10; † Fprob < 0.10; * Fprob < 0.05; ** Fprob < 0.01; *** Fprob <0.001

A summary of the abbreviations can be found in Table 1; [a] ml O_2/g/hr [b]; J/g/hr ; [c] counts/hr

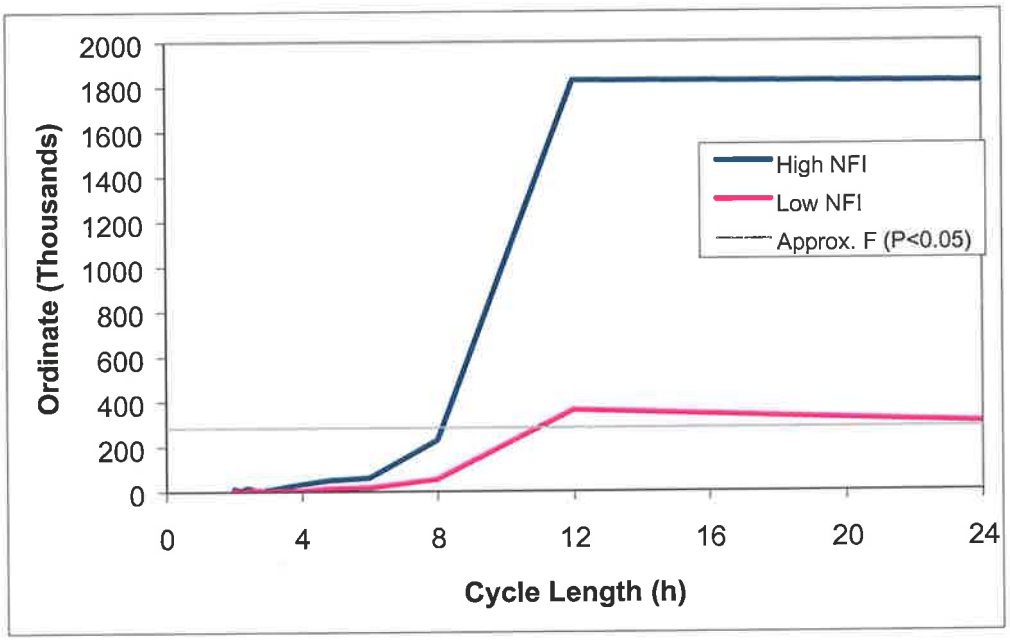


Figure 4.4 **Periodogram for the high and low NFI selection lines**

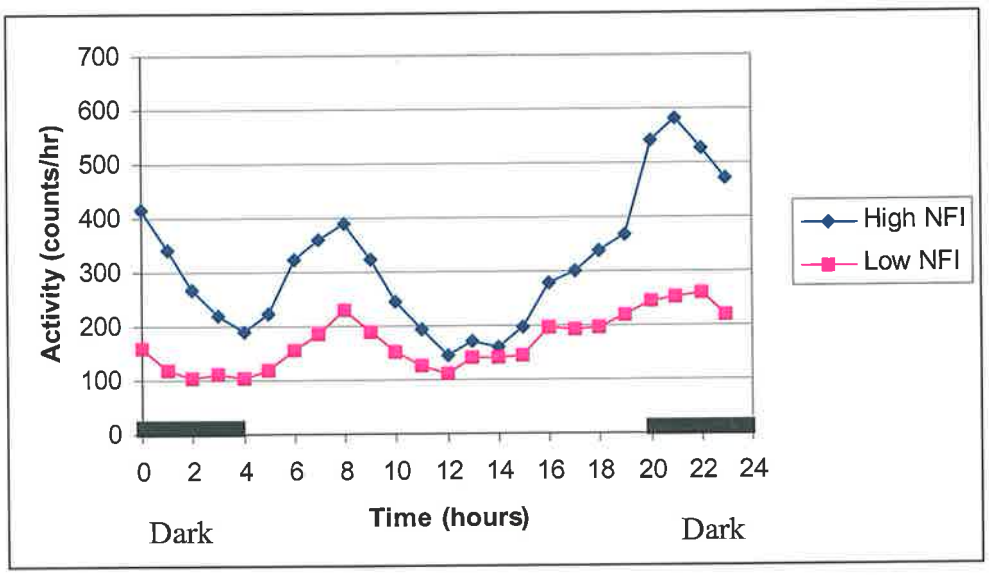


Figure 4.5 **Mean activity of each of the NFI selection lines**

4.3.4 Estimated energy balance

The metabolisable energy intake when expressed as Joules per gram of body weight per day was 20% different between the selection lines (Table 4.6). Energy retention was small and not different between the lines. Thus, heat production was very similar to the metabolisable energy intake (20% difference). Resting metabolic rate was also 20% greater in the high NFI line when expressed in joules per gram of body weight per day. When the energy cost of activity was estimated, the high NFI line used approximately 40% more energy on activity. There were no differences in energy used for protein synthesis, degradation or fat synthesis between the lines. When metabolisable energy intake was taken into account, differences between the NFI lines were observed in activity only (Table 4.7).

Table 4.6 Energy balance means (± standard error) for each selection line and the group of animals used to estimate each component

J gbwt^{-1} day^{-1}	High NFI	Low NFI	Group	% Dev.	F Probability
MEI	2925 ± 65	2443 ± 68	2	20	***
ER	46 ± 17	40 ± 17	2	15	n.s.
HP	2879 ± 94	2403 ± 70	2	20	***
RMR	1768 ± 93	1474 ± 73	3	20	**
EC_a	1019 ± 10	731 ± 6	3	40	***
EC_{ps}	98 ± 7	94 ± 8	1	4	n.s.
EC_{pd}	4 ± 1	4 ± 1	1	13	n.s.
EC_{fs}	26 ± 14	26 ± 15	2	0	n.s.

n.s.; Fprob > 0.10; † Fprob < 0.10; * Fprob < 0.05; ** Fprob < 0.01; *** Fprob <0.001

% Dev., percentage deviation of the high NFI line from the low NFI line. Group refers to the group of animals that the measurements were recorded from (as per section 4.2.1).

A summary of the abbreviations can be found in Table 1.

Table 4.7 Energy balance means (± standard error) adjusted for metabolisable energy intake.

J gbwt^{-1} MEI^{-1} day^{-1}	High NFI	Low NFI	% Dev.	F Prob.
HP	0.982 ± 0.025	0.982 ± 0.028	0	n.s.
RMR	0.660 ± 0.035	0.749 ± 0.047	-12	n.s.
EC_a	0.348 ± 0.108	0.293 ± 0.082	19	***

n.s.; Fprob > 0.10; † Fprob < 0.10; * Fprob < 0.05; ** Fprob < 0.01; *** Fprob <0.001

% Dev., percentage deviation of the high NFI line from the low NFI line.

A summary of the abbreviations can be found in Table 1.

4.3.5 Gastric emptying

The 20 most extreme mice for NFI from generation 12 of both NFI selection lines were analysed for differences in their rate of gastric emptying. The difference between the 20 high intake mice and the 20 low intake mice in both NFI and DFI was 48% (Table 4.8), with no differences between the two groups of mice in average daily gain or body weight. The high intake mice had significantly lower (21%) gastric emptying of solid material. They also took less time to grind food in their stomach (25%) and reach their maximum speed of emptying (23%). For both the lipid and solid meals, the high intake mice had a more rapid decline (34%) in the gastric emptying curve (Figure 4.6). The height of the curve for the high intake line was in general 50% greater than the low intake line for the lipid meal but not the non-nutrient or the solid meal. The gastric emptying of the non-nutrient liquid meal was not different between the lines. The mixed model analysis indicated that the differences observed between the selection lines was not due to a sire effect but most likely due to real differences between the lines. Surprisingly there was a significant sex by line interaction for $t_{1/2}$, t_{max}, and k of the non-nutrient meal. The high intake females and the low intake males had faster $t_{1/2}$ and t_{max} and rate of decline (k) was also significantly greater.

Table 4.8 Differences between the selection lines in gastric emptying of different meals; non-nutrient, intra-lipid and solid (egg yolk), means ± se.

Trait	High NFI	Low NFI	% Difference	F-Prob.
Non-nutrient				
$t_{1/2}$	13.95 ± 1.46	13.65 ± 1.46	2	n.s.
K	6.69 ± 0.49	5.68 ± 0.41	18	n.s.
maxPDR	156.2 ± 9.79	143.6 ± 10.32	9	n.s.
Intra-lipid				
$t_{1/2}$	20.3 ± 2.6	23.1 ± 2.6	-12	n.s.
K	3.50 ± 0.25	2.56 ± 0.25	37	*
maxPDR	148 ± 11	95 ± 11	56	**
Solid (egg yolk)				
$t_{1/2}$	31 ± 2	39 ± 2	-21	*
t_{lag}	6.1 ± 0.6	8.0 ± 0.7	-24	*
t_{max}	16.3 ± 1.4	20.9 ± 1.5	-22	*
K	2.55 ± 0.2	2.25 ± 0.2	13	n.s
maxPDR	80.23 ± 9.00	71.28 ± 11.88	13	n.s.

n.s.; Fprob > 0.10; † Fprob < 0.10; * Fprob < 0.05; ** Fprob < 0.01; *** Fprob <0.001.
A summary of the abbreviations can be found in Table 1.

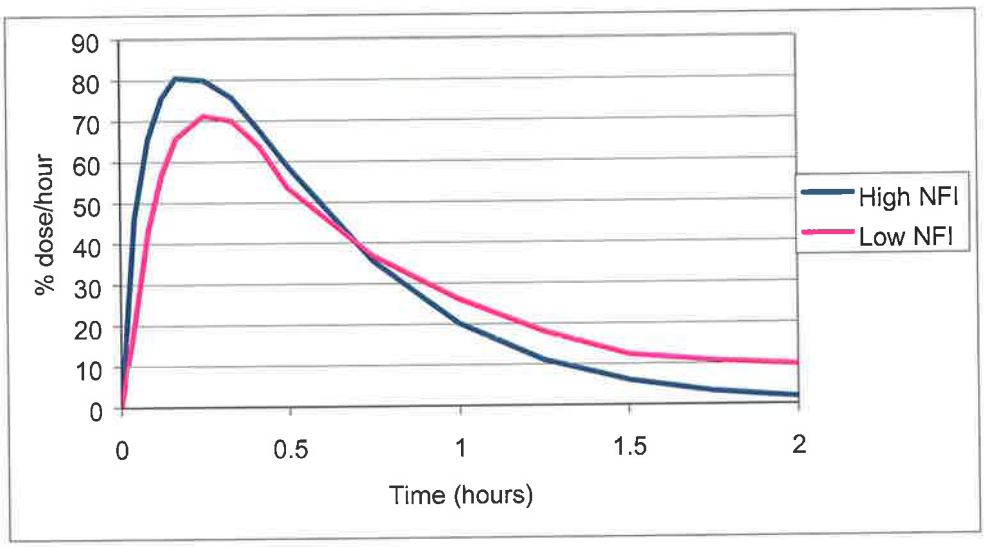


Figure 4.6 Differences between the NFI selection lines in gastric emptying curves.

4.4 Discussion

The experiments described in this chapter were designed to obtain a better understanding of net feed intake with the aim of eliminating or detecting candidate genes for this trait. Divergent selection for post-weaning NFI resulted in the high NFI line mice consuming significantly more feed than the low NFI line with little or no difference in growth and body weight in all generations of mice used in these studies. These results are consistent with the work of Hughes (2003) on earlier generations of these mice. There have been a number of other studies examining the efficiency of feed utilisation in mice (Archer and Pitchford 1996; Bishop and Hill 1985; Hastings et al. 1997; Nielsen et al. 1997a & b) that support these findings.

Bishop and Hill (1985) and Hastings et al. (1997) studied mice selected on 4-6 week feed intake adjusted for 4 week body weight and mice selected on voluntary food intake between eight and ten weeks corrected for phenotypic body weight. Results from the selection experiments performed on the younger mice indicated that the high intake mice were larger, ate more, were slightly leaner, and had higher maintenance requirements compared to the low intake mice (Bishop and Hill 1985). Moreover the selection performed in adult mice, resulted in the high intake line gaining more weight, and eating more and having a higher fasting heat production than the low intake mice (Hastings et al. 1997). Divergent selection for heat production or loss in 9- to 11-week-old male mice was conducted by Nielsen et al. (1997a & b). After 15 generations of selection, feed intake had changed in the same direction as heat loss such that the difference in intake between the high and low heat loss lines was 20.6%. No difference in body mass was observed up to generation 15.

4.4.1 Differences in activity

Since the energy expenditure during locomotion can be very high relative to expenditure at rest, the activity of the mice was continuously monitored by infra-red detectors. From this study, female mice were significantly more active than male mice. In the small number of studies evaluating sex differences in mice (Mousel et al. 2001) and cattle (Voisinet et al. 1997), females were more active (temperamental) than males. Contrary to these findings,

Moruppa (1990) observed more active male mice. However, mice and rats tend to have increased nocturnal activity (Mount and Willmott 1967; Anderson and Smith 1987) and the mice in the above study were measured during the day for only three hours. It is possible then that these differences could be reversed during the night when the mice are more active.

Mice from the high NFI line were almost twice as active as their low NFI counterparts when based daily activity (counts/hr). The circadian rhythm of activity was similar for the selection lines but the amplitude was greater in the high NFI line. Both lines had a large peak in activity at the beginning of the dark period, followed by a smaller peak at 8 am, 4 hours after the lights were turned on. During periods of low activity, the lines were not different in activity. These results are supported by a number of studies in mice (Moruppa 1990; Mousel et al. 2001), poultry (Luiting et al. 1994), pigs (Henken et al. 1991) and cattle (Richardson et al. 1999) selected for and against efficiency of feed utilisation. Moruppa et al. (1990) found that mice selected for increased food intake adjusted for body weight were more active for 3 hours during the light part of the day compared to mice selected for a decrease in intake. Also mice selected for high heat loss were more active than their low heat loss counterparts (Mousel et al. 2001). After 1 generation of selection for divergent NFI in cattle, Richardson et al (1999) found that activity accounted for 10% of the variation observed in NFI, which was supported by the work of Henken et al. (1991) on swine. In laying hens selected for divergent net feed intake, Luiting et al. (1994) found that high-intake hens spent 28% of their daily heat production on activity compared with only 18% in the low-intake hens. Also Dauncey (1986) showed that C57BL *ob/ob* mice were less active than the lean C57BL 6/6 mice. It would appear that animals selected for more efficient food utilisation decrease their energy requirements in part, through decreased activity.

4.4.2 Body composition differences between the NFI selection lines

The energy cost of fat deposition (53 kJ/g) is much greater than that of lean tissue (11kJ/g) (Pullar and Webster 1977). Surprisingly, selection for low NFI resulted in mice that were generally fatter. Hughes (2003) demonstrated after seven generations of selection for divergent NFI that the low NFI mice were 23% fatter post-weaning. These findings are also consistent with the work of Bishop and Hill (1985) and (Nielsen et al. 1997a), who

have found correlated increased body fat in mice selected for low feed intake adjusted for body weight and for low heat loss, respectively. Although these results were supported by the work of Bishop and Hill (1985) and Nielsen et al. 1997a, in similar experiments, they were unexpected. It is commonly accepted that fat tissue is energetically more expensive to deposit than lean tissue (Brody 1945; Pullar and Webster 1977; and Webster 1980). It was thought that if there were any differences between the NFI selection lines in body composition that the high NFI, less efficient mice would be fatter. However, lean tissue is thought to be energetically more expensive to maintain than fat once deposited. Thus the maintenance requirements during growth would be greater for leaner animals and only fat deposited during the feed intake test period would be expensive in feed requirements (not fat laid down before the test starts).

Genetic correlations between feed efficiency and body composition, especially fatness have been published for a variety of species. In poultry, Gabarrou et al. (1997) found that efficiency was associated with increased fatness, supporting the above studies in mice. In contrast Mrode and Kennedy (1993) and Von Felde et al. (1996) in pigs and Arthur et al. (1996) in beef cattle found that efficiency was associated with decreased fatness. Since there is variable evidence for the relationship between feed efficiency and body fatness, it may be wise to include a measure of body composition in the multiple regression model when calculating net feed intake.

4.4.3 Protein turnover

Since muscle represents the largest protein store in the body and it is important in animal production systems, its synthesis and degradation has been widely studied. There was no significant difference between the lines in fractional synthesis rates in either the liver or the muscle. This was surprising given that other studies in mice (Bernier et al. 1987; Bernier and Calvert 1987) , and pigs (Reeds et al. 1980), have shown a positive relationship between the energy cost of protein synthesis and metabolisable energy intake. However, Bernier and Calvert (1987), and Bernier et al. (1987) studied two lines of mice that varied in growth rate. Generally, animals with higher growth rates eat more. Therefore, the relationship between metabolisable energy intake and protein synthesis could have been due to the differences in growth rates and not just intake. The mice from the present study differed in feed intake independent of growth rate and body weight. Although the lines

were not different in growth or body weight, their body composition was different. It is also possible that the protein synthesis measurements were inaccurate. The estimates of fractional protein synthesis rates in the muscle, and therefore, absolute protein synthesis rates appear to be lower in this experiment than those previously published for mice (6 – 8 %/day compared with 18%/day for an adult mouse, Garlick 1980, and 25% in 6 week old mice, Bernier and Calvert 1987). It is most likely that this difference is due to the small amount of labelled sample, which meant that the HPLC was detecting at its lower limit. Pooling the muscle samples within each line may have provided enough sample for more accurate fractional synthesis estimates. Re-analysing the muscle samples using an enzymatic method could also be done. It is also possible that the estimates of fat and protein gain taken from Pullar (1977) and Reeds (1991) are not accurately reflecting what is happening in this mouse model.

It would appear that there was a difference in nutrient partitioning between the lines. The high NFI mice partitioned more protein to the skin and viscera, with the low line partitioning more protein to the carcass. Interestingly, the low NFI line had significantly larger carcasses than the high intake mice. This is most likely because the high intake line were more active than the low line and increased activity is thought to induce changes in growth performance and muscle characteristics. Studies in growing sheep (Pethick and Rowe 1996), pigs (Essen-Gustavsson and Lindholm 1983; Essen-Gustavsson et al. 1992) and other species (Henriksson 1995) have shown that exercise decrease both carcass weight and subcutaneous fat.

The work of Tomas et al. (1988, and 1991) showed that genetic selection for weight, intake or efficiency of growth affects protein turnover in poultry. The authors found no differences in muscle fractional synthesis rates in broiler chickens selected for increased 5 to 9 week weight gain or increased 5 to 9 week food consumption or decreased 5 to 9 week feed conversion ratio. However, selection for improved food utilisation for growth resulted in a concomitant decrease in muscle protein breakdown, and subsequently, a reduction in muscle protein turnover. In the weight and intake selected poultry lines, muscle protein breakdown was significantly correlated with feed conversion ratio. If differences between the lines in protein turnover do exist, then they would most likely be attributable to protein breakdown in the more rapidly growing phase of growth. Differences observed between

the selection lines in feed conversion when the mice were younger, and rapidly growing coupled with the findings in broiler chickens selected for either high intake or improved feed conversion ratio Tomas et al. (1988, and 1991), support this hypothesis. Although the present study was not designed to accurately determine differences between the selection lines in muscle protein breakdown, estimates were crudely calculated. These muscle protein breakdown estimates did not differ between the NFI selection lines. Since there was no difference between the NFI selection lines in growth rate or muscle protein synthesis, differences between the lines in muscle protein degradation, and therefore, protein turnover would seem unlikely. However, it must be remembered that these estimates and the protein synthesis measurements were obtained when the animals were at the end of their growing period. Garlick et al. (1973) concluded that all of the growth in young rats could be accounted for by a change in the rate of protein synthesis. Consequently, measurements of both protein synthesis and breakdown rates in the NFI selection mice between three weeks (weaning) and eight weeks of age may provide a better insight.

Protein synthesis is an energetically expensive exercise that can be crudely broken into two phases. The first phase of protein synthesis is the transcription of mRNA from DNA. The ratio of mRNA to DNA provides an indication of the efficiency of transcription, that is, the amount of mRNA transcribed per unit of DNA. The second phase is the translation of mRNA into protein. The ratio of protein to mRNA gives and indication of translational efficiency, the amount of protein produced per unit of RNA. Because only total RNA concentration was measured in this experiment and not the concentration of the different forms of RNA, the efficiency of translation and transcription could not be estimated. However, the ratio of protein to DNA was higher in the low NFI selection line. Since the concentration of DNA remains relatively constant and the ratio of RNA to DNA was not different between the lines, the differences between the lines in the ratio of protein to DNA is most likely due to differences in protein degradation. The ratio of protein to RNA is a more complex ratio that is not easily interpreted because both the concentrations of protein and RNA are continually changing. However, it appears that the protein to RNA ratio (which was also significantly higher in the low NFI line) also follows this trend, where the low NFI mice appear to have more stable proteins (lower protein degradation) or increased translation.

4.4.4 The impact of restricting feed intake

Intake is thought to be one of the greatest modulators of protein synthesis in both the liver and muscle. When an animal is deprived of food, it rapidly loses weight. The initial weight loss is most likely due to the loss of gut contents. However, the response in different tissues of the body is variable. Garlick et al. (1975) found that the liver and jejunal mucosa decreased significantly, whilst muscle, brain and kidney change very little when rats were starved for 2 days. It was not surprising then that the amount of protein in the muscle and the fractional rate of protein synthesis were not different between *ad libitum* fed and restricted mice, considering that the mice were restricted and not starved. The absolute rate of protein synthesis in the muscle was 32% higher in *ad libitum* fed mice compared with restricted mice. This was not expected given that the absolute rate of protein synthesis was calculated from the amount of protein present in the muscle and the fractional synthesis. On closer examination, the *ad libitum* fed mice had slightly greater total muscle protein and slightly faster fractional synthesis rates, resulting in greater rates of absolute synthesis.

There are conflicting reports in the literature, on the change in concentration of DNA and RNA under food restriction. Concentrations have been shown to increase (Winick and Noble 1966; Robinson and Lambourne 1970) and decrease (Howarth and Baldwin 1971; Bernier et al. 1987) when food is restricted. In this experiment, the concentration of RNA did not change, but the concentration of DNA increased in mice that were restricted. Since the concentration of RNA was not different between the fed versus restricted mice, but the weights of the legs were different, the total amount of RNA was greater in the fed mice. Lobley (1993) stated that in well-fed rodents the limitations on protein synthesis seem to relate to the total RNA present. The results herein support this supposition as the amount of RNA was reduced in the restricted mice and the rate of absolute synthesis was also decreased. Interestingly, there were greater amounts of protein and RNA produced per unit of DNA in *ad libitum* fed mice. This increase in protein is most likely due to increased RNA concentration. However, because the mRNA, rRNA and tRNA were not measured separately, it is not possible to rule out differences in the rate of protein degradation as the source of difference.

The fractional synthesis rates in the liver decreased by 19% following a 2-day restriction in feed intake. The rates of absolute synthesis in the liver could not be estimated because the amount of protein in the liver was not measured. However, it is most likely that the size of the liver and the total amount of protein in the liver were reduced in the restricted mice, so these mice would have had a lower absolute rate of protein synthesis. McNurlan et al. (1979) in a study on rats found that starving rats for 2 days decreased the amount of protein produced in the liver by 40%.

The interaction between the level of feeding and selection was not significant for any of the traits measured. This would indicate that the differences observed between the *ad libitum* and restricted mice were the same for both of the selection lines. When the interaction was observed more closely, restricting feed intake to 60% reduced the fractional synthesis rates in the liver in both of the selection lines. However, the reduction was greater in the high NFI line (a 20% reduction in the high NFI line compared to 13% in the low line). It is possible that if the restriction were greater than 60% of *ad libitum* intake or for a longer period then these differences would have significant and greater. While the interaction was not significant this trend supports earlier work on the lines used herein (Fenton 1997), where pre-weaning nutrient restriction resulted in mice from the low NFI line having greater body weight than the high NFI mice. When the mice were not restricted there was no difference between the selection lines in body weight. This is understandable since mice from the high NFI line require more food to maintain the same body weight; therefore, when feed is limited these mice would be most affected. These findings suggest that mice with low NFI may be better able to cope with fluctuations in nutrient supply.

4.4.5 Metabolic rates

There was a 20% difference in basal metabolic rate observed between the high and low intake mice. Unfortunately, there was no method for detecting movement within the calorimetric chambers. Hughes (2003) found there was no difference in basal metabolic rate, when adjusted for activity within the calorimeter, in an earlier generation of mice from the same selection lines. In that study, Hughes (2003) also recorded visceral organ weights and found that there was no significant difference between the selection lines in liver weight. Selman et al. (2001) reported that in mice selected for and against feed intake corrected for body mass, liver mass was the most significant trait linked to changes in

resting metabolic rate. In a recent review of selection experiments for net feed intake in pigs, poultry and cattle, Tixier-Boichard et al. (2002) concluded that the main sources of variation of heat production appear to be diet-induced thermogenesis and activity, rather than basal metabolic rate. The current experiment was not designed to measure diet-induced thermogenesis. Given this information and the activity results from this experiment, the differences observed in basal metabolic rate were most likely due to differences in activity whilst the mice were in the chambers. Assuming that there is no difference between the selection lines in basal metabolic rates, the differences observed herein would be due to activity within the measuring chamber, and would support the findings on protein turnover in this experiment.

4.4.6 The energy balance

The largest contributor to the difference between the selection lines in energy utilisation was activity. The large differences detected in activity between the lines remained after adjustment for metabolisable energy intake. This would indicate something beyond intake is driving the differences observed between lines in activity. A possible explanation for this could be that selection for and against NFI has indirectly selected for and against the rate of maturity, such that at any point in time, the low NFI line was physiologically older. Hughes (2003) suggested that the small negative genetic correlation of net feed intake with growth rate and body weight could result in the high net feed intake line reaching its mature weight slower. The results observed in body composition would support this explanation, as the low NFI mice were generally fatter than the high NFI mice. To further investigate this explanation, a standard three parameter growth curve (logistic) was fitted to the data. This indicated that there was no difference between the lines in rate of maturity (Figure 4.7). However, the data available were limited to mice that were between 32 and 85 days of age (near the asymptote of the curve) so the parameters of the curve were poorly estimated.

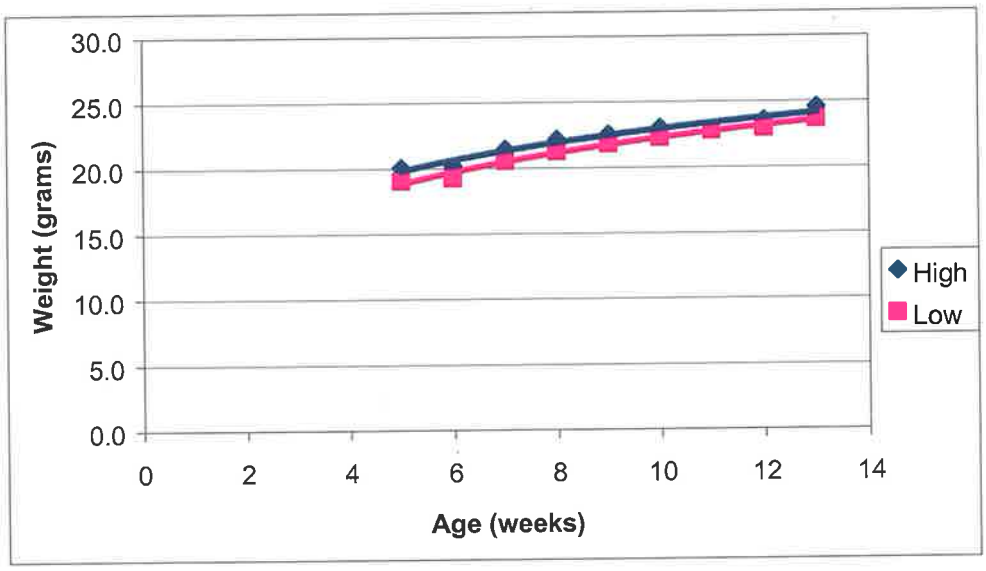


Figure 4.7 Growth curves of the NFI selection lines

4.4.7 Does gastric emptying play a role in the efficiency of food utilisation in mice?

The previous sections have concentrated on the differences in energy going into the system and briefly on the energy coming out. There are other potential sources of variation between the NFI selection lines that could contribute to the physiological differences observed between the NFI selection lines. The rate at which food moves through the intestinal tract is critical to the body's digestive processes (Tivey & Butler 1998). Gastrointestinal motility is a complex process that is influenced by motor characteristics such as gut muscles for movement of food as well as neurophysiological regulators such as gut peptides, which provide feedback mechanisms. The development of breath tests for the assessment of gut function has been used as a medical diagnostic tool. The technique is relatively new and its full potential not yet reached. However, the early indications for its use in cattle (McIeary et al. 1997), horses (Bracher et al. 1995), pigs (Zentek 1992) and mice (Symonds et al. 2002) are promising.

In general, the motility of solid food in the high NFI line was faster than low NFI mice when adjusted for the amount of feed consumed during the test. There was no difference between the lines in the gastric emptying of the non-nutrient liquid meal or the lipid liquid meal. However, both the gastric half-emptying time and the solid lag phase were faster in the high intake mice. The emptying of solids and liquids is dependent on different parts of the stomach. One of the major factors controlling emptying of liquids is the pressure

gradient across the gastroduodenal junction, which is dependent on the tone of the proximal stomach (Horowitz et al. 1984). In contrast, digestible solids are emptied by a dual process of grinding and mixing, which occurs in the antrum and an emptying process by propulsive forces in the fundus, which is dependent on fundal tone. The solid lag phase gives additional information on the early pattern of gastric emptying of solids. It would appear that the flow of food through the stomach to the pyloric caeca was faster in the high intake line, indicating that these mice do not break down digesta to the same extent as the low intake line.

The rate of decline of the gastric emptying curve was also significantly higher in the high intake line for both lipid and solid meals. Differences in the rate of decline of the curve (k) are thought to be due to the second half of gastric emptying (Symonds pers. com.) and could potentially be caused by differences in nutrient induced feedback from the small intestine. It is believed that feedback from the small intestine is one of the regulatory processes for gastric emptying, to prevent an overload of nutrients to the small intestine (Weber and Ehrlein 1998). The degree of inhibition depends on the concentration of nutrients. Cholecystokinin (CCK) is a potent inhibitor of gastric emptying and plays an important role in the intestinal feedback regulation of gastric emptying. The precise mechanisms by which CCK is released in response to luminal stimuli are unknown, however protein, amino acids and lipolytic products of fat digestion are important stimuli (Mayer 1994). Gastrin, motilin, secretin and peptide YY are other hormones that contribute to the regulation of gastric emptying. Severe disruption or delay of the negative feedback mechanisms results in malabsorption, which is known as "dumping syndrome". However, malabsorption seems unlikely because the maximum amount of recovery of labelled carbon in the breath is not different between the mice lines.

There was a significant difference between the lines in the height of the gastric emptying curve when the mice were given a lipid meal. The height of the curve is believed to reflect post-gastric events (most likely liver metabolism) (Tivey and Butler 1998). Since the high intake line is more active than the low line, it is possible that their glycolytic pathway could be up regulated to maintain their higher energy requirements. Consequently, the load on their liver would be increased. However, there was no difference between the lines in the height of the gastric emptying curves when administered with a non-nutrient liquid

meal. This would indicate that there are differences in the breakdown of the long chain fatty acids and not in the efficiency of the Krebs cycle because the non-nutrient meal utilises ^{13}C acetate whilst the lipid meal contained ^{13}C octanoic acid as the tracer molecule. The difference between these two molecules is that octanoic acid requires the cleavage of the first two carbon atoms (thus liberating the ^{13}C-acetic group) in addition to acetate metabolism (Braden et al. 1995).

Interestingly, the differences between the lines in the height of the emptying curve were diminished when the mice were given the more complex meal (the solid meal was also labelled with ^{13}C octanoic acid). This is most likely due to the more complex pathways (which have slower emptying rates) involved in the breakdown of solid foods. It is also possible that there was an interaction between proteins and fats in the solid meal.

Gastric emptying is a complex process driven by several factors. The consistency and caloric density of the meal, hormone secretion, neural regulation, and motor activity of the stomach all play important roles. It would appear that the high intake line passes food through the gut more rapidly than the low intake line. This food (or digesta) would most likely contain larger particles that are more difficult to absorb than the smaller particles (because they have a faster lag time). Also the feedback mechanisms that signal the gut to slow the rate of gastric emptying appear to be slower in the high intake mice. CCK, a gut peptide, is known to act by negative feedback to slow the rate of gastric emptying and may be involved. However, it is possible that the increased rate of gastric emptying is a result of high NFI and not a cause of high NFI.

4.5 Conclusion

Selection for low post-weaning NFI in mice will decrease post-weaning food intake with little or no impact on body weight and growth rate. This study investigated physiological factors that may be correlated with selection for divergent NFI in mice that could act as candidate genes for NFI. After 11 generations of selection it appears that the largest difference between the lines was in activity, with smaller but significant differences in body composition, and gastric emptying (generation 12). Although there was a large difference between the NFI selection lines in activity, there are a number of genes controlling activity leaving a large number of potential candidate genes. However, CCK,

gastrin, motilin, secretin and peptide YY are hormones that contribute to the regulation of gastric emptying and could act as candidates for NFI.

Using the flooding dose method to measure protein synthesis did not reveal any differences between the lines in protein synthesis or turnover. However, genes involved in protein degradation and turnover should not be ruled out as candidates for NFI. In conclusion, the differences between the lines in activity, body composition and gastric emptying along with QTL positions will provide valuable information when searching for candidate genes for NFI.

Chapter 5.

Mapping quantitative trait loci for feed efficiency in mice

5.1 Introduction

In Chapter 4, two lines of mice selected for and against net feed intake (NFI) were used to determine the underlying biology of NFI, and consequently any physiological indicators for the trait. The results from the breath analysis study revealed a possible association between gastric emptying and NFI. However, the largest difference between the NFI selection lines was in activity. Significant differences were also observed in body composition, indicating a possible difference in fat metabolism. These results combined with the location of loci controlling NFI reduce the number of candidate genes to be searched for.

Generating enough progeny per sire to detect QTL and to measure feed efficiency in cattle is both time consuming and expensive. Hence, mice have been widely used as models for domestic species such as cattle, sheep and pigs for both genetic and biological studies. Mammals all have a highly conserved genome size and through the Human Genome Project it is has been estimated that they share between 30,000 – 50,000 genes. Therefore, mice can provide a valuable source of genetic information for mapping in domestic species by observing regions of the genome that are conserved between the species of interest (cattle) and the mouse. Because humans and mice have the most comprehensive genetic and physical maps, they are used as the reference for farm animal genetic map development. This is known as comparative mapping. Conserved syntenies or regions of the genome are established by using type I markers as anchors across the species maps (Obrien et al. 1993). Traits of economic importance are usually mapped using markers that are specific to a species by linkage mapping. Radiation hybrid, linkage and physical maps provide the tools to integrate these markers with conserved genes, allowing homologous chromosomal locations for specific traits in other animal genomes to be identified (Gellin et al. 2000). There are a number of comparative maps between mice and domestic species currently available to locate regions of genomes conserved between these species.

In this chapter, regions of the mouse genome associated with feed efficiency are reported using the unique mouse NFI selection lines. Candidate genes within these regions have also been investigated. These regions will be used to comparatively map the gene(s) in cattle and other species in subsequent chapters.

5.2 Materials and Methods

5.2.1 Experimental design and phenotyping

Mice from the eighth generation of selection for divergent NFI were used in an F_2 cross. These lines were 20% different in intake with little or no differences in growth and body weight traits (Hughes 2003). Fifteen high NFI males were mated to fifteen low NFI females and fifteen low NFI males were mated to fifteen high NFI females to produce 62 F_1 progeny. These F_1 mice were then put on a three-week post weaning feed intake test. Four F_1 males were randomly selected and mated to 32 F_1 females (one F_1 male joined to eight F_1 females). Approximately 200 mice were generated from this first mating and they were put on a five-week feed post-weaning feed intake test, followed by body composition analysis. The same four F_1 males were joined to the same F_1 females to produce larger full sib families. The progeny from the second mating were also measured for feed intake, body weight, weight gain and body composition. A total of 442 progeny were generated, approximately 110 progeny per F_1 sire.

Feed intake was measured for a total of five weeks in the F_2 progeny, as described in Chapter 3. When establishing the selection lines, intake measurements were recorded for three weeks post-weaning only. An extra two weeks of intake measurements were recorded to ensure an accurate measure of daily feed intake for mapping.

NFI was then calculated as described in Chapter 3 using the daily feed intake estimate measured over five weeks for all F_2 progeny. The variation observed in NFI within sire families was different between the four F_1 sires (Figures 5.1 and 5.2). Progeny from sires 1021 and 1027 only were genotyped because the progeny from these sires had the largest amount of variation in NFI. Sixty progeny per sire were chosen from four dams. Each dam family contained 15 progeny that were genotyped consequently; in two dam families all progeny were genotyped. Selective genotyping was done to reduce the cost by reducing the number of genotypes without compromising the power to detect QTL. There was an 18% difference between animals from the high NFI tails and the low NFI tails for NFI and DFI with no significant difference in fat %, ADG and MMWt (Figure 5.3).

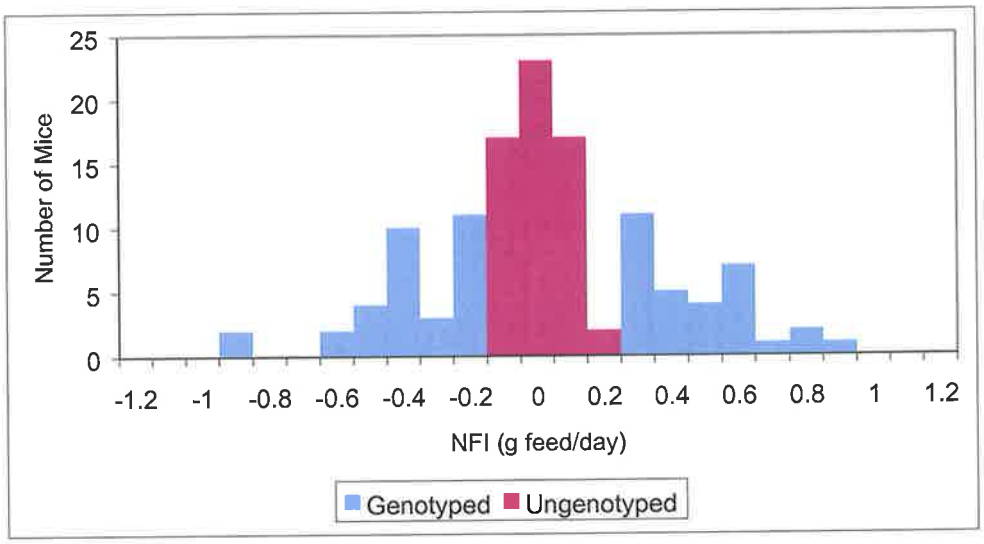


Figure 5.1 **Distribution of NFI for Sire 1021 progeny**

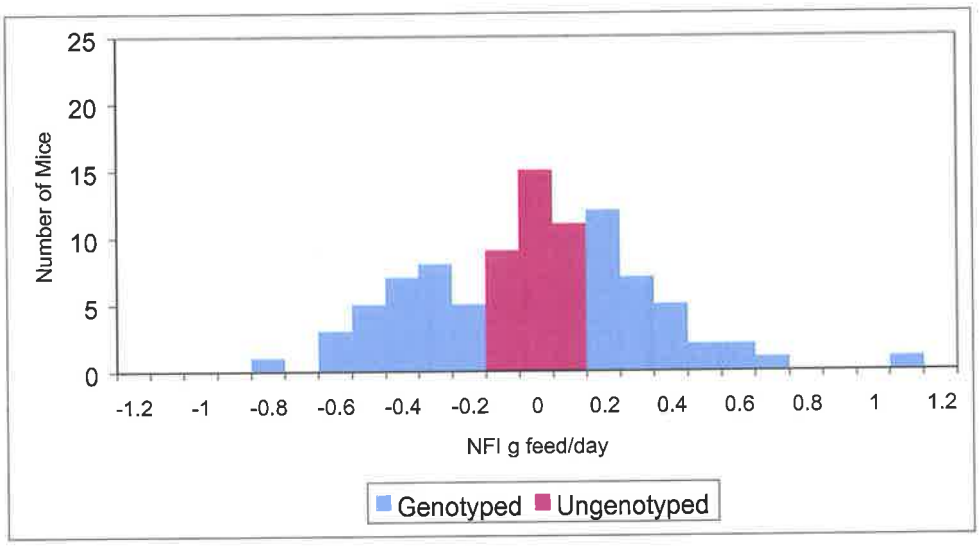


Figure 5.2 **Distribution of NFI for Sire 1027 progeny**

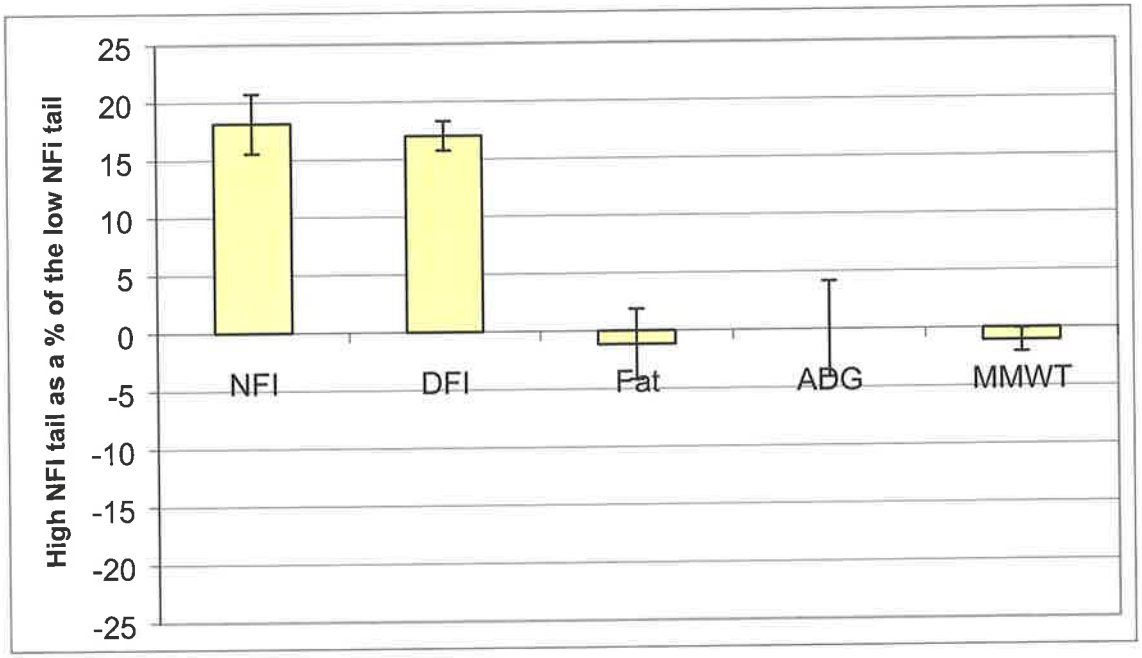


Figure 5.3 **Percentage difference between the high and low NFI tails**

5.2.2 Genotyping

DNA was extracted using a standard phenol chloroform protocol (Moore 2001). The extracted DNA was sent to the Australian Genome Research Facility (Melbourne, Australia) where DNA amplification and genotyping were performed Ewen et al. (2000). Eighty microsatellite markers (Table A.1, Appendix A) that were multi-allelic for the initial inbred mouse lines (BALB/c and C57) were chosen. Two of these markers were completely uninformative, leaving 78 microsatellite markers spaced at approximately 20 cM intervals. Using CRIMAP (Green et al. 1990), the average spacing of markers was 21 cM, ranging from 6.6 cM to 40 cM (Table A.1, Appendix A).

5.3 QTL Analysis

5.3.1 Selective genotyping

It is widely accepted that selective genotyping individuals with extreme phenotypes for the quantitative trait increases the power per individual genotyped (Lander and Botstein 1989; Darvasi and Soller 1992; Muranty and Goffinet 1997; Ronin et al. 1998; Johnson et al. 1999a; Henshall and Goddard 1999; and Xu and Vogl 2000). However, phenotypic values of all ungenotyped individuals must be included in the analysis with the marker genotypes treated as missing to prevent biasing of QTL effects (Lander and Botstein 1989). If genotyped individuals only are used in the analysis, then upwards biasing of the estimated

allele effect occurs. This is due to the positive relationship between residual effects and the QTL effect in the pooled tails population, which magnifies the allelic effect (Johnson et al 1999a).

The regression method proposed by Haley and Knott (1992) has been widely accepted and applied to QTL mapping studies because of its ease of computation and it provides similar or identical estimates and test statistics as the more complex maximum-likelihood (ML) estimation by expectation-maximization (EM) algorithms (Haley and Knott, 1992; Xu 1998 a, b). Regression interval mapping regresses the quantitative trait value on the conditional expected genotypic value. However, this method does not take into consideration all individuals, genotyped and ungenotyped, and therefore, suffers from upward biasing when selective genotyping is applied. Darvasi and Soller (1992) investigated an analysis of variance approach when ungenotyped individuals are excluded from the analysis to estimate QTL effects with a bias correction. The estimates of gene substitution effects from the regression method could be adjusted using the formula from Darvasi and Soller (1992). However, this adjustment assumes truncation selection and equal representation from the tails of the distribution. Johnson et al. (1999a) demonstrated that the likelihood profiles are similar between the two methods and so the regression method could be used as a quick tool for screening for QTL to locate areas of interest for more detailed analysis. Johnson et al. (1999a) proposed an EM algorithm implemented via Monte Carlo sampling using a Gibbs sampler for handling missing data.

Henshall and Goddard (1999) altered the roles of genotypes and phenotypes in the likelihood function. Phenotypes were treated as independent variables and genotypes as dependent variables with the logistic regression approach. The advantage of this method is that it only requires a standard statistical package to analyse the data and estimates are not affected by selective genotyping. However, this approach does not suit F_2 populations because there are more than two segregating genotypes.

Xu and Vogl (2000) developed an exact ML approach to map QTL under selective genotyping using phenotypic values of genotyped individuals only. This method provided estimates that were slightly lower than using the complete data set. They found that QTL

parameters were almost identical with the estimate of residual variance subject to a relatively large error.

As a preliminary analysis tool, the regression approach appears to provide a quick method for locating areas of interest for a more detailed analysis when selective genotyping is applied. Alternatively, if the experimental design has only two segregating genotypes (as in the backcross design) and composite interval mapping is not required, the logistic regression approach is appropriate. It can be applied by standard statistical software and takes into account missing genotype data. If this is not the case and the full data set can be analysed, then the full ML analysis should be performed. If it is impossible or difficult to analyse the full data set (e.g. sample size is too large, phenotypes of ungenotyped animals are missing or composite interval mapping is to be performed), then the method proposed by Xu and Vogl (2000) could be used.

5.3.2 Preliminary analyses – Regression approach

To make the most efficient use of marker data, maximise experimental power and minimise the risk of biased estimates, it is necessary to take into account information from all of the informative markers in a linkage group (Haley et al. 1994). Because the parental lines were not genotyped, the phase of the F_2 progeny was determined from CRI-MAP (Green et al. 1990) *chrompic* outputs. The F_2 progeny were assigned either a H (an allele coming from the high NFI line) or an L (an allele coming from the low NFI line) according to the parental line origin of the allele. This process assumes that the NFI lines are segregating at marker loci and are fixed for NFI alleles at the major QTL affecting NFI (Haley et al. 1994). According to this assumption, all F_1 progeny would be heterozygous with genotypes of HL (receiving the high NFI allele from the grand-sire) or LH (receiving the high NFI allele from the grand-dam). This procedure also assumes that there is no difference between HL and LH heterozygous individuals. A simple linear regression was fitted adjusting for the fixed effects of sex (male/female), genotype (HL/LH) and the interaction between sex and genotype with the number of animals weaned included as a covariate to test whether there was a difference between the two F_1 heterozygotes.

As a preliminary analysis on the F_2 progeny, the effect of marker genotype on phenotype was evaluated by ANOVA using the Proc MIXED procedure in SAS (1988). Fixed effects

included marker genotype and sex, with sire and dam nested within sire the random effects to adjust for polygenic effects. A simple linear regression was also fitted using QTL Cartographer (Basten et al. 2001) single marker analysis procedure where the F statistic is used to support or disprove the hypothesis that the marker is unlinked to the quantitative trait. For each marker in turn, the phenotypic data was fitted to the simple linear model:

$$y = ß_0 + ß_1 x + e$$

Equation 5.2

where:

y = the phenotype from the trait of interest

$ß_0$ = regression parameter

$ß_1$ = regression coefficient associated with marker genotype

x = indicator variable for marker genotype (HH, HL/LH, LL)

e = residual/error variation, assumes normal distribution.

To confirm and better define the locations and effects of the QTL influencing the traits, regression interval mapping was done using QTL Express (Seaton et al. 2002; QTL Express, http://qtl.cap.ed.ac.uk; accessed 2001 - 2003). First, a single QTL model was fitted with the fixed effects of sex and litter size and sire-dam combination. The sire-dam combination was included as a fixed effect to adjust for polygenic effects. Following the single QTL model, a sex by QTL interaction and two QTL models were fitted including the same fixed effects.

5.3.3 Maximum likelihood interval mapping

As discussed earlier (section 5.3.1) the maximum likelihood (ML) approach is the preferred method of analysis to detect QTL when selective genotyping is applied. Composite interval mapping was performed on body composition, growth and body weight traits and multiple interval mapping were applied to the feed intake and efficiency data using QTL Cartographer (Basten et al. 2001). The same model was fitted to each of the mapping techniques, which accounted for sex differences and the sire-dam combination. Multiple-interval mapping (MIM) was performed following interval and composite interval mapping. MIM requires several steps 1) composite interval mapping using a subset of significant markers selected by forward and backwards stepwise regression to determine

candidate positions, 2) identify candidate epistatic terms for the initial model by pooling markers and marker pairs together in a combined forward stepwise regression analysis, 3) re-evaluate the significance of each QTL effect in the current model, 4) re-evaluate the significance of each QTL effect 5) re-optimise current positions. Based on the work of Zeng et al. (1999), the model selection (criterion for comparing different models) used was Bayes information criterion (BIC) = 2ln(n) option.

5.3.4 Threshold values

To obtain overall significance levels a permutation test as discussed by Churchill and Doerge (1994) and Doerge and Churchill (1996) was applied to the data. This is a re-sampling procedure that replicates the original analysis many times on data sets generated by reshuffling the original data. Trait values of individuals' are randomly shuffled over individuals' marker genotypes. The new sample contains the individuals' original marker information but with a randomly assigned genotype. The test statistic is then calculated on the new sample, with the procedure being repeated many times to create an empirical distribution of the test under the hypothesis of no marker-trait associations. Churchill and Doerge (1994) suggest 1000 re-samplings are required for a 5% significance level or at least 10 000 re-samplings for a 1% significance level.

5.4 Results

5.4.1 Phenotypic analysis

An F_2 intercross between divergently selected high and low NFI mouse lines generated 442 F_2 progeny from 27 F_1 dams and 4 F_1 sires. After eight generations of selection, the parental selection lines were approximately 20 % different in both NFI and DFI (Table 5.1). The selection lines also differed in body fat percentage (19%), but no difference in weight or average daily gain. Daily feed intake in the F_1 and F_2 population was lower than either of the selection lines, and growth was faster in the F_1. This is not surprising because the parental lines, F_1 and F_2 populations all come from different management groups, where there are environmental differences such as different batches of feed.

Table 5.1 Means (± standard errors) for each generation

	Traits									
	NFI	**DFI**	**GE**	**Fat%**	**ADG**	**Lean**	**WW**	**Wt6**	**MWt**	**MMWt**
High	0.28	4.46	0.06	14.4	0.30	19.9	10.7	23.3	21.5	9.98
	±0.02	±0.03	±0.00	± 0.3	±0.01	±0.4	±0.2	±0.3	±0.1	±0.73
Low	-0.29	3.73	0.08	17.8	0.32	18.5 ±	11.45	22.5 ±	20.2	9.53
	±0.05	±0.06	±0.01	±0.6	±0.02	0.3	±0.2	0.2	±0.3	±0.73
F_1	0.00	3.67	0.11	nm	0.37	nm	11.1	21.9	19.31	8.66
	±0.03	±0.44	±0.01		±0.19		±2.1	± 0.3	±0.4	±0.93
F_2	0.00	3.51	0.07	14.2	0.24	19.1	12.2	22.3	20.8	8.53
	±0.03	±0.43	±0.00	±1.9	±0.19	±2.6	±2.0	±0.2	±0.3	±0.92

High – high NFI line; Low – low NFI line; nm – not measured

A summary of the abbreviations can be found in Table 1.

A simple linear regression was fitted to the F_1 data to determine whether there was a difference between reciprocal cross mice that had inherited a high NFI allele from their grand-sire versus grand-dam (ie HL genotypes versus LH genotypes). There was a significant ($P<0.05$) difference between inheriting a high NFI allele from the grandsire versus the grand-dam in intake and NFI (Figure 5.4). Male mice receiving a high NFI allele from the grand-dam (LH) had a 11% higher NFI compared to those male mice receiving a low NFI from the grand-dam. This difference was only 4% in females. The same trend was observed in daily feed intake for the males but was not observed in the females. There was no difference between LH and HL genotypes in the other measured traits.

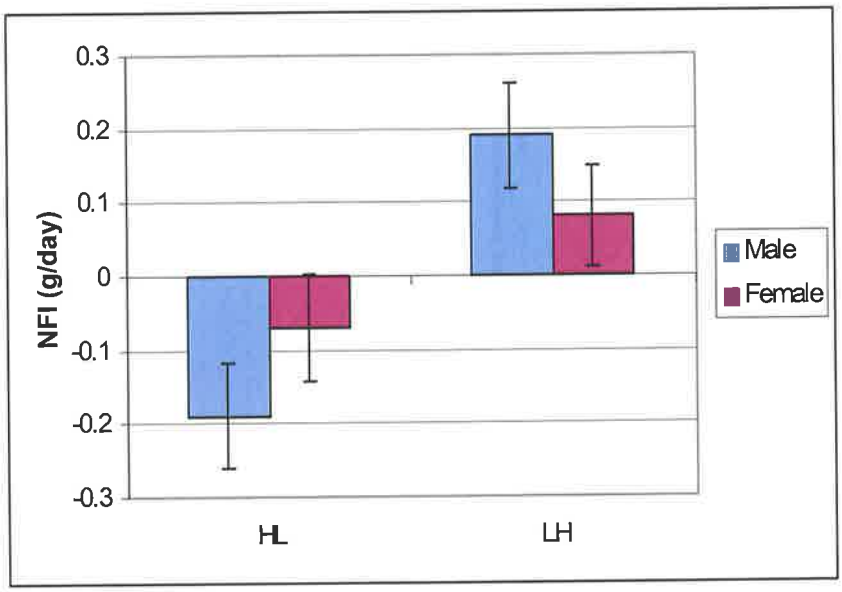


Figure 5.4 **NFI Least Squares Means for the two F_1 genotypes**

As expected, NFI was highly correlated (0.74) with DFI but not with any of the other growth traits in the F_2 generation (Table 5.2). DFI was moderate to highly correlated with all of the weight traits but not with gross efficiency. Gross efficiency was most strongly correlated (0.56) with ADG.

Table 5.2 **Phenotypic correlations between traits measured in the F_2 generation**

	NFI	DFI	GE	Fat%	ADG	Lean	WW	Wt6	MWt
DFI	0.74								
GE	-0.26	-0.18							
Fat%	-0.12	0.10	0.15						
ADG	-0.00	0.16	0.56	0.18					
Lean	0.03	0.55	0.13	0.16	0.47				
WW	-0.04	0.30	0.01	0.02	-0.08	0.53			
Wt6	0.09	0.58	-0.10	0.22	0.26	0.85	0.54		
MWt	0.00	0.56	0.33	0.29	0.53	0.98	0.56	0.86	
MMWt	0.00	0.58	0.04	0.23	0.21	0.90	0.70	0.86	0.93

A summary of the abbreviations can be found in Table 1.

There were no significant differences between the F_2 progeny of the four F_1 sires in net or daily feed intake, body fat percentage or six-week body weight (Table 5.3). However, progeny from sire 1027 grew 18.5% slower than progeny from sire 1002 (Table 5.4). Consequently, there was a 20% difference in gross efficiency between the two sires' progeny. By definition there was no significant difference between the sexes in NFI. Males were greater than females for all of the weight traits, body fat percentage and growth. Males also ate more than the females. Interestingly, the mice that were born in the second parity ate significantly less than mice from the first parity and were heavier and leaner (Table 5.5). Unfortunately, management group and parity are confounded consequently the differences observed in parity could be associated with variation in feed quality or any other management group differences.

Table 5.3 Tests of significance for main effects

Trait	Sex	Parity	NW	Sire	Dam(Sire)
NFI	n.s.	n.s.	n.s.	n.s.	***
DFI	***	*	*	n.s.	***
GE	***	n.s.	n.s.	**	***
Fat%	***	*	*	n.s.	***
ADG	***	n.s.	n.s.	***	***
Lean	***	***	***	**	***
WW	***	***	***	***	***
Wt6	***	***	***	n.s.	***
MWt	***	***	***	**	***
MMWt	***	***	***	**	***

*** P<0.001; ** P<0.01 ; * P<0.05; n.s. not significant

A summary of the abbreviations can be found in Table 1.

Table 5.4 Least squares means (±se) for each F1 sire family

Trait	S1002	S1011	S1021	S1027
NFI	-0.04 ± 0.02	-0.03 ± 0.03	0.02 ± 0.03	0.04 ± 0.03
DFI	3.49 ± 0.03	3.53 ± 0.04	3.48 ± 0.04	3.54 ± 0.04
GE	0.08 ± 0.00^{a}	0.06 ± 0.00^{b}	0.07 ± 0.00^{ab}	0.06 ± 0.00^{b}
Fat%	14.22 ± 0.18	14.37 ± 0.22	14.18 ± 0.17	13.99 ± 0.19
ADG	0.27 ± 0.01^{a}	0.22 ± 0.01^{b}	0.25 ± 0.01^{ab}	0.22 ± 0.01^{b}
Lean	19.41 ± 0.24^{a}	19.19 ± 0.30^{ac}	18.92 ± 0.21^{bc}	18.99 ± 0.26^{bc}
WW	12.04 ± 0.12^{a}	12.89 ± 0.20^{b}	11.56 ± 0.17^{c}	12.61 ± 0.25^{b}
Wt6	20.76 ± 0.30	20.78 ± 0.34	20.56 ± 0.25	20.72 ± 0.33
MWt	22.62 ± 0.29^{a}	22.49 ± 0.36^{ac}	22.02 ± 0.26^{bc}	21.99 ± 0.31^{b}
MMWt	8.54 ± 0.08^{ab}	8.71 ± 0.10^{a}	8.41 ± 0.07^{b}	8.53 ± 0.10^{b}

Means within a row with different superscripts differ ($P<0.05$).

A summary of the abbreviations can be found in Table 1.

Table 5.5 Sex and replicate least squares means (±se)

Trait	Female	Male	Parity 1	Parity 2
NFI	0.00 ± 0.02	0.00 ± 0.02	0.00 ± 0.32	0.00 ± 0.02
DFI	3.35 ± 0.03	3.66 ± 0.03	3.58 ± 0.02	3.45 ± 0.03
GE	0.06 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
Fat%	13.48 ± 0.140	14.86 ± 0.10	14.51 ± 0.13	13.94 ± 0.13
ADG	0.21 ± 0.01	0.28 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Lean	17.29 ± 0.12	20.87 ± 0.13	18.48 ± 0.18	19.62 ± 0.16
WW	11.61 ± 0.12	12.77 ± 0.13	11.20 ± 0.15	12.96 ± 0.01
Wt6	18.66 ± 0.16	22.66 ± 0.17	20.25 ± 0.22	21.03 ± 0.20
MWt	19.94 ± 0.14	24.49 ± 0.16	21.54 ± 0.22	22.83 ± 0.20
MMWt	7.93 ± 0.05	9.11 ± 0.05	8.28 ± 0.07	8.72 ± 0.06

A summary of the abbreviations can be found in Table 1.

5.4.2 Marker analysis

Although the parental lines were divergently selected on NFI for eight generations, not all markers were fixed for the NFI alleles. This meant that some individuals from the high (H) and low parental (L) lines shared common marker alleles. When this was the case, the origin of the allele in the F_2 individual could not be assigned, resulting in loss of informativeness for those markers. The informativeness of a marker is the proportion of F_1 meioses for which the allelic line of origin could be determined (Table A.1, Appendix A). For autosomes, the average level of informativeness of the markers (as opposed to the informativeness of the location) was 71% and 84% for MMU X.

5.4.3 QTL analysis

5.4.3.1 Feed intake and efficiency QTL

The LOD scores for both the feed intake and efficiency traits and the growth and body composition traits were surprisingly high. It is possible that in searching for the best combination additive dominance and epistatic effects over many possible cominations has lead to artificially low error variance and artificially high LOD scores, percentage variance explained and significance values. However, multiple interval mapping detected nine NFI QTL across eight mouse chromosomes (Table 5.6). Not all additive estimates for NFI were in the same direction, indicating that the NFI alleles were not fixed within the selection lines. The QTL on MMU 11 appears to be additive in gene action, the heterozygote value is approximately the average of the homozygote values. The QTL on MMU 16 (12.6cM) shows a significant dominant effect. The remaining QTL appear to be a combination of dominant and additive effects. The QTL with the largest LOD score (LOD = 4.3) on MMU 1 accounted for a surprising large proportion of the difference between the parental lines.

There were one significant ($P<0.01$) and seven suggestive ($P<0.05$) QTL detected for DFI, six with epistatic effects. Multiple interval mapping identified the total additive and dominance effects of the eight QTL segregating for DFI explained 50% of the total phenotypic variance and the six epistatic effects explain the remaining 50% of the phenotypic variance. As was the case with NFI, not all of the additive effects were in the same direction indicating that not all DFI alleles are fixed within the selection lines. Of the eight QTL detected for DFI, four appear to be segregating in regions that contain NFI

QTL. Three of these common QTL regions share the same sign and similar magnitude of the additive and dominance effects.

5.4.3.2 Body composition, growth, and body weight QTL

Five significant ($P<0.01$) and one suggestive ($P<0.05$) ADG QTL were detected by composite-interval mapping. Of these six QTL, five were consistent with the regression analysis. QTL Express did not detect the QTL located on MMU 14 only (Table 5.6). Unfortunately, this chromosome had only two microsatellite markers on it. There were seven suggestive body fat percentage QTL and 12 body weight QTL detected. The NFI QTL were not always detected near the same QTL of other traits. However, the NFI QTL detected on MMU 14 and MMU 16 (12.6 cM) were within 8 cM of QTL detected for ADG. Also, the sign of the additive effects were opposite and the sign of the dominance effects were the same at each location. The body fat percentage and NFI QTL detected on MMU 12 were 3 cM apart and in opposite sign. This was an interesting location in that both the NFI and body fat percentage QTL were additive whilst the DFI QTL also found in that region was largely dominant in gene action.

Table 5.6 QTL position and size in phenotypic standard deviations from Multiple Interval Mapping using QTL Cartographer

Chrom.	Trait	Position cM	LOD	Additive	Dominance
1	DFI	20.6	2.6*	-0.15	0.02
	Fat%	48.1	3.3*	-0.13	0.45
	ADG	57.1	4.0*	-0.24	-0.75
	NFI	**75.3**	**4.6****	**-0.84**	**-1.17**
	DFI	87.3	2.3*	-0.25	-0.21
2	DFI	15.0	3.5*	0.18	-1.08
	ADG	31.2	4.6**	-0.23	-1.08
3	Fat%	17.0	2.0*	0.18	0.45
	Wt6	35.6	6.5**	0.56	0.99
	DFI	47.6	2.1*	1.00	-0.80
4	Wt6	10.0	2.9*	0.31	-0.21
	WW	61.5	4.5**	-0.80	1.00
5	**NFI**	**9.3**	**3.1***	**0.60**	**0.72**
	WW	72.8	2.1*	0.82	-0.47
6	WW	20.0	2.6*	0.13	0.25
7	GE	30.1	4.0*	-0.22	0.84
	DFI	33.0	2.1*	0.38	-0.03
	Fat%	36.6	3.0*	0.60	0.86
	ADG	42.6	6.4**	1.03	-1.85
10	GE	15.0	2.9*	0.16	-0.31
	ADG	18.7	4.3**	0.25	0.43
	Wt6	32.6	2.3*	0.09	0.77
11	Wt6	38.3	6.9**	0.38	-0.23
	NFI	**46.0**	**4.1***	**0.32**	**-0.25**
	Fat%	71.9	2.5*	0.67	0.95
12	DFI	41.9	3.1*	-0.17	-0.76
	Fat%	44.8	2.6*	-0.89	-0.20
	NFI	**47.9**	**2.5***	**0.49**	**-0.06**
13	GE	2.0	4.9**	-0.57	-0.29
	DFI	37.8	4.5**	0.22	-0.24
	NFI	**38.8**	**3.2***	**0.22**	**-0.16**
14	GE	0.1	6.3**	-0.06	0.24
	NFI	**13.4**	**3.0***	**0.77**	**-0.81**
	ADG	40.0	2.1*	-0.44	-0.06
15	**NFI**	**33.3**	**2.9***	**0.32**	**0.45**
	WW	40.7	2.5*	0.15	-1.08
16	**NFI**	**12.6**	**3.0***	**-0.18**	**0.94**
	ADG	15.0	4.5**	0.03	0.42
	NFI	**42.6**	**4.3****	**-0.47**	**0.61**
	DFI	43.0	5.8**	-0.33	0.08
17	Fat%	46.9	2.6*	0.07	-0.11
X	Fat%	22.0	3.1*	-0.10	-0.70
	Wt6	22.0	4.3**	-0.10	-0. 94
	GE	45.9	5.1**	0.69	-0.29
	WW	69.5	2.0*	0.45	-0.31
	Wt6	69.5	3.6*	0.41	-0.68

Phenotypic standard deviations (ADG = 0.088 gbwt/day, DFI = 0.42 g/day, Fat = 1.93 %, GE = 0.026 gbwt/gfeed, NFI = 0.33 g/day, WW = 2.00 g, Wt6 = 3.14 g); * Genome-wide significance threshold of P < 0.05 (suggestive linkage); ** Genome-wide significance threshold of P < 0.01 (significant linkage)

5.5 Discussion

The main objective of this study was to detect the loci that control feed efficiency in mice. A lot of mapping studies have been performed using mice as a model species for both humans and livestock species. However, there are no published studies spefically on mapping for feed efficiency in mice. The only species to have a published estimate of the position of feed efficiency traits is poultry. Van Kaam and colleagues (1999) have published several QTL for feed intake at a fixed age and weight.

5.5.1 Sex linkage or genomic imprinting?

There appeared to be a significant difference in the F_1 generation between heterozygotes that inherited a paternal high allele compared with a maternal high allele. Mice that inherited a high maternal allele had a higher NFI and daily feed intake compared with mice that had inherited a paternally high allele. This could be indicative of sex linkage or more likely, imprinting. Genomic imprinting causes maternal and paternal alleles of the same genes to function unequally due to gene inactivation during gametogenesis. Interestingly, both insulin and serotonin receptor 2A are imprinted (Kato et al. 1998). It is also possible that the differences observed between reciprocal crosses was due to maternal effect.

5.5.2 Detecting the undetected

Currently, QTL studies are beginning to unravel the genetic basis of phenotypic differences. However, the number of crosses, individuals and markers limits most of these studies. Consequently, QTL studies are known to underestimate the total number of loci involved. Estimating the total number of loci allows the proportion of the total QTL detected to be calculated. This information becomes useful when further genetic studies (such as fine and comparative mapping) into the QTL detected are being considered particularly when the proportion of detected QTL is small.

Historically, the number of genes underlying a quantitative trait has been estimated using methods developed by Castle (1921) and Wright (1968), known as the Castle-Wright estimator;

$$\bar{\eta}_{cw} = \frac{(\Delta\bar{z})^2}{8\mathrm{Var}(S)}$$

Equation 5.3

Where, $\Delta\acute{z}$ is the difference between parental lines in the trait of interest and Var(S) is the amount of segregation variance. Based on this estimator the number of loci underlying NFI is 10 (9.4).

The Castle-Wright estimate, however, assumes unlinked loci, equality of allelic effects, and that all genes with a positive effect are sorted into one line and all those with a negative influence are sorted into the other line (Lynch and Walsh 1998). From the results of the interval mapping, however, the sign of the additive effects were mixed in each line, violating an assumption of the Castle-Wright Estimator. If the assumptions underlying the Castle-Wright Estimator are not met, the equation tends to underestimate the true number of genes (Jones 2001). To overcome these restrictive assumptions Zeng (1992) improved the equation by studying the impact of linkage and unequal allelic effects;

$$\bar{\eta}_{cwz} = \frac{2\check{c}\bar{n}_{cw} + C^2(\bar{n}_{cw} - 1)}{1 - \bar{n}_{cw}(1 - 2\check{c})}$$

Equation 5.4

Where č is the average recombination rate between loci, and C is the coefficient of variation of the distribution describing additive effects. The equation of Zeng (1992) gave an estimate of 14 (13.4) loci. This latter estimate is likely to be more accurate given that nine putative NFI QTL were found in this study alone.

5.5.3 Feed intake and efficiency

Initially, interval mapping using the regression approach was performed to determine regions that contain QTL. Multiple-interval mapping (MIM), a maximum likelihood approach was also performed because selective genotyping had been applied. The main advantage of MIM over previous QTL mapping procedures is that it uses a multiple-QTL

model, which allows epistatic terms to be included, compared with a single-QTL model for each interval analysed by the other procedures. MIM is believed to more precise and powerful than the single-QTL mapping procedures (interval and composite interval mapping) at estimating QTL positions and size (Zeng et al. 2000). The major disadvantage of this method is the computational requirements and time that it takes to perform.

QTL mapping analysis of a cross between high and low lines divergently selected for NFI for eight generations identified nine chromosomal regions containing loci with a significant effect on NFI (Table 5.6). Six of the nine additive effect estimates have the same sign, which indicates that one line has more positive alleles and the other more negative alleles, but the mouse lines are not likely to be fixed for all of the NFI alleles. This contradicts one of the assumptions of the F_2 analysis made in this study, that the alleles for NFI were fixed in each line. Further interval mapping was performed using the half-sib analysis of QTL Express. This analysis confirmed all the QTL detected in the F_2 analysis, but the QTL were not always segregating in each sire family.

In a preliminary study on mice divergently selected for heat loss, Moody et al. (1998) revealed significant linkage for two QTL and suggestive linkage for a further two QTL influencing heat loss. A QTL for feed intake was also detected. The suggestive QTL detected for feed intake was located on MMU 7 at approximately 40 cM. This appears to match the QTL detected by MIM in the study herein on MMU 7 at 33 cM. However, in a later study by Moody et al. (1999) using a similar resource population, this QTL was not detected. Interestingly, one of the significant QTL detected by Moody et al. (1998) and Moody et al. (1999) for heat loss (*Hlq1*) and one of the NFI QTL detected in the current study were observed on the distal arm of MMU 1 (Figure 5.5). Also, the current study observed a QTL for NFI on MMU 11 at 46 cM and one of the four suggestive QTL detected by Moody et al. (1999) was observed on MMU 11 at 68 cM. Although these pairs of QTL are over 20 cM apart, it is possible that the genes underlying these traits are the same and they are both segregating for the efficiency of food utilisation (since heat loss and NFI are both indicators of feed efficiency). A further two suggestive QTL were located on MMU 5 and 14 in a fine mapping study in mice from the same selection lines (Elo et al. 2002). NFI QTL were also detected on MMU 5 and 14 in the current study in similar regions to those of Elo et al. (2002).

Van Kaam et al. (1999) performed a genome scan in poultry and detected two significant QTL for feed intake at a fixed age, one significant QTL for feed intake at a fixed weight and one QTL for feed efficiency. The QTL for feed intake at a fixed weight, was detected on poultry chromosome 2 at 41 cM. By observing the comparative maps between poultry and mice (Groenen et al. 2000), this region in poultry appears to be homeologous to mouse MMU 5 at approximately 15 cM. MIM revealed in the study herein a QTL for NFI at 13 cM on MMU 5. The poultry study also found a feed efficiency QTL on poultry chromosome 2 at 417 cM, this appears to be homeologous to MMU 15 at 25 - 40 cM. The present study detected a QTL for NFI on MMU 15 at 33cM, but there was no QTL for gross efficiency on this chromosome. There was also a fat percentage, weaning weight and a metabolic-mid-weight QTL detected on MMU 15 at approximately 40 cM in the current study (Table 5.6).

A QTL for feed intake at a fixed age was detected on poultry chromosome 1 at 235 cM (Van Kaam et al. 1999). This region in poultry appears to be conserved with MMU 6 around 55 – 60 cM. There were no feed intake or NFI QTL detected on MMU 6. However, the second QTL detected for feed intake at a fixed weight was located on poultry chromosome four. There have been several chromosomal rearrangements between mouse and poultry within this region. One of the genes underlying this trait could be located on MMU5 between 40 and 56 cM or MMU 3 between 20 and 69 cM, based on the comparative map published by Groenen et al. (2000). The current study did not locate any QTL for NFI within either of these regions in the mouse. However, there was a daily feed intake QTL detected by MIM on MMU 3 at 47.6 cM.

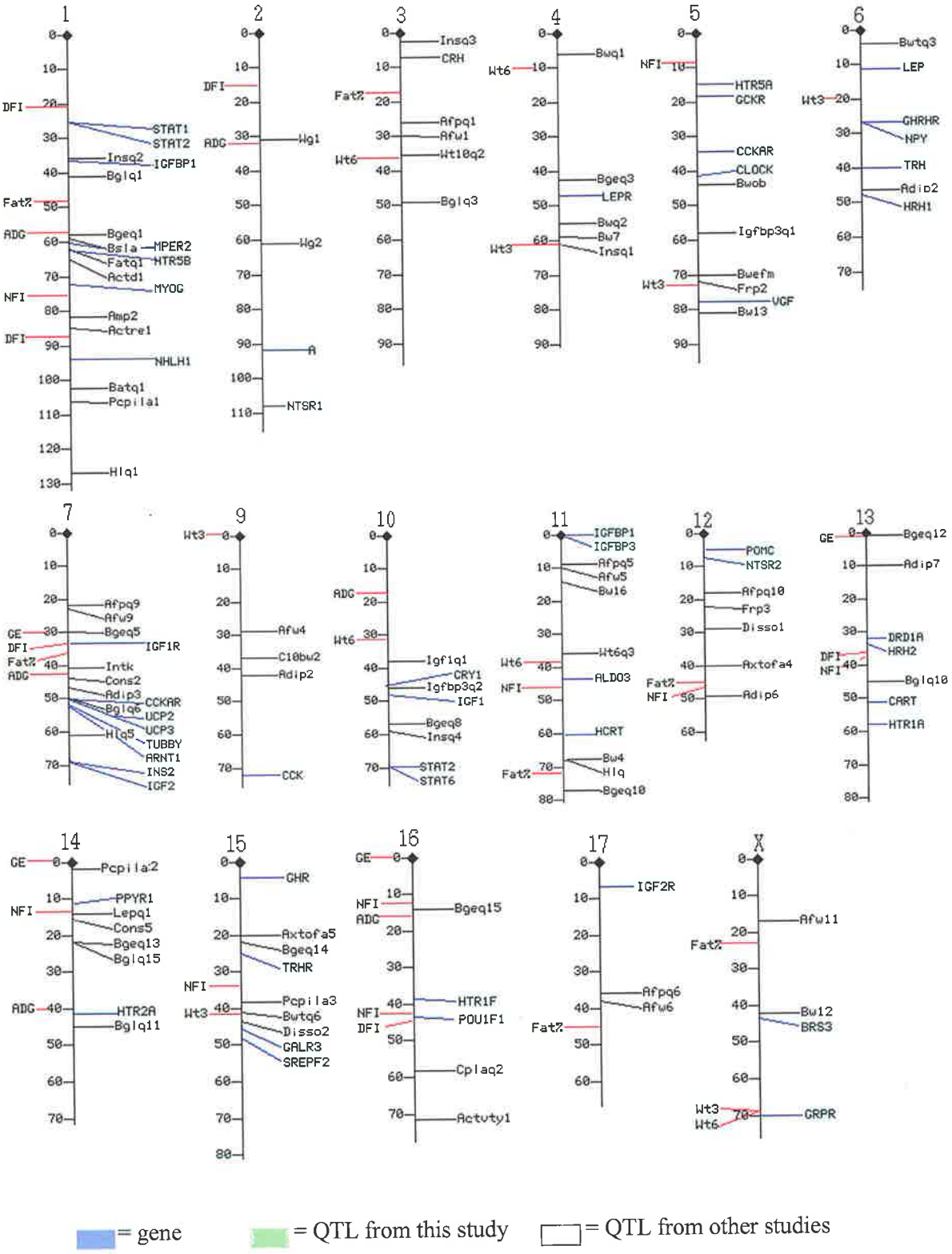
When interpreting and comparing the traits measured in the above poultry study and the traits of this study, feed intake at a fixed weight is comparable with NFI and feed intake at a fixed age is analagous to daily feed intake. Thus, it is encouraging that the region containing poultry feed intake at a fixed weight was homeologous with one of the regions observed to contain NFI in the mouse, and one out of the two QTL detected for feed intake at a fixed age in poultry was homeologous with one of the regions containing daily feed intake in this mouse study.

5.5.4 Activity QTL

In Chapter 4, very large differences between the parental lines in activity were observed (Table 5.6). A number of different 'activity' QTL have been mapped in mice. The positions of these QTL were compared with the regions detected for NFI in this study. A number of QTL for basal/open field activity have been mapped on the distal portion of MMU 1 (*Actd1*, Koyner et al. 2000; *Actre1* Demarest et al. 2001; *Amp2* Shimomura et al. 2001; *Pcpila1* Alexander et al. 1996; Syntenic QTL Flint et al. 1995; Gershenfield et al. 1997; Talbot et al. 1999; Gill et al. 2000 and Hitzemann et al. 2000). From these studies, it is possible that there are two regions containing QTL segregating for basal activity on MMU 1) is centered at approximately 60-70 cM (Talbot et al. 1999; Koyner et al. 2000; Gill et al. 2000, and Hitzemann, et al. 2000) and 2) appears to be centered at 90-100 cM (Demarest et al. 2001; Flint et al. 1995; Gershenfield et al. 1997 and Shimomura et al. 2001). Unfortunately, the resolution from these studies was not sufficient to discount that the same QTL was being detected. Also located on the distal region of MMU 1 is a QTL for the amplitude of circadian rhythm 2. The spectral analysis carried out in chapter 4 revealed that the parental lines' circadian rhythms were the same but the amplitude of the rhythm was different.

Of the other QTL detected for NFI, three are in similar regions to activity related QTL detected in other mouse studies. Alexander et al. (1996) detected a QTL for PCP induced locomotor activity (*Pcpila2*) on MMU 14 10 cM from the QTL for NFI in this study and another QTL for the same trait (*Pcpila3*) 4 cM from the QTL for NFI on MMU 15. The activity QTL detected on MMU 16 (*Actvty1*) by Shimomura et al. (2001) was 30 cM from the NFI QTL whilst the QTL on MMU 5 for free running period (*Frp3*) was over 60 cM from the NFI QTL. It is possible that the genes responsible for activity in the above studies are the same genes that are influencing NFI in this study.

Figure 5.5 **Candidate genes and QTL from other studies**



5.5.5 Growth QTL

This project involved selective genotyping. This increased the power to detect QTL for NFI and highly correlated traits for the number of animals genotyped. However, the power to detect QTL for growth, body fat percentage and body weight traits, which are not correlated with NFI, is reduced to the number of animals actually genotyped. Composite interval mapping (CIM) using maximum likelihood was performed on growth, body composition and body weight because the time required to map these secondary traits using MIM was too great. Despite this, CIM revealed a number of QTL associated with growth, body composition and body weight. The reason for observing the number of QTL for these traits was most likely due to the low selective pressure applied to the genotyping.

Three suggestive and three significant QTL for average daily gain were detected by CIM in this study. It was encouraging that support for all of these QTL could be found in a number of other mouse studies (Figure 5.5). With the exception of the QTL located on MMU 10, all QTL in this study were detected within an 8 cM region to previously reported QTL. The difference on MMU 10 was 40 cM and it is possible that these are not the same QTL.

Growth is a complex trait that is genetically determined by many genes that control nutrient turnover and energy balance. Keightly and Hill (1992) concluded that as many as one-quarter of catalogued mouse mutants have a pleiotrophic or main effect on some aspect of growth, such that the number of candidate genes for growth is very large. Because there is such a large number of candidate genes, only those limited to the somatotropic axis (*db, lep, Gh, Ghr, Ghrhr, Igf1, Igf1r, Igf2,Igf2r, tub, ad, dw and hg)* were investigated. Of the 13 candidate genes investigated, only the insulin-like growth factor 1 receptor (*Igf1r*) and the insulin-like growth factor 1 (*Igf1*) gene have been mapped close to average daily gain QTL detected in this study. The regions in question cover about 20 cM, highlighting the need to fine map these regions to confirm the candidate genes. Fine mapping is important for locating candidate genes as demonstrated by the fine mapping of the *hg* (high growth) gene by Horvat and Medrano (1995 and 1996). The authors revealed that the *hg* mutation, which was originally mapped close to the *Igf1* locus was not an allele at this locus and so *Igf1* was discounted as a likely candidate. Associations have been found between growth in mice and the growth hormone (*gh*) and

insulin-like growth factor 2 loci (*Igf2*) (Winkelman and Hodgetts 1992), neither of which shows an association with growth in the present study.

5.5.6 Body weight QTL

Body weight is another complex trait that comprises the weight of protein and fat compounds as well as bones and body fluids. Twelve QTL for the three weight traits (three and six week body-weight and metabolic mid-weight) were detected on 10 chromosomes (Table 5.6). Support for the QTL detected on nine of the 10 chromosomes can be found in other mouse mapping studies (Rance et al. 1997; Brockmann et al. 1998; Le Roy et al. 1999; Moody et al. 1999; Anunciado et al. 2000; Ishikawa et al. 2000).

The estimated position of QTL affecting both body weight and body fat provide information on both chromosomal position and on the likely nature of candidate genes effecting growth and/or obesity. The body weight (metabolic mid-weight) QTL located on MMU 15 would appear to contain the receptor for murine growth hormone receptor (*Ghr*) (Mouse Genome Informatics, http://www.informatics.jax.org/; accessed October 2003). The growth hormone gene is located on MMU 11 approximately 25 cM from the body weight QTL detected in this study. Also the QTL influencing three-week body weight on MMU 4 and 6 map closely to the leptin receptor (*db/lepr*) and the leptin gene (*ob/lep*), respectively. Leptin is a peripheral signal modulating the appetite and food consumption in interaction with the central nervous system (Brockmann et al. 2000). The two mouse models of obesity, ob and db, are recessively inherited mutations of the leptin gene and its receptor, respectively (Brockmann et al. 2000). The growth hormone releasing hormone receptor gene (*Ghrhr*) is also located on MMU 6 close to both the leptin gene and the three-week body weight QTL detected in this study.

5.5.7 Body fat QTL

Studies into the pathways regulating body mass and body composition have increased over the past decade. Several mutations have been observed in the laboratory mouse including obese/leptin (*ob/lep*), diabetes (*db*), fat (*fat*), lethal yellow/agouti (*a*) and tubby (*tubby*) that are associated with pronounced obesity (Taylor and Phillips 1996). This study revealed seven suggestive QTL for body fat percentage that are supported by a number of previous mouse QTL studies (Keightley et al. 1998; Moody et al. 1999; and Le Roy et al. 1999). Of

the five known obesity-influencing genes, only the tubby gene on MMU 7 is found near a QTL from this study. The *tubby* gene was initially identified in mice as the gene involved in the tubby syndrome. The tubby syndrome results in late-onset obesity, insulin resistance, hearing loss and retinal degeneration (Noben-Trauth et al. 1996; Koritschoner et al. 2001 and references therein). There was no statistical support in this study for QTL located near the agouti, diabetes, leptin or the fat genes. Interestingly, two insulin-like growth factor binding protein genes (2 and 5) and insulin-growth factor 1 receptor were located near the QTL for fat percentage on MMU 1 in this study.

The body fat percentage QTL and the NFI detected on MMU 12 were only 3 cM apart. It was not determined whether these QTL were two distinct QTL or one QTL having a pleiotrophic effect. However, the sign of the additive effects were opposite, in line with the findings of Chapter 4, where low NFI mice were fatter. There were no genes mapped to this region that could act as a candidate for both feed efficiency and/or body fat percentage.

5.5.8 An interesting observation

It was of interest to note that by generation nine the high NFI, low efficiency line were all white in colour, whereas in the low NFI line, there was a mixture of black, white and agouti mice. The agouti gene is of interest because it has been found to play a role in obesity. Dominant mutations in the mouse *agouti* gene confer a pleiotrophic syndrome characterized by obesity, mild hyperphagia, decreased thermogenesis, hyperinsulinemia, peripheral insulin resistance, impaired glucose tolerance, hyperglycaemia in males, increased susceptibility to cancer and yellow hair (Michaud et al. 1997). It is possible that the agouti gene and the agouti related protein gene could act as candidate genes for feed efficiency. However, there was no NFI QTL detected on MMU 2 or 8. Also, in the F_2 generation of the present study, there were a mixture of agouti and white coloured mice in both the high and low tails of the NFI distribution. It is most likely that the observation made in generation nine was just due to chance (random genetic drift).

5.5.9 Candidate genes for feed efficiency

Candidate genes are genes located in the region of the QTL and whose function is likely to affect the trait(s) of interest (Kinghorn and Van der Werf, 2000). Studying candidate genes of known biological action is a useful method to identify genes controlling feed efficiency.

These candidate genes can then be used to improve feed efficiency through the adoption of marker (gene)- assisted selection. There were a large number of possible candidate genes for feed efficiency identified in this study (Table 5.7 and Table 5.8). They can be classified in four broad groups; 1) biorhythms, 2) growth regulation and body composition, 3) energy regulation, and 4) neuropeptide signalling.

Table 5.7 Candidate genes for feed efficiency

Gene	MMU	Abbrev.
Biorhythms		
Period homolog 2	1	*Per2*
Circadian locomotor output cycles kaput	5	*Clock*
Period homolog 1	5	*Per1*
Growth regulation and body composition		
Insulin-like growth factor binding protein 2	1	*Igfbp2*
Insulin-like growth factor binding protein 5	1	*Igfbp5*
Myogenin	1	*Myog*
Somatostatin receptor 2	11	*Sstr2*
Somatastatin receptor 1	12	*Sstr1*
Calpastatin	13	*Cast*
Growth hormone receptor	15	*Ghr*
Peroxisome proliferator activated receptor alpha	15	*Ppara*
Ribosomal protein R3	15	*Rpl3*
Somatostatin receptor 3	15	*Sstr3*
Sterol regulatory element binding factor 2	15	*Srebf2*
Pituitary specific transcription factor 1	16	*Pou1f1*
Somatostatin	16	*Sst*

Table 5.8 Energy regulation and neuropeptide candidate genes for feed efficiency

Gene	MMU	Abbrev.
Energy regulation		
ATPase Ca^{++} transporting plasma membrane 4	1	*Atp2b4*
Aldolase 3 isoform	11	*Aldo3*
ATPase H^+transporting, lysosomal V0 subunit isoform 1	11	*Atp6v0a1*
Phosphofructokinase muscle	15	*Pfkm*
ATPase H^+ transporting, lysosomal 70kD, VI subunit A isoform 1	16	*Atp6v1a1*
Neuropeptide signalling		
Serotonin receptor 5B	1	*Htr5b*
Cholecystokinin A receptor	5	*Cckar*
Dopamine receptor D5	5	*Drd5*
Serotonin receptor 5A	5	*Htr5a*
Hypocretin	11	*Hcrt*
Pancreatic polypeptide	11	*Ppy*
Solute carrier family 6 A4	11	*Slc6a4*
Pro-opiomelanocortin-alpha	12	*Pomc*
Cocaine and amphetamine regulated transcript	13	*Cart*
Dopamine receptor 1A	13	*Drd1*
Serotonin receptor 1A	13	*Htr1a*
Solute carrier family 6 A3 (neurotransmitter to dopamine)	13	*Slc6a3*
Serotonin receptor 2A	14	*Htr2a*
Pancreatic polypeptide receptor 1	14	*Ppyr1*
Galanin receptor 3	15	*Galr3*
Dopamine receptor 3	16	*Drd3*
Serotonin receptor 1F	16	*Htr1f*

5.5.9.1 Biorhythms

Many biochemical, physiological, and behavioural processes display daily rhythms generated by an internal time keeping mechanism referred to as the circadian clock. The central oscillator driving this clock is located in the hypothalamus and is thought to be composed of interlocking, auto-regulatory feedback loops involving a set of clock genes

(Oster et al. 2002). Among the components driving mammalian circadian clock are period 1 and 2 (*Per1* and *Per2*) and cryptochrome 1 and 2, and the *clock* gene. The period 1 and 2 genes are located on chromosomes that contain NFI QTL. Both *Per1* and *Per2* are expressed in a circadian pattern in the suprachiasmatic nucleus (Albrecht et al. 1997) and maintain a pattern of circadian expression in constant darkness. A mutation in *Per2* leads to a gradual loss of circadian rhythmicity in mice kept in constant darkness (Oster et al. 2002). The *Clock* gene is a semidominant mutation that lengthens circadian period and eliminates the persistence of rhythmicity (Vitaterna et al. 1994). These genes are of interest since large differences were observed between the NFI selection lines in the amplitude of their activity levels in (Chapter 4). It is possible that differences in the expression of these genes could increase the activity levels of the high NFI mice, and consequently, increase NFI and reduce body fat percentage.

5.5.9.2 Growth regulation and body composition

Muscle

Growth is an energetically expensive process carried out by all living organisms. Although there was no significant difference between the NFI selection lines in growth, it is possible that there is a significant difference between the lines in the efficiency of this energetic process. There were a number of genes that regulate growth observed near NFI QTL detected in this study include growth hormone receptor (*Ghr*), pituitary specific transcription factor 1 (*Poulf1*) and myogenin (*Myog*). *Myog* is a basic helix-loop-helix transcription factor that belongs to the muscle regulatory factor (MRF) family. Members of the MRF family play an important role in regulating skeletal muscle development and growth. *Poulf1* is a member of the POU-domain family gene and is positive regulator of growth hormone, prolactin and thyrotrophin-stimulating hormone β (TSHβ). Ontogenetic studies have shown that *Poulf1* is the critical cell-specific transcription factor for activating expression of the prolactin and growth hormone genes in the anterior pituitary gland (reviewed in Tuggle and Trenkle 1996).

Growth hormone is a growth enhancer that has been under commercial development to improve meat quality, productive efficiency and milk production in domestic animals. Growth hormone has two distinct types of effects: 1) direct, the result of growth hormone binding to its receptor on target cells, and 2) indirect effects, which are mediated primarily

by IGF-1 in response to growth hormone. Growth hormone mediated improvement in productive efficiency of livestock are generally associated with the up-regulation of hepatic *Ghr*, increases in plasma insulin-like growth factor-1 (*Igf1*) levels, and inhibition of lipogenesis (Mao et al. 1998).

Somatostatin (*Sst*) was first identified as a member of a family of hypothalamic hormones that inhibited secretion of growth hormone (Brazeau et al. 1973). It has also been shown in mammalian intestines to inhibit motility, the release of enteric hormones, and secretion of digestive enzymes, growth and reduce splanchnic blood flow (Yamada and Chiba 1989). It is believed that *Sst* is a regulator of glucagon-like peptide -1 (GLP1) secretion Hansen et al. (2000). GLP1 is a peptide hormone secreted from the small intestine and colon in response to meal ingestion (Hansen et al. 2000). *Sst* and its receptors (*Sstr1, Sstr2* and *Sstr3*) also influence the secretory response of cAMP- and Ca^{2+} in the colon (Samson et al. 2000) and consequently, could possibly act as a candidate gene for feed efficiency.

The insulin-like growth factors (IGF-1 and IGF-2) are the major growth-promoting factors present in the circulation. IGFs modulate their activities through IGF-binding proteins. There are six known IGF binding proteins that control the distribution, function and activity of IGFs by binding IGFs in various cells, tissues and body fluids, and thereby, regulating their metabolic and mitogenic effects (Baxter 2000). The insulin-like growth factor binding proteins 2 and 5 (*Igfbp2* and *Igfbp5*) are located on MMU 1 in the same region as one of the QTL for NFI. *Igfbp2* is one of the most predominant binding proteins in the serum and can bind IGF-1 and IGF-2 with high affinity. Mice selected for low body weight have been shown to display increased serum levels of *Igfbp2* (Eckstein et al. 2001). Eckstein et al. (2001) also found that over-expression of *Igfbp2* negatively regulates bone growth and mediates growth hormone action via IGF-1. *Igfbp5* is the most conserved IGF-binding protein across species. It has been identified as an essential regulator of physiological processes in bone, kidney and mammary gland (Schneider et al. 2002).

Another candidate gene that influences growth, and therefore, feed efficiency is calpastatin (Cast). The calpain/calpastatin system is an endogenous, Ca^{2+} dependent proteinase system, believed to initiate *in vivo* muscle protein degradation. This system, especially the calpastatin component, appears to be related to meat tenderness. *Cast* is a powerful

inhibitor of calpain activity. Associations between *Cast* activity and muscle hypertrophy, and meat tenderness have been shown previously in lambs and cattle (Whipple et al. 1990; Pringle et al. 1995; Wulf et al. 1996). After one generation of selection for divergent NFI in beef cattle, McDonagh et al. (2001) found a 13% difference in *Cast* levels between high and low NFI steers. The increased level of *Cast* in the more efficient steers is most likely a correlated response to selection rather than direct selection. Therefore, *Cast* may not be an appropriate candidate gene, but it could be a good biochemical marker. This relationship requires further investigation as increased levels of *Cast* negatively affect meat tenderness.

Fat

The brain controls energy expenditure, in part, via the sympathetic nervous system, which innervates brown adipose tissue (Tartaglia et al. 1995). However, McDaneld et al. (2002) concluded that the response to selection for increased energy expenditure in mice was not mediated by increased expression or function of *Ucp1*. Brown adipose tissue is a specialised form of adipose tissue that functions as a thermogenic organ in rodents. Uncoupling protein –1 (*Ucp1*) is specific to brown adipose tissue and generates energy by dissipating the mitochondrial proton gradient (Garlid et al. 1998). It is believed that the functional role of uncoupling proteins -2 and -3 (*Ucp2* and *Ucp3*) is to regulate lipids as a fuel substrate rather than as mediators of regulatory thermogenesis (Samec et al. 1998). Raimbault et al. (2001) observed significant differences between lines of chickens selected for NFI for the avian homologue of *Ucp2* and *Ucp3*. It was suggested that these proteins could explain most of the differences in heat production between the divergently selected lines. The uncoupling proteins 2 and 3 are located in the same position on MMU 7. There was a QTL for DFI but not NFI near these genes.

There are a number of transcription factors involved in regulating lipid metabolism. One of these transcription factors, peroxisome proliferator activated receptor alpha (*Pparα*) is a member of the nuclear receptor super family that regulate the expression of several genes encoding proteins involved with adipocyte differentiation and fat deposition (Schoonjans et al. 1996). *Pparα* was also located near one of the QTL for NFI. It is involved in fatty acid oxidation by up-regulating the expression of the enzymes acyl-CoA oxidase and carnitine palmitoyltransferase (Ding et al. 2000). *Ppara* has been shown previously (Ding et al.,

2000) to be highly expressed in the adipose tissue of pigs and to up-regulate Ucp2 expression in hepatocytes through increased transcription in rodents (Nakatani et al. 2002).

There were other candidate genes that influence adipose tissue located near QTL for NFI. Sterol regulatory element-binding proteins (*Srebp*) belong to the basic helix-loop-helix leucine sipper (bHLHLZ) family of transcriptional regulatory proteins. Sterol regulatory element-binding protein-2 (*Srebp2*) is one of the proteins that control cholesterol homeostasis by stimulating transcription of sterol-regulated genes (Miserez et al. 1997). Interestingly, over-expression of *srebp1* and *2* has been documented in the liver and adipose tissue of leptin-deficient mice and the obese Zucker rat (in a review by Osborne 2001). The over-expression of *Srebp2* has also been shown to result in the accumulation of both cholesterol and fatty acids (Le Lay et al. 2001).

Allan et al. (2000) evaluated the gene expression in the hypothalamus and brown adipose tissue of mice divergently selected for heat loss. In both the hypothalamus and brown adipose tissue, the ribosomal protein L3 (*Rpl3*) gene was expressed at higher levels in mice from the low heat loss line. Ribosomal protein L3 is the largest protein of the 60S subunit of the ribosome in eukaryotes and acts as the centre channel through which new peptides emerge (Allan et al. 2000). The authors speculated that the ribosomal protein L3 could be involved in the transcription of genes involved in the gylcolytic pathway, which would result in an increase in glycolysis. This in turn would lead to excess energy production, making the animal more efficient in energy substrate utilisation. The authors further hypothesized that this positive energy balance could lead to increased fat deposition and signal for decreased energy consumption. This gene is found on MMU 15, however, its exact location on that chromosome is not published. Since a QTL for NFI was detected on MMU 15, ribosomal protein L3 could act as a candidate for feed efficiency.

5.5.9.3 Energy regulation

The candidate energy regulatory enzymes are include the glycolytic enzymes Aldolase C *(Aldo3)*, and phosphofructokinase (*Pfkm*), and ATPases *Atp2b4, Atp6v0a1,*and *Atp6v1al.* Aldolase C is found in the brain and other tissues of neurological orgin (Lebherz et al. 1969). It is the third isoform in the aldolase family. Aldolases typically catalyse the 'splitting of sugar' during glycolysis, where the six carbon sugar fructose biphosphate is

broken down to form two three carbon sugars. *Pfkm* is the enzyme responsible for catalysing the second step of glycolysis in the muscle. Both of these glycolytic steps require energy, in the form of ATP. As hypothesised by Allan et al. (2000) increased glycolysis could lead to excess energy production, making the animal more efficient in energy substrate utilisation. Subsequently, this positive energy balance could lead to increased fat deposition and signal decreased energy consumption.

ATP synthesis provides the majority of cellular energy in eukaryotes. Generally, ATP synthesis is catalysed by a group of enzymes known as ATPases. Three ATPases were located near NFI QTL. The vacuolar H^+ -ATPases (*Atp6v0a1* and *Atp6v1a1*) translocate hydrogen across the membranes of organelles including vacuoles, lysosomes, endosomes, golgi apparatus, and coated vesicles (Murata et al. 2002). *Atp2b4* is a plasma membrane Ca^{2+} pump. The main function of *Atp2b4* is to maintain the calcium gradient across the plasma membrane via regulated active extrusion of calcium ions from the cell (Shull 2000).

5.5.9.4 Neuropeptide signalling

Several factors contribute to the regulation of energy balance, including neurotransmitters/ neuromodulators and neuropeptides in the brain and proteins produced in the peripheral fat tissues or muscles (Inui 2000). To regulate body mass, animals need to monitor energy stored as fat and adjust food intake and energy expenditure accordingly. A number of candidate molecules controlling energy intake, expenditure, and/or partitioning have been identified by conventional pharmacological and transgenic studies Inui (2000) (Figure 5.6).

Positive regulators

Of the neuropeptide candidate genes located near NFI QTL, two positively regulate body adiposity: orexin (*Ox*) and galanin receptor 3 (*Galr3*) (Figure 5.6). Orexin, also known as hypocretin, is a neuropeptide located on lateral hypothalamus cells. It is believed to play a significant role in the regulation of food intake and sleep-wakefulness, possibly by coordinating the complex behavioural and physiologic response of these homeostatic mechanisms (Sakurai et al. 1998; Yamada et al. 2000). It has also been suggested that orexin plays a broader role in the homeostatic regulation of energy metabolism, autonomic function, hormonal balance and the regulation of body fluids.

Galanin is a neuropeptide that is widely expressed in the nervous system and has important physiological, neuroendocrine and behavioural effects. It regulates growth hormone secretion, stimulation of appetite, modulation of memory and learning, analgesia action and anti-seizure activity (Smith 1998). The galanain receptor 3 (*Galr3*) activates potassium channels linked to the regulation of galanin release.

Negative regulators

The remaining neuropeptide candidate genes negatively regulate body adiposity. Serotonin is a monoamine neurotransmitter involved in the regulation of appetite and emotion. It mediates a wide range of physiological functions by activating multiple receptors (Hannon and Hoyer 2002). Interestingly, of the nine QTL detected for NFI, five are located near serotonin receptors (*Htr5b, Htr5a, Htr1a, Htr2a, Htr1f*) and another near solute carrier family 6 A4, a neurotransmitter to serotonin. *Htr1a* receptors have been implicated in the neuroendocrine regulation of adrenocorticotrophic hormone (ACTH) secretion, whilst decreased blood pressure and heart rate, and increased locomotor responses can be induced by *Htr1a* activation (Hannon and Hoyer 2002). *Htr1a* receptors have also been shown to increase body temperature, stimulate appetite and relieve anxiety. *Htr2a* receptor activation also stimulates ACTH secretion, as well as corticosterone, oxytocin, renin and prolactin. The actions of *Htr1f*, *Htr5a*, and *Htr5b* receptors are not well defined.

Dopamine is another neurotransmitter that regulates a variety of physiological functions. It acts through D1- and D2- like receptors to regulate neuronal motor control, cognition, event prediction, and emotion (Demchyshyn et al. 2000). The D1-like receptor gene family is made up of two members: D_1 and D_5 receptors (*Drd1* and *Drd5,* respectively). Both *Drd1* and *Drd5*, and dopamine receptor D_3 (*Drd3*) were located near QTL for NFI. Also, the solute carrier family 6 A3 (*Slc6a3*) neurotransmitter to dopamine is located on mouse MMU 13 near a NFI QTL. *Slc6a3* also known as *Dat1* is the gene responsible for pre-synaptic reuptake of dopamine and a major site of action of psycho-stimulant drugs (Gelernter et al.1995). Interestingly, the content of dopamine in the brain of the American cockroach has been shown to follow daily rhythm (Pree and Rutschke 1983). High dopamine contents were synchronised with maximum locomotor activity. Also, the dopamine content was higher in females than in males. There was a significant difference

between the high and low NFI mouse lines in activity, with females more active than males.

Cocaine and amphetamine regulated transcript (*Cart*) and pro-opiomelanocortin (*Pomc*) are peptides regulated by leptin (Yeo et al. 2000, in a review by Pritchard et al. 2002). *Cart* is expressed in the hypothalamus within the region that has been implicated with feeding behaviour. It has been shown to be an important determinant of body weight homeostasis in normal and obese animals. *Pomc* is expressed in the pituitary gland, skin, immune system and brain (Yeo et al. 2000, in a review by Pritchard et al. 2002). It is cleaved post-translationally to generate a range of bioactive peptides. The effects of *Pomc* are mostly mediated through melanocortin (MC) receptors (MC1R, MC2R, and MC5R) that effect skin pigmentation, adrenal steroidogenesis, and thermoregulation. *Pomc* also influences appeptite through the interaction between itself, its bioactive peptides and MC3R and MC4R (Pritchard et al. 2002). A number of observations demonstrate that the MC4R plays an important role in controlling feeding behaviour, whilst MC3R plays a role in energy homeostasis.

It was established in Chapter 4, that there were differences between the NFI lines in gastric emptying rates. It was proposed that some of these differences could be due to differences in feedback mechanisms and more specifically, through differences in cholecystokinin (CCK). Cholecystokinin is believed to be a satiety signal and evidence suggests that the physiological role of CCK is as a meal termination signal. Interestingly, the cholecystokinin A receptor gene (*Cckar*) has been mapped on MMU five near a NFI QTL from this study. However, the *Cck* and *Cckbr* genes were not located on chromosomes that contained NFI QTL. It is possible that the *Cckar* gene is influencing gastric emptying and inadvertently feed efficiency.

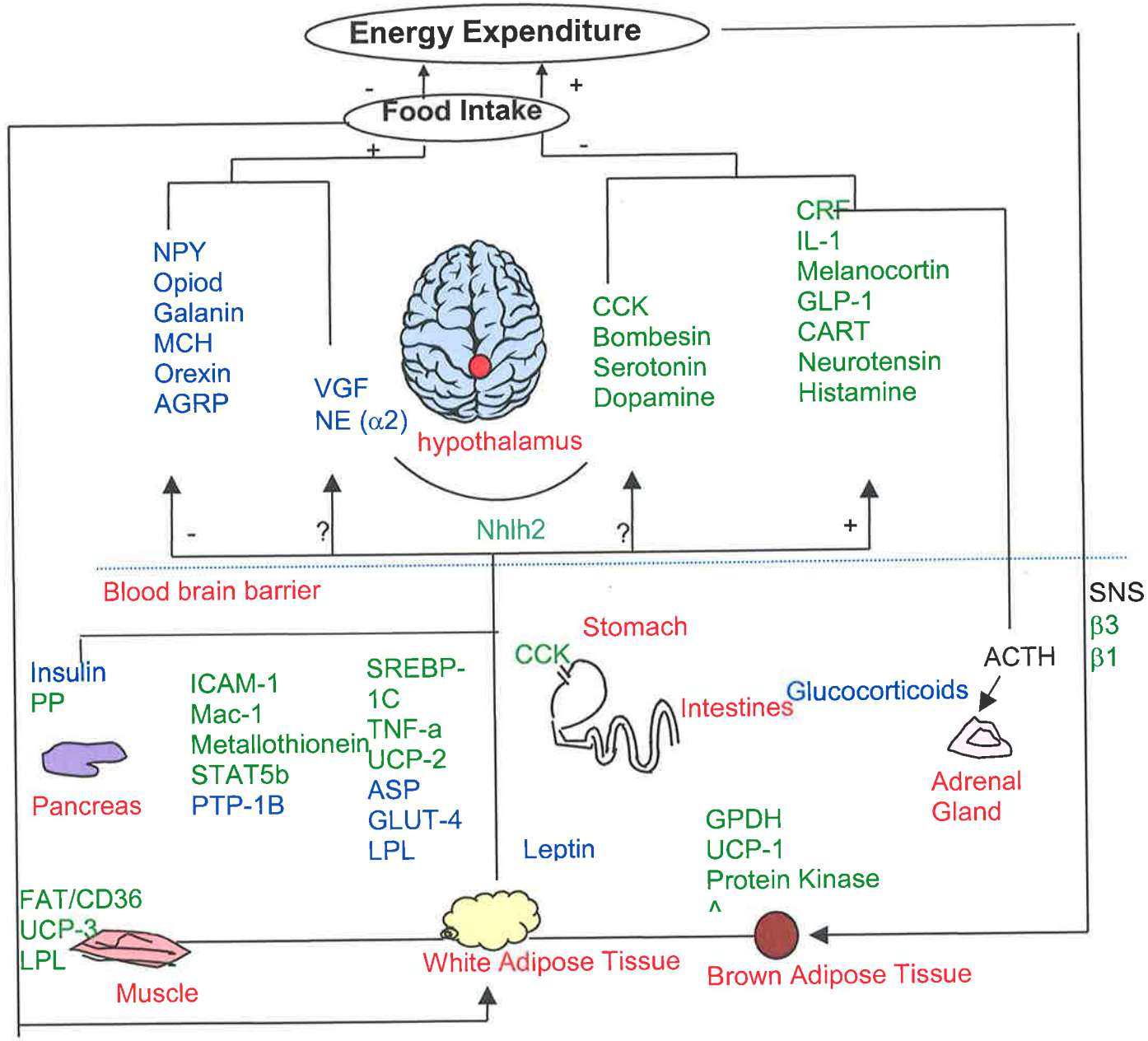


Figure 5.6 **Candidate molecules for feed efficiency, green represents positive regulators of body adiposity and blue, negative regulation of body adiposity (adapted from Inui, 2000)**

Finally, there were two other candidate genes that play a role in gastric motility located near QTL for NFI. Pancreatic polypeptide (*Ppy*) is a peptide that belongs to a family that includes neuropeptide Y and peptide YY. *Ppy* is produced by endocrine cells located in the periphery of pancreatic islets and is released into circulation after ingestion of food and exercise. *Ppy* and its receptor 1 (*Ppyr1*) regulates intake not only through the modulation of gastric acid secretion and gastrointestinal motility, but also through the inhibition of pancreatic exocrine secretion, gall bladder contraction, and simulation of glucocorticoid secretion (Asakawa et al. 1999; Katsuura et al. 2002; Yang et al. 2002). Both *Ppy* and its receptor were located near different QTL for NFI.

5.6 Conclusion

The aim of this chapter was to locate the regions of the mouse genome containing QTL for NFI and to investigate any possible candidate genes in these regions. Nine QTL influencing NFI were detected across eight mouse chromosomes. Of these nine QTL, four were close to QTL detected previously in mice for heat loss, a further two were comparative with QTL detected for feed efficiency in poultry. Also, a number of activity related QTL from previous mouse studies have been located near NFI QTL. Given the large differences between the NFI selection lines in activity reported in Chapter 4, it is possible that the genes responsible for activity could act as candidate genes for NFI.

There were 28 candidate genes identified in nine regions containing NFI QTL. These candidate genes influence energy balance through a variety of different mechanisms; 1) biorhythms, 2) growth and body composition, 3) glycolytic enzyme acitivity and 4) neuropeptide signalling. Of these 28 candidate genes, 22 were neuropeptides. Fine mapping of the regions containing NFI QTL could reduce the number of candidate genes to be investigated. Comparative mapping with other species that have NFI QTL mapped could also help reduce the number of potential candidate genes for NFI which will be discussed in later chapters.

Chapter 6.

The effect of incomplete data on cattle feed intake estimates

6.1 Introduction

As has been mentioned previously, the cost of measuring feed intake in cattle has limited its use as a trait in selection indices. Mapping the gene(s) associated with intake and developing a commercial DNA based test is one way of reducing the cost of identifying animals with high or low intake. This is the focus of chapters 5 and 8. However, reducing the number of days that animals are on feed may also provide an alternative solution. If the beef industry can identify and use animals with superior feed efficiency the cost of production could be significantly reduced. Barwick et al. (1999) demonstrated that the inclusion of feed intake in a selection criteria could increase the accuracy of selection for breeding objectives aimed at maximising profitability by 3 - 42%.

With recent advances in electronics and computing, automatic feeders are available which enable easy, accurate delivery of feed and recording of intake. Studies in other livestock species such as swine indicate that automatic feeders do not always function properly (de Haer et al. 1992 and Knap and Van der Steen 1994). In manual feeding and recording operations, human error is always a source of inaccuracy. Therefore, the effect of missing feed intake data on actual feed intake records for genetic evaluation needs to be investigated and data correction strategies developed. This is important not only for industry but for the following chapters, where both phenotypic analysis and QTL mapping for feed intake in cattle has been performed. Accurate trait estimates are required to confidently map the QTL associated with daily and net feed intake traits.

Results of research by Archer et al. (1997) and Archer and Bergh (2000) indicated that for beef cattle, 35 days is required for accurate estimates of feed intake, while 70 days is required for feed efficiency estimates which utilises both feed intake and growth measurements. On the basis of these results, the currently recommended length of the test for feed intake and feed efficiency for Australian beef cattle is 70 days (Exton 2001). In a study with pigs, Eissen et al. (1999) suggested that it might be possible to record feed intake for only part of the test period. Therefore, exploring the possibility of measuring feed intake for only part of the test period as a means for cost-efficient use of facilities is worth pursuing.

The three objectives of this study were: 1) to evaluate simple models to describe feed intake, 2) to examine the effect of missing data on feed intake records in beef cattle, and 3) develop corrective strategies and schemes to explore the possibility of measuring feed intake for only part of the test period. The purpose of these aims was two-fold, we need accurate trait estimates when mapping and also industry requires methods for overcoming missing or poor quality data during an intake test.

6.2 Materials & Methods

6.2.1 Animals and Data

Two data sets containing feed intake measurements from cattle were analysed. The first data set (post-weaning) consisted of daily feed intake and weekly weight measurements from 180 Angus males and females located at the Agricultural Research Centre, Trangie, New South Wales, Australia. The measurements were taken post-weaning when the animals were approximately 300 days of age and weighed between 140 and 430 kilograms (mean weight (± standard deviation) of 283 ± 44 kg, Figure 6.1) at the start of the test. The average growth rate (± standard deviation) and daily feed intake (± standard deviation) during the test period were 1.30 ± 0.30 and 9.64 ± 1.04 kg/day respectively. The between animal variation (1.52 kg/day) in feed intake appeared to be slightly higher at the end of the test period when the animals were eating more (Figure 6.2).

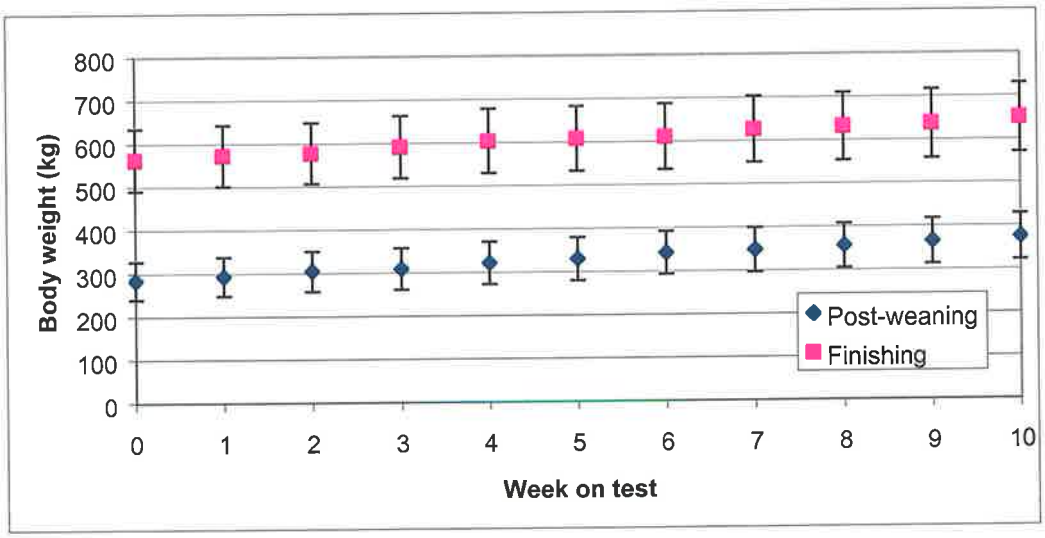


Figure 6.1 Average body weight (±standard deviation) for each week

The efficiency test was conducted using an automated feeding system developed at Trangie Agricultural Research Centre, which allowed measurements of individual daily intakes of each animal. A 21-day pre-test adjustment period was allowed for animals to adapt to the feeding system and diet, and was followed by a 70-day test period. The animals were weighed weekly during the efficiency test. All animals had *ad libitum* access to a pelleted ration consisting of 70% lucerne hay and 30% grain with approximately 10.5MJ metabolisable energy per kg dry matter and 16% crude protein.

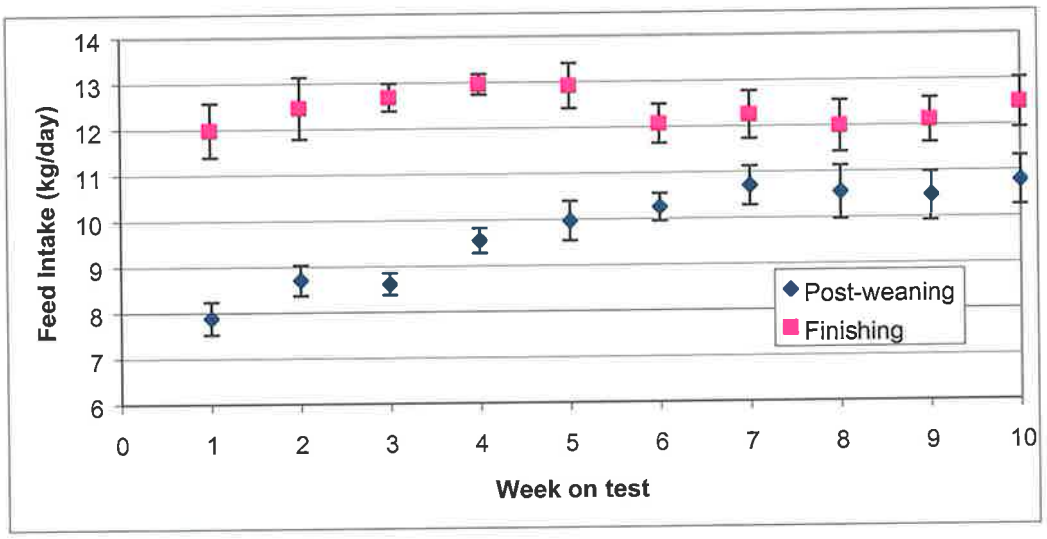


Figure 6.2 Average feed intake (±standard deviation) for each week

The second data set (finishing, older cattle being finished for slaughter) comprised 80 Limousin/Jersey crossbred cattle (either ¾ Limousin ¼ Jersey or ¾ Jersey ¼ Limousin) that were fed at the University of New England, Tullimba Research feedlot located near Armidale, New South Wales, Australia. These animals were the first cohort of the animals described in Chapter 3.5 and were of mixed sex, ranging between 425 and 735 kilograms live weight (mean 563 ± 65) and were approximately 900 days of age at the start of the test. The mean growth rate (± standard deviation) and feed intake (± standard deviation) during the test period was 1.05 ± 0.90 and 12.0 ± 0.6 kg/day respectively. The between animal variation in feed intake (2.36 kg/day) did not appear to differ significantly throughout the test period. However, the between animal variation in this data set is 55% greater than in the post-weaning data set. Prior to the feed intake test the animals were grain fed for 150 days. The cattle had access to a pelleted ration with approximately 11.4 MJ metabolisable energy per kg dry matter and 17% crude protein *ad libitum* for 80 days.

The data sets were analysed separately. Both data sets were analysed only over the first 70 days of the test, after the pre-test period (21 days). A 70-day test period was chosen because the current recommendation for measuring net feed intake (feed intake independent of growth and body weight) is for a 21-day pre-test adjustment period followed by a 70-day test period with weight measured every 2 weeks (Exton 2001). Any animals that had missing intake measurements during the full test period were removed from the data set, which decreased the number of animals in each test to 129 and 50, respectively.

6.2.2 Estimating Eliminated Daily Feed Intake Records

Various feeding scenarios were simulated, with practical applications considered, by deleting intake records from both data sets (Table 6.1). The random schemes were derived by arbitrarily deleting a proportion of an animals' feed intake records in steps of 10% to simulate random missing values, which may occur through lost electronic ear tags or short power failures. It was assumed that such failures did not result in animals being unable to feed as usual, just that invalid or no data was recorded by the automated feed system.

Table 6.1 Description of the different intake scenarios evaluated for their affect on accuracy of measurement of individual feed intake

Scheme	Description of Missing Intake Scenarios
Day1…Day6	Progressively deleting data daily (1day per week, 2 days per week … 6 days per week)
Week1S…Week9S	Progressively deleting data weekly from the beginning of the test period
Week1M…Week9M	Progressively deleting data weekly from the middle of the test period
Week1E…Week9E	Progressively deleting data weekly from the end of the test period
Wee1R…Week9R	Randomly deleting data in weekly blocks
1 wk on/off	1 week of measurement 1 week off measurement throughout the test period
2 wk on/off	2 weeks of measurement 2 weeks off measurement throughout the test period

6.2.3 Analyses

Before any feed intake records were removed first-, second-, and third – degree polynomial functions and a non-linear function were fitted to both data sets. The identification (tag) of each animal was fitted as a fixed effect and day as a covariate. The interaction between tag and the polynomial functions of day were also fitted to each of the linear models to allow for the differences between animals in the increase or decrease of intake over time. The effect of temperature and humidity was included as a fixed effect in the form of a temperature humidity index. However, the range in the temperature humidity index was small and had no effect on intake so, the index was left out of the final model. The polynomial models fitted can be found in Table 6.2, while the non-linear function fitted was:

$$ADFI(t) = a + b \times \ln(c + t) \qquad \text{(Equation 6.1)}$$

Where ADFI(t) represents the average daily feed intake at test day t, and a, b, and c are function parameters, tag is the ear tag number or identification of the animal and ln is the

natural logarithm. These functions were chosen because polynomial functions are commonly used for fitting feed intake and growth data (Kanis and Koops 1990; Eissen et al. 1999). The non-linear function was chosen to take into account the potential that feed intake could plateau at the later stages of the test.

Average daily feed intake was calculated for each animal for each intake scheme (Table 6.1) using least squares means from the linear regression adjusted for tag and the tag by day interaction. Pearsons correlation and pair-wise T-tests of significance were calculated between true daily feed intake (ADFI from full data set) and the estimate of daily feed intake for each scheme (ADFI from each scheme) to determine whether missing data was having a significant effect on estimating daily feed intake using proc means (SAS 1998).

Results

There was no evidence to suggest non-linearity in either of the data sets (Figure 6.3 and Figure 6.4) with only 19% of the observed variation in intake accounted for by the non-linear model in both data sets. The quadratic term whilst being significant in both data sets accounted for only a small amount (an extra 2.5% and 1.3%) over the linear model. The cubic terms were not significant in either of the data sets. The analysis was allowed for different starting intake levels (y-intercepts) for each animal by fitting tag and the different rates of change in intake (slope) using the tag by day interaction (Table 6.2).

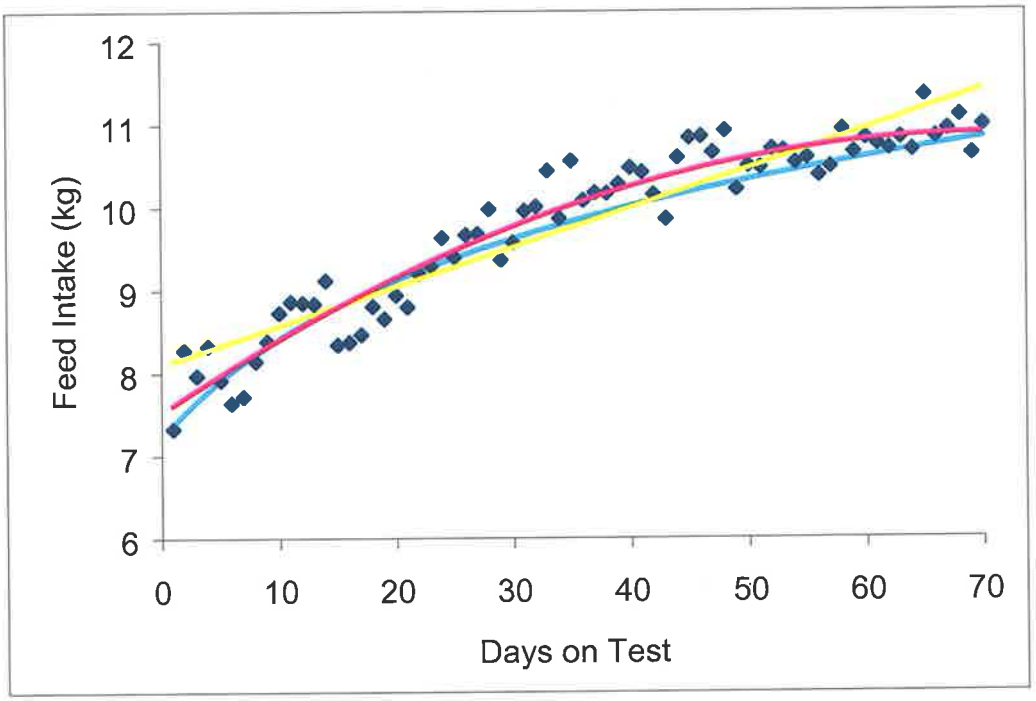


Figure 6.3 True and estimated records of feed intake per day in post weaning using linear, quadratic and non-linear functions plotted against days on test for one animal in the post-weaning data sets (♦ = true record; linear model estimates; quadratic model estimates; non-linear model).

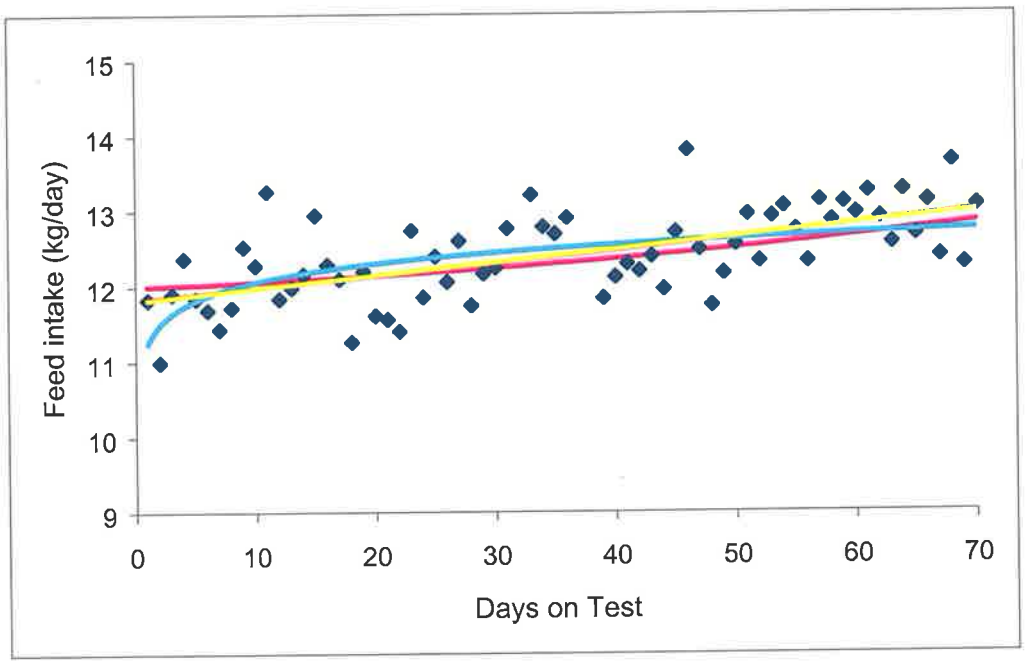


Figure 6.4 **True and estimated records of feed intake per day in feedlot finishing test using linear, quadratic and non-linear functions plotted against days on test for one animal in the finishing data sets (♦ = true record; ——— linear model estimates; ——— quadratic model estimates; ——— non-linear model).**

Table 6.2 **ANOVA for the post-weaning and finishing data sets**

	Post-Weaning			Finishing		
Source	DF	SS	F-Prob.	DF	SS	F-Prob.
Tag	128	13919	< 0.0001	49	8390	< 0.0001
Day	1	7297	< 0.0001	1	349	< 0.0001
Day x Tag	128	1456	< 0.0001	49	1060	< 0.0001
Day^2	1	628	< 0.0001	1	94	< 0.0001
Day^2 x Tag	128	425	< 0.0001	49	256	< 0.0001
Day^3	1	2	0.3542	1	133	0.1472
Day^3 x Tag	128	180	0.9963	49	220	0.3916
Error	8514	17275		3366	15109	
Total	8514	17275		3366	15109	
N. Lin. Err.	9029	41181		3565	25623	

DF - degrees of freedom; SS = type 1 sums of squares; F-Prob. = F probability.

N. Lin. Err. – non linear error term

For the young cattle, missing 3 or more days intake measurements per week, during a 10 week test period resulted in a significantly different estimate ($P<0.05$) of intake than the full data set (Table 6.3, Figure 6.5). However, missing 1 or 2 days per week was not significantly different from the full test period. Measuring intake 5 days per week (missing approximately 30% of the intake records) only reduced the Pearson correlation to 0.998. In the finishing data set only the Day6 scheme was significantly different from the full test period, missing between one and five days of intake measurements per week was not significantly different from measuring intake for the full test period. The correlation was reduced to 0.94 when measuring only 1 day per week in the finishing data set.

Table 6.3 T-values and significance for missing data schemes with the full data set

Scheme[a]	Post-Weaning	Finishing
Day1	0.64 ns	0.30 ns
Day2	1.23 ns	0.36 ns
Day3	2.35 *	0.47 ns
Day4	4.40***	0.72 ns
Day5	7.34***	1.13 ns
Day6	14.30***	2.54 *
1wk on/off	5.46***	0.28 ns
2wk on/off	1.43 ns	1.03 ns

[a] schemes as described in Table 6.1

ns = not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; $P < 0.001$.

Table 6.4 T-values and significance between the true estimate of daily feed intake and estimates when data is missing in weekly blocks from the start, middle, end and randomly

Weeks	Start		Middle		End		Random	
Missing	**PW**	**Finish**	**PW**	**Finish**	**PW**	**Finish**	**PW**	**Finish**
1	5.15 ***	0.41 ns	1.17 ns	0.05 ns	2.94 ***	0.25 ns	0.15 ns	0.04 ns
2	8.58 ***	0.40 ns	2.11 *	0.02 ns	5.39 ***	1.19 ns	0.06 ns	0.21 ns
3	13.54 ***	1.43 ns	4.07 ***	0.47 ns	8.77 ***	2.34 *	0.26 ns	0.32 ns
4	16.52 ***	3.12 **	5.56 ***	1.06 ns	13.70 ***	3.14 **	0.27 ns	0.14 ns
5	19.04 ***	5.13 ***	6.67 ***	1.23 ns	19.04 ***	5.13 ***	0.10 ns	0.46 ns
6	20.55 ***	4.71 ***	6.37 ***	1.58 ns	24.76 ***	4.68 ***	0.25 ns	0.90 ns
7	20.50 ***	5.45 ***	8.17 ***	1.22 ns	31.59 ***	3.34 ***	0.56 ns	1.01 ns
8	21.57 ***	4.77 ***	9.97 ***	2.93 **	34.30 ***	3.60 ***	0.30 ns	0.78 ns
9	26.42 ***	2.20 **	9.90 ***	6.18 ***	46.36 ***	3.66 ***	2.29 **	1.96 *

ns = not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; $P < 0.001$.

PW – Post-weaning data set; Finish. – Finishing data set.

In the post-weaning data set, missing the first week of the test period (10% of intake records) gave a significantly different average intake value from the full test period (Figure 6.6). For the finishing data set, missing up to 3 weeks at the beginning of the test period (30% of intake records) was not different from the full test. Thus, 70 days was required for growing cattle but only 50 days for finishing animals. Missing intake records weekly gave higher T-values than missing intake records daily for the same proportion of data missing

(Table 6.3, and Table 6.4). Missing data daily was more closely correlated with the full test period than missing the equivalent proportion weekly (Figure 6.5). For both daily and weekly missing intake schemes, T-values were significantly higher in the post-weaning data set than the finishing data set.

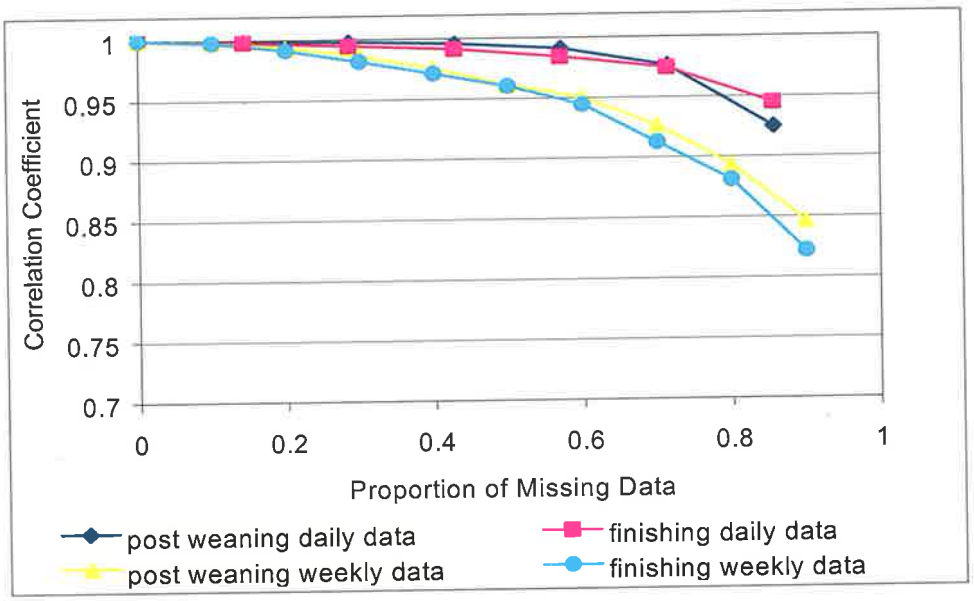


Figure 6.5 Correlation Coefficients for both data sets when data is missing daily and in weekly blocks

There was a significant difference between missing data at the start, middle, end and randomly throughout the test period (Figure 6.6, and Figure 6.7). For both data sets, missing intake at the start has a greater influence on the intake measurement compared with the end. Missing intake measurements at both the start and end of the test resulted in a less accurate measure of feed intake than having intake measurements missing randomly or in the middle of the intake test. Measuring feed intake in 2-week blocks over the 10-week test period was also not significantly different from measuring intake for the entire test period in both data sets.

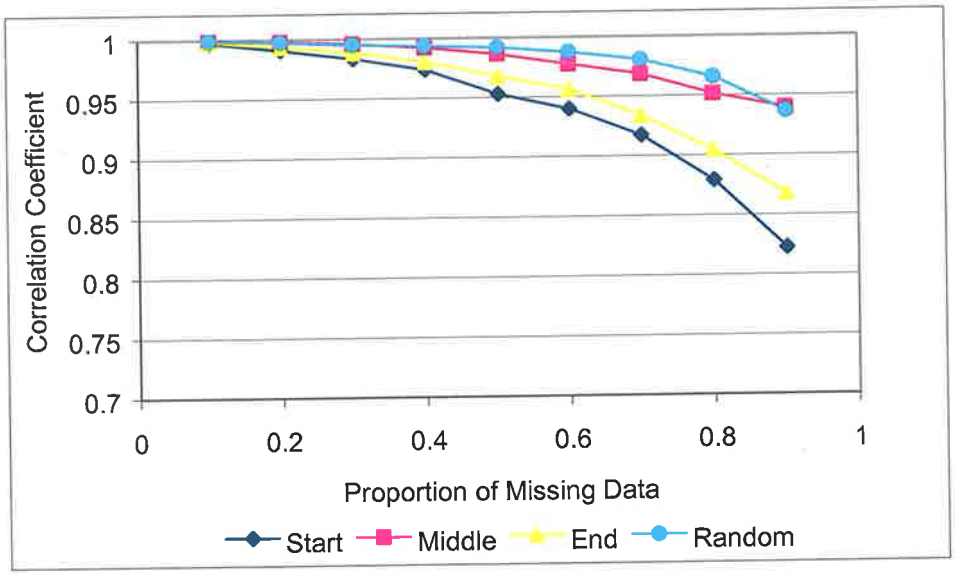


Figure 6.6 **Correlation coefficients for the 300d data set when data is missing in weekly blocks from the start, middle, end and randomly throughout the test period.**

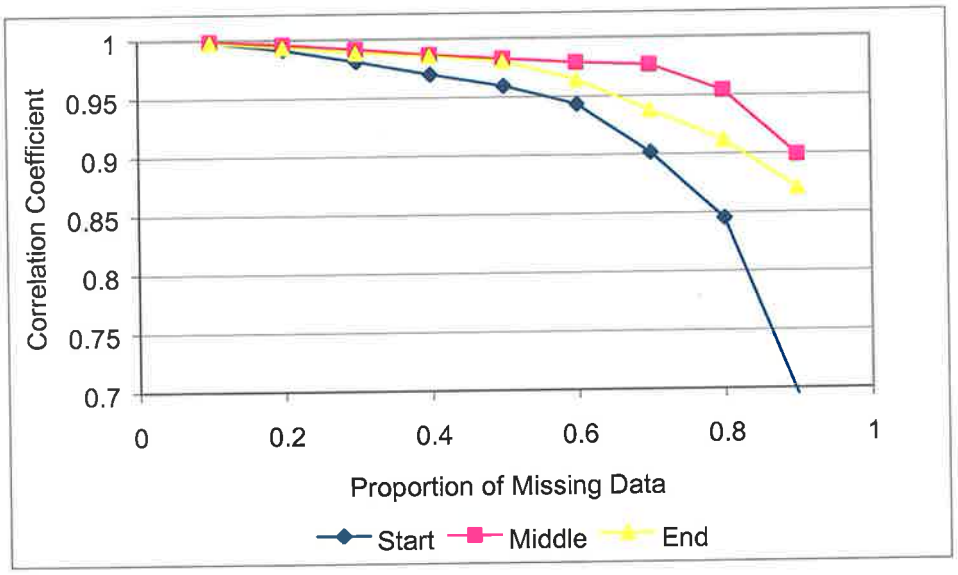


Figure 6.7 **Correlation coefficients for the 900d data set when data is missing in weekly blocks from the start, middle and end of the test period.**

6.3 Discussion

Determining the optimum duration for measuring feed efficiency requires criteria by which optimal length of measuring can be assessed. Archer et al. (1997) used variance components, heritabilities, phenotypic and genetic correlations in addition to the efficiency of selection of a shortened test period from standard feed efficiency test to assess the optimum length of feed intake test. The results indicated that a 35-day efficiency test was sufficient for measuring feed intake, however 70 days were required to get an accurate measure of growth rate, feed conversion, and net feed intake. The accuracy of measuring feed intake in this study was 93% for a 70-day test, using a repeatability of 0.35 and a heritability of 0.39 (Arthur et al. 2001).

When feed intake measurements are missing from 70 day feed intake test, it would appear that a linear model is a suitable model for estimating daily feed intake with little to be gained by using a non-linear function. These findings generally support the work of Eissen et al. (1999), who recommended the linear function over more complex polynomial and non-linear functions to estimate daily feed intake for growing pigs when feed intake data is missing. This was because the linear function could handle incidental as well as other types of missing feed intake periods better.

The results of this study suggest that the effect of missing data was greater in the post-weaning data set compared with the finishing data set. The differences between the two data sets were: (1) the age of animals, (2) the number of animals in the data, and (3) the environment which included slight differences in the feeding systems. The most likely reason for the difference in the effect of missing data would be the age of the animals. The effect of differences in the number of animals was tested by randomly dividing the post-weaning data set into three smaller data sets of approximately 50 animals each. The three smaller data sets were analysed separately for a direct comparison with the finishing data set. The results were virtually identical to the full post-weaning data set, indicating that the difference was due to the age of the animals and not due to differences in the number of animals in the data sets. The environmental differences appear to be small as temperature and humidity were shown to have no effect on feed intake in these data sets and the feed composition did not differ significantly. It appears from these results that losing feed intake records when animals are still growing has a larger impact on estimating daily feed intake

compared to missing intake records for the older animals that were at a later stage of maturity (and had probably reached their mature intake).

Generally, it was clear that missing weekly blocks of data in the middle or randomly deleting data gives a more accurate estimate of daily feed intake than missing the same proportion of data at the start or end of the test. This is most likely because there are measurements flanking the missing records. This supports Eissen et al. (1999) who found that deleting intake records from growing pigs in blocks had a greater impact on estimated daily feed intake than randomly deleting a similar number of feed intake records. Also, deleting records in weekly blocks had a larger impact on estimated daily feed intake than deleting records daily, or randomly deleting the same number of records. This clearly showed that missing data in a weekly block at the start and end of the test period had a greater impact on the between animal variance. Therefore, if there was a machinery breakdown in the middle of a 70 day test, 1 week of missing values for cattle that are still growing and 7 weeks in cattle that are closer to their mature intake could be missed without compromising the estimate of daily feed intake, provided the animals were maintained on the same feed ration.

The results generally agree with Archer et al. (1997) that feed intake can be measured accurately using much less data than the recommended 70 days of continuous feeding. However, to measure net feed intake an accurate measure of feed intake, growth and body weight are needed which requires a 70 day test, weighing cattle fortnightly (Archer et al. 1997; and Archer and Bergh 2000). If an animal can be maintained on the same diet and managed in the same way, NFI can be accurately calculated by recording weight every two weeks and intake on a two weekly basis over a 10-week period for a total of six weeks (i.e. 2 weeks on, 2 weeks off). Measuring feed intake every second week was found to be sufficient in growing pigs (Von Felde et al. 1996 and Eissen et al. 1999). However, for this to be successful, measurements should be recorded over the entire test period and not concentrated during the first or second half of the test period. This could lead to more efficient utilization of automated feeders as at least 2 groups of animals could be measured at the same time.

6.4 Conclusion

The current recommendation for performance tests for growth rate, feed conversion, and net feed intake for Australian beef cattle is 70 days after a 21-day pre-test adjustment period. If intake records are missing during the test period, using a linear function to represent average daily feed intake appears to give an accurate estimate. During the active growing phase, animals are more sensitive to missing feed intake records than are older animals. However, loss of feed intake data for a day or two, any time during the test period, or in weekly blocks in the middle of the test period has little effect on the accuracy of the feed intake data. Further feed intake experiments should be undertaken to investigate the potential to optimise the use of automatic feeders using these feeding strategies, to verify measuring intake for two weeks on and two weeks off, over 10 weeks. Also, it should be noted that the study herein concerns missing data due to failure to record intake. This implies that during the period of missed data the animals had access to the same diet and continued to eat. The results of this study might not necessarily be applied to the situation where the animals did not have access to feed during the period of missed data or where animals need to be shifted to different feed bunks and then back again.

It should be noted that the study herein concerns missing data due to failure to record intake. This implies that during the period of missed data the animals had access to the same diet and continued to eat. The results of this study might not necessarily be applied to the situation where the animals did not have access to feed during the period of missed data, where animals were either moved to different locations, or where cattle are mixed with unfamiliar animals. However, these calculations provide confidence that the estimates of feed intake obtained in the proceeding two chapters where feed intake and net feed intake have been estimated with incomplete data.

Chapter 7.

Relationships between feed intake, behaviour and production traits in cattle

7.1 Introduction

When mapping QTL and genes, a clear phenotypic measurement and information on the physiology and biology of the mapped trait are required. The biology of net feed intake in mice was examined in Chapter 4. The results from that chapter indicated that selection for divergent net feed intake in mice had produced a concomitant shift in activity levels, gastric emptying and body composition. The more efficient mice had a higher body fat percentage, lower activity levels and a slower rate of gastric emptying, with very little difference in growth or body weight. In Chapter 6, the accuracy of daily feed intake measurements was examined for beef cattle when feed intake values were missing. It was determined that the small amount of data that was missing in the gene mapping data set would not affect the accuracy of the feed intake and efficiency measurements. However, the underlying biology of feed intake and efficiency was not examined. It is important to gain a better understanding of net feed intake from the primary species of interest, cattle.

Feed intake is a major factor influencing body weight and growth in beef cattle. Although feed intake is controlled by rumino-reticular fill and physiological mechanisms, it can be modulated by management factors such as grouping strategy, feeding and housing facilities, and social interactions that occur throughout the day. Substantial research has been done in dairy cattle to optimise feeding behaviour to maximise milk yield (Dado and Allen 1994; Grant and Albright 2001; Olofsson 2000). Dado and Allen (1994) observed intense feeding behaviour generally occurred in higher producing cows that were older, consumed more feed, ate larger meals more quickly, ruminated longer and more efficiently, and drank more water than lower producing cows that were typically younger. The 'behaviours' that have been measured in dairy cattle that are known to affect productivity of cows in confinement and fed ad libitum are; number of daily meals, meal size, chew rate, total chewing time, time eating, ruminating time, ruminating bouts, competition for feed space, competition for water space, drinking session volume, number of drinking sessions, drinking time, and drinking rate.

Group feeding of cattle, for example in a feedlot, inevitably results in some degree of competition for feed. In this situation, cattle form dominance hierarchies particularly at the feed and water troughs. When there is competition for food and water, the dominant cows spend more time eating than cows of lower social rank, resulting in higher daily feed

intake (Olofsson 2000). The early work of Dickson et al. (1970) indicated that social dominance was strongly correlated with age, body size, and seniority in the herd in dairy cattle. Both social hierarchies and competition for feed, affect feeding behaviour, such that feed intake and water consumption can be reduced when cows are introduced to a new group whilst dominance is being established. Consequently, both group strategy and group feeding behaviour have the potential to impact on cow productivity, animal well-being, herd health, and farm profitability (Grant and Albright 2001). The correlation between dominance, competition for feed, and performance is most pronounced in situations where feeding space makes feed a defensible resource (Fraser 1995).

In beef cattle, temperament has been shown to influence not only animal performance but also meat quality (Tulloh 1961; Fordyce and Goddard 1984; Voisinet et al. 1997; Fordyce et al. 1988). The early work of Tulloh (1961) indicated a favourable relationship between growth rate and temperament in both heifers and steers grazed at pasture. The more recent findings of Voisinet et al. (1997) support this work, where cattle that were quiet and calm during handling, had greater average daily gains than cattle that became more agitated.

The aims of this chapter were to determine the relationships between feed intake, behaviour, and production traits in the Davies Gene Mapping Herd to gain a better understanding of feed intake and efficiency.

7.2 Materials & Methods

7.2.1 Traits Analysed

The traits analysed herein are described in detail in the animal resources and general methods, Chapter 3. The temperament traits analysed were docility score (DS) and flight distance (FD, Fisher et al. 2000). Total time spent feeding (FT), feeding rate (FR), the number of feeding sessions per day (FS), average length of each feeding session length (AS), and the average meal size (MS) were included in the eating behaviour trait group. Feed intake traits were those that were recorded whilst the animal was in the feedlot and included net feed intake (NFI), daily feed intake (DFI), average daily gain (ADG), and mid-weight (MWt). Production traits were measured immediately following the feed efficiency test, when the animals were slaughtered (Table 7.1). The Warner Bratzler peak force was recorded on each animal, 1, 5, 12, and 26 days after slaughter. The natural logarithm of the peak force measurement was then regressed against the day of ageing, for each animal. The intercept became the initial peak force (ln kg) and the slope was the ageing rate (ln kg/day). It should be noted that the temperament traits, were measured when the animals were 8 months old for the 1998 drop and 20 months old for the 1996 and 1997 drops. All other measurements were taken when the animals were on average 30 months old and had been subjected to a significant amount of human handling in the feedlot.

Table 7.1 List of trait groups, abbreviations and definitions of the traits used in throughout this chapter

Trait Groups	Definitions	Animals
Temperament		
DS	Docility Score	362
FD	Flight Distance (m)	357
Eating Behaviour		
FS	Number of times the animal fed per day	314
FT	Total amount of time (secs) spent feeding per day	314
AS	Average length (secs) of each feeding session	314
FR	The rate at which feed was consumed (kg/sec)	314
MS	Average size (kg) of each meal eaten during the day	314
WAT	Weighted average of time spent feeding	314
Production		
ADG	Average daily gain on feed test (kg/day)	324
MWt	Average weight during feed test (kg)	324
HSCW	Hot standard carcass weight (kg)	356
P8	Subcutaneous fat depth (mm) at the P8 rump site	356
Marb	Ausmeat marbling score	355
IMF	Intra-Muscular Fat %	355
EMA	Eye Muscle Area (cm^2)	355
Meat	Total weight of meat (kg)	329
Fat	Total fat weight (kg)	329
Bone	Total weight of bones (kg)	329
Offal	Weight of visceral organs (kg)	323
PF_{LD}	Peak force required to shear the *longissimus dorsi* (ln kg/day)	355
Age_{LD}	Aging rate of *longissimus dorsi* (ln kg/day)	355
PF_{ST}	Peak force required to shear the *semitendinosis* (ln kg/day)	351
Age_{ST}	Aging rate of *semitemdinosis* (ln kg/day)	351
Intake		
DFI	Total amount of feed consumed each day (kg/day)	323
NFI	Feed intake independent of body weight and growth (kg/day)	319
GE	Gross Efficiency (kg gain/kg feed)	319
MR	Maintenance Requirements (kg feed/kg mid-weight)	319

7.2.2 Statistical Analysis

Residual correlations were calculated using Proc Corr (SAS 1998) between eating behaviour, intake, and temperament and production traits, using the residuals from a linear model adjusting for both breed and cohort. To confirm these phenotypic correlations, NFI was regressed on each of the intake traits. Cohort, breed, and sire were fitted as fixed effects. Before any further analyses were performed, daily feed intake was adjusted for the differences between cohorts (a combination of year of birth and sex) by using the residuals from a model that included cohort only. Feed test differences between the cohorts include different geographical locations, climates, seasons, years and diets as detailed in Chapter 3. The stepwise regression technique (entry < 0.2 and cut-off P<0.01) of the REG procedure (SAS, 1998) was used to determine the animal traits that were influencing daily feed intake. The traits in the initial model for daily feed intake included all production and temperament traits. The final model from the regression step was then run using the general linear model (GLM) procedure to allow the fixed effects of breed and sire to be fitted. NFI was also modelled using stepwise regression followed by the GLM procedure of SAS (1998). However, average daily gain and mid-weight were not included in the model as these traits were used to calculated NFI. The hot standard carcass weight was also removed from the model because it was very highly correlated with mid-weight.

To determine if differences in feeding patterns were influencing feed efficiency, the amount of food eaten during the day light was compared with that consumed when it was dark. This separation of daily feed intake was estimated for the 98' drop heifers and steers only. Since the amount of daylight was not the same when the heifers and steers were measured, because they were fed at different times of the year the amounts of feed consumed during daylight and darkness was expressed in kg of feed consumed per hour. Also, a weighted average of time was calculated to determine if the cattle had developed a feeding pattern or dominance hierarchy within each pen. The weighted average of time was calculated as

$$WAT = \frac{\sum T_e \times T_f}{T_t}$$

Equation 7.1

Where WAT is the weighted average of time, Te is the time of day at which the animal entered the feeder, Tf is the time spent feeding and Tt is the total time spent feeding on that day.

7.3 Results

7.3.1 Correlations

There was a strong positive phenotypic correlation between net feed intake and daily feed intake (r=0.81, Table 7.2) and maintenance requirements (r=0.93, Table 7.4). By definition, average daily gain and mid-weight were not correlated with net feed intake. Of the feeding behaviour traits, the amount of time spent feeding (r=0.37), eating rate (r=0.32) and the number of feeding sessions per day (r=0.30) were all moderately and positively correlated with net feed intake. More efficient cattle spent less time feeding, ate slower and had fewer visits to the feeding unit. The temperament traits, FD and DS were not correlated with NFI. Interestingly, the size of each meal showed no relationship with NFI (r=0.02) whilst, the relationship between average length of each session and NFI (r=-0.13) was very low and negative.

Table 7.2 Phenotypic (residual) correlations between feed intake and behavioural traits

	NFI	DFI	FR	FS	FT	AS	MS	FD
DFI	**0.90**							
FR	***0.34***	**0.43**						
FS	***0.26***	***0.23***	0.12					
FT	**0.40**	**0.40**	**-0.54**	0.06				
AS	-0.09	0.06	***-0.36***	**-0.50**	**0.41**			
MS	0.06	0.14	0.15	**-0.55**	0.04	***0.77***		
FD	-0.05	-0.14	-0.04	0.00	-0.06	-0.01	-0.06	
DS	0.05	0.09	-0.04	0.02	0.09	0.11	0.11	-0.42

A summary of abbreviations can be found in Table 1.

0.0-0.19 very low; ***0.20-0.39 low***; **0.40-0.59 moderate; *0.6-0.79 high;* 0.8-1.0 very high.**

Table 7.3 further confirms the residual correlations where animals with lower net feed intakes (more efficient) ate less, and more slowly, they spent less total time feeding and

had fewer eating sessions. To decrease net feed intake by 10% would require a reduction in; daily feed intake by approximately 1 kg/day, eating rate 0.6 g/second, total time spent feeding 1 minute, and one less feeding session per day. The length of each feeding session would also be increased by 35 seconds with no significant influence on the size of each meal, flight distance or docility score.

Table 7.3 Table of F probabilities, simple regression parameters and the % change in each trait with a 10% increase in NFI

Trait	F Prob.	Parameters	% change
DFI	***	0.74	7.4
FR	***	1.11	26.4
FT	***	0.03	1.0
FS	***	0.16	9.6
AS	***	-0.03	3.5
MS	n.s.		
FD	n.s.		
DS	n.s.		

A summary of abbreviations can be found in Table 1.

*** P<0.001; ** P<0.01; * P<0.05; n.s. not significant.

The residual correlations between eating behaviour traits and maintenance requirements were of the same magnitude and direction as for net feed intake (Table 7.4 and Table 7.2). Surprisingly, there was no relationship between gross efficiency and daily feed intake (r=0.01 Table 7.4), indicating that there was more variation in average daily gain (after adjusting for differences between breed and cohorts) than daily feed intake. In addition, heavier animals ate significantly more and faster than lighter animals. The heavier animals were more 'flighty' than the lighter animals.

Table 7.4 Phenotypic (residual) correlations between feed intake and behavioural traits, and carcass and growth traits

	NFI	DFI	FR	FS	FT	AS	MS	FD	DS
ADG	0.07	***0.32***	0.12	0.07	0.17	0.04	0.06	-0.08	0.01
MWT	0.16	**0.62**	**0.49**	0.00	0.06	0.05	***0.30***	***-0.22***	0.17
GE	***-0.20***	-0.01	-0.02	-0.01	0.04	0.07	0.03	-0.01	-0.05
MR	**0.95**	**0.83**	***0.22***	***0.27***	**0.40**	-0.12	-0.01	0.01	0.00
HSCW	0.18	**0.48**	**0.47**	0.03	0.04	0.03	***0.25***	-0.17	0.19
P8	0.00	0.12	0.05	-0.02	-0.03	0.00	0.01	-0.16	0.08
Marb	0.06	0.09	-0.15	0.07	0.04	-0.03	-0.10	0.08	-0.03
IMF	0.05	0.06	***-0.20***	0.07	0.04	-0.07	-0.14	0.00	-0.03
EMA	0.08	***0.38***	**0.41**	-0.01	-0.04	0.01	***0.21***	-0.09	0.11
Meat%	-0.03	***0.20***	***0.28***	0.01	-0.07	-0.02	0.09	-0.03	0.09
Fat%	0.07	0.18	***-0.23***	0.00	0.14	0.07	-0.02	-0.06	0.01
Bone%	-0.07	***-0.34***	-0.16	-0.02	-0.12	-0.10	-0.15	0.16	***-0.21***
Offal%	0.17	***0.27***	***-0.25***	-0.07	-0.03	-0.04	-0.16	0.04	-0.09
Meat	0.15	**0.52**	**0.45**	0.03	0.03	0.02	***0.21***	-0.18	0.18
Fat	0.15	***0.37***	0.11	0.06	0.18	0.08	0.13	-0.19	0.14
Bone	0.16	**0.50**	**0.47**	0.02	0.01	-0.02	***0.21***	-0.17	0.13
Offal	0.07	**0.40**	***0.31***	-0.03	0.04	0.04	0.19	-0.19	0.10
PF_{LD}	***0.21***	0.16	0.15	0.03	0.06	0.06	0.13	-0.06	0.03
Age_{LD}	0.04	-0.06	-0.04	0.10	-0.01	-0.07	-0.10	-0.06	0.06
PF_{ST}	0.02	-0.19	-0.20	0.02	0.06	0.03	-0.06	-0.07	-0.06
Age_{ST}	-0.02	-0.02	0.10	0.08	-0.06	-0.08	-0.08	-0.02	-0.02

A summary of abbreviations can be found in Table 1.

0.0-0.19 very low; ***0.20-0.39 low***; **0.40-0.59 moderate;** ***0.6-0.79 high;*** **0.8-1.0 very high.**

7.3.2 Traits describing feed intake and efficiency

A multivariate regression analysis was performed to determine the animal production and behavioural traits that best describe daily feed intake. The final model describing daily feed intake (adjusted for differences between cohorts) included average daily gain, and body composition traits offal, fat, meat and bone weights and the peak force of the *longissimus dorsi* (Table 7.5). Both sire and breed were not significant when these traits

were included in the final model. This indicates that body weight component traits explain the differences between breeds and sires. The final model described 35% of the variation observed in daily feed intake. For every extra kilogram of feed consumed, the cattle were 39 grams fatter, contained 13 grams more meat, and 12 grams more offal and had a 1.5 kg greater peak force of the *longissimus dorsi* muscle.

Table 7.5 ANOVA for animal production traits explaining approximately 35% of the variation in daily feed intake adjusted for differences between cohorts

Source	DF	Type III S.S.	Pr > F	Parameters
Intercept		317.6	***	-12.10
ADG	1	64.1	***	0.088
Offal	1	67.1	***	0.016
Fat	1	42.0	***	0.039
Meat	1	106.9	***	0.022
PF_{LD}	1	36.7	***	1.211
Residual	280	816.2		
Total	285	1133		

A summary of abbreviations can be found in Table 1.

*** P<0.001; ** P<0.01; * P<0.05; n.s. not significant.

The peak force of the *longissimus dorsi*, meat, fat and bone weights, and the eye muscle area explained only 17% of the variation in net feed intake (Table 7.6) The final model describing net feed intake in Table 7.6 is similar to the model describing daily feed intake (Table 7.5), in that meat, and fat weights, and the peak force of the *longissimus dorsi* muscle were all included in the model. In contrast to the model in Table 7.6, eye muscle area was included in the model describing net feed intake. Animals with higher net feed intake, tended to be fatter, contain larger or heavier bones, have larger eye muscle area but less kilograms of meat and a higher peak force of the LD muscle. Also, animals that had higher net feed intake spent significantly more time feeding but the number of feeding sessions throughout the day was not different.

Table 7.6 ANOVA for production traits accounting for 17% of the variation of in net feed intake

Source	DF	Type III S.S.	Pr > F	Parameters
Intercept			***	-6.31
PF_{LD}	1	94.4	***	2.657
Meat	1	82.4	***	-0.027
EMA	1	47.3	***	0.051
Bone	1	38.9	***	0.057
Fat	1	13.0	*	0.023
Residual	280	727.1		
Total	285	1003.1		

A summary of abbreviations can be found in Table 1.

*** P<0.001; ** P<0.01; * P<0.05; n.s. not significant.

Table 7.7 ANOVA for feeding behaviour traits explaining 15% of the variation in net feed intake

Source	DF	Type III S.S.	Pr > F	Parameters
Intercept			***	-3.00
FS	1	21.6	**	0.08
FR	1	21.2	**	0.40
AS	1	119.1	***	0.09
Residual	306	883.1		
Total	309	1267		

A summary of abbreviations can be found in Table 1.

*** P<0.001; ** P<0.01; * P<0.05; n.s. not significant.

7.3.3 Feeding Behaviour

There was no significant difference between the amounts of feed consumed during dark versus daylight hours. There was a small negative correlation (r=-0.30) between the amounts of feed consumed during the night versus day, it would seem that animals eating more during the day ate less at night and had higher net feed intakes. The cattle ate 8% faster, spent 50% more time feeding and had 48% more feeding sessions during the day but did not eat more food (Table 7.8). Consequently, the average session length was 23%

lower and the average meal size was 30% lower during the day. The correlation between net feed intake and the amount of feed consumed during the day was high and positive (r= 0.68) compared with a moderate negative correlation with the amount of feed consumed at night (r= -0.38).

Table 7.8 Day versus night feed intake means and level of significance

Trait	Day	Night	Significance	% Difference
Intake (kg/hour)	0.71 ± 0.02	0.67 ± 0.02	n.s.	6
FR	2.14 ± 0.03	1.99 ± 0.03	**	8
FS	0.31 ± 0.01	0.21 ± 0.01	***	48
FT	9.37 ± 0.16	6.23 ± 0.16	***	50
AS	24.4 ± 0.86	31.5 ± 0.86	***	-23
MS	2.29 ± 0.09	3.18 ± 0.09	***	-30

*** P<0.001; ** P<0.01; * P>0.05; n.s. not significant.

% Difference, the percentage difference of the traits during the day compared with the night.

In both the 1998-drop heifers and steers, there was a significant effect of tag (animal) nested within pen for both the weighted average of time (Table 7.9) and the time at which the animal first entered the feed station. In both cases the interaction between date and tag nested within pen was not significant. This indicates that within each pen there was some form of hierarchy that had been established and that this hierarchy did not change significantly between days in both cohorts. The repeatability of this weighted average of time and the minimum rank (first visit to the feeder) during the 70-day test period was 28% for the 1998-drop heifers and 38% for the 1998-drop steers. Although, there was no information for the first three weeks while the animals were adjusting to their new environment, the repeatability of WAT during the first week of the test period was much lower 13% and 16%.

Table 7.9 Weighted average of time

Source	98 Heifers			98 Steers		
	DF	S.S.	Pr>F	DF	S.S.	Pr>F
Date	1	0.121	*	1	0.635	***
Animal nested within pen	58	2.386	**	62	4.171	***
Date*Animal nested within pen	58	2.055	n.s.	62	1.865	n.s.
Residual	1977	56.138		2241	80.30	
Total	2094	60.70		2366	86.97	

*** P<0.001; ** P<0.01; * P<0.05; n.s. not significant; S.S. – type 3 sums of squares; Date is the measurement date.

7.4 Discussion

7.4.1 Traits describing feed intake and efficiency

Net feed intake was highly correlated (r_p = 0.84) with daily feed intake but not with average daily gain or mid-weight (r_p=0.00). The low correlation between net feed intake and average daily gain, and mid-weight was by definition because they are used to calculate net feed intake. These results are supported by the work of Herd and Bishop (2000) and Arthur et al. (2001 b,c) in cattle tested immediately post-weaning. The phenotypic correlation between net feed intake and daily feed intake in these studies was 0.70 and 0.60 respectively. In pigs (de Haer et al. 1993), poultry (Luiting and Urff 1991a & b) and mice (Hughes 2003) net feed intake is also highly correlated with daily feed intake but not with average daily gain or body weight. The studies of Herd and Bishop (2000) and Arthur et al. (2001b) both calculated genetic correlations for daily feed intake (r_g=0.69, 0.79), average daily gain (r_g=0.09, 0.10) and mid-weight (r_g=0.22, 0.32) with net feed intake. The heritability of net feed intake in beef cattle is moderate with published estimates ranging from 0.17-0.31 (chapter 2). From these findings it has been concluded that selection for improved net feed intake should reduce the cost of feeding cattle without compromising growth performance (Arthur et al. 2001).

Thirty five percent of the variation in daily feed intake among the beef cattle in this study was related to the variation in average daily gain and body weight. Average daily gain accounted for 11% while component traits that make up body weight (meat, fat, bone and offal weight) accounted for 20% of the variation. For every extra kilogram that an animal

ate, it grew 0.088 kg/day faster, its offal, fat, and meat was 16, 39, and 22 grams heavier respectively. Surprisingly, the peak force of the LD muscle also accounted for a small but significant proportion of the variation in intake. Those animals with a higher daily feed intake had tougher LD muscles. The difference between the three sires and between the Limousin and Jersey backcrosses in net and daily feed intake was explained by the differences in growth rate and body composition because when the fixed effects of sire and breed were fitted to the multiple regression model, they were not significant.

Only 17% of the variation in net feed intake could be explained by the combined differences in the peak force of the LD muscle, and body composition traits, meat, fat, and bone weight and the eye muscle area. Animals with higher net feed intake that were less efficient had higher peak forces, more fat, heavier or larger bones and larger eye muscle areas. However, they produced significantly less kilograms of meat. This would suggest that cattle that are more efficient (have low net feed intake values) had a greater retail beef yield (meat %). This supports the mouse study in chapter 4 where the low NFI selection line had significantly more (7%) more carcass protein than the high NFI line.

It was interesting that fat weight remained in the model describing net feed intake given the phenotypic correlation was very low (r=0.14). Richardson et al. (2001) found an improvement in net feed intake was accompanied by small changes in body composition towards greater lean mass and less fat in the carcass after one generation of selection for divergent post-weaning net feed intake in cattle. There was also a genetic association between body composition and net feed intake, with fatness being associated with higher residual feed intake. However, the differences between the lines in body composition accounted for less than five percent of the variation in residual feed intake. In the same group of animals, McDonagh et al. (2001) found slightly less sub-cutaneous fat over the rib and rump, but no difference in the intra-muscular fat of the LD muscle.

The same trend has been observed in pigs where body composition is highly correlated with net feed intake (r_p = 0.33, r_g = 0.67, Johnson et al. 1999c). In contrast, it has been shown in mice (Hughes 2003 and chapter 4) and poultry (Luiting 1990) that selection for improved net feed intake at a young age results in animals that are fatter. Fat is considered to be energetically more expensive to deposit but less expensive to maintain. It could be

argued then that it would make more sense energetically for those animals with low net feed intake to be leaner immediately post-weaning while they are still growing, but to be fatter at maturity. This could explain the differences observed between the mouse and cattle selection lines. Mice, which are a fast maturing species may have been measured at a later stage of development than both the cattle and the pigs and were therefore fatter. In contrast, the cattle were measured after a post-weaning test but almost certainly not have been through the "fattening" stage of growth. However, this is an unlikely explanation because the cattle in this study were mature and the trends observed in this study were similar to those observed in young beef cattle by Richardson et al. (2001).

The trend towards a greater lean content in low net feed intake animals agrees with the negative genetic correlation (r_g=-0.43) between post-weaning net feed intake and estimated lean carcass content reported by Herd and Bishop (2000) for British Hereford cattle. It has been suggested (Richardson et al. 2001) that this could be due to differences in protein turnover. It has been shown that genetic selection for growth and other traits is accompanied by variation in protein metabolism in domestic animals (reviewed by Oddy, 1999). In a separate study on the first generation of selection for divergent net feed intake McDonagh et al. (2001) found steers from the low, more efficient line had lower rates of myofibre disassembly and higher levels of calpastatin. This suggests that the rate of protein breakdown in the low net feed intake steers is lower. Associated with this increase in calpastatin and decrease in myofibril fragmentation is the potential for a negative influence on meat tenderness. McDonagh et al. (2001) reported no difference between the selection lines in the peak force of the *longissimus dorsi*. Whilst the protein synthesis studies in chapter 4 did not reveal any significant differences between lines of mice selected for divergent net feed intake, it would be worthwhile investigating protein turnover by measuring both synthesis and breakdown in cattle with extreme net feed intakes.

In this study, it was surprising that the peak force of the *longissimus dorsi* explained a significant proportion of the variation in both feed intake and net feed intake. Animals with higher net feed intakes that were considered less efficient, had higher peak force measurements. The peak or shear force of meat is related to the pressure required in the tasters mouth to shear the beef sample and is used as an indicator of meat toughness, the higher the peak force, the tougher the meat sample. In contrast, McDonagh et al. (2001)

did not reveal a difference between the selection lines in peak force. There are three major differences between the current study and that of McDonagh et al. (2001); 1) the animals in the current study were mature in terms of feed intake, at approximately 30 months of age compared with animals post-weaning, 2) the animals in this study represented animals from a reciprocal back-cross between Jersey and Limousin cattle whereas the animals in the above study are from the first generation of selection for divergent net feed intake using Angus, Shorthorn, Hereford and Poll Hereford cattle and 3) the current study included both heifers and steers in the analysis, where as the study by McDonagh et al. (2001) was conducted on steers only.

Peak force measurements are believed to reflect both the myofibrillar and connective tissue components of muscle (Harris and Shorthose 1988). The actual measurement of peak force is primarily associated with the myofibrillar component of meat, while the difference between peak force and the initial yield force is believed to reflect the connective tissue features (Harper 1999). There are many factors that influence the toughness of muscle fibres. These include; variation in the inextricable linkage between muscle fibre type, connective tissue content and cross-linking, proteolytic activity, glycogen content and the potential rate of glycogenolysis (Harper 1999). It is possible that differences exist in the types of muscle fibres in the LD. In a review of skeletal muscle fibres as factors for meat quality Karlsson et al. (2000) concluded that tenderness may be related to the oxidative capacity and fat content of muscle fibres. This was based on the work of Essén-Gustavsson and Fjelkner-Modig (1985) who found that Hampshire pigs contained muscles with a higher oxidative and lower glycolytic capacity, were more tender than Yorkshire pigs. Also in humans, Helge et al. (1999) found that body fat was inversely correlated to the proportion of type I (slow, oxidative fibres). Therefore, cattle that were less efficient and had tougher meat may have had LD muscles with lower oxidative and higher glycolytic capacity. However, Cameron et al. (1999) did not find any significant differences in pigs selected for and against lean growth. Further studies investigating differences in protein turnover, and muscle biology are required on animals that are divergent in net feed intake.

7.4.2 Feeding behaviour

The efficiency of feed utilisation has a number of variable components, including: feed intake; feeding rate; efficiency of nutrient digestibility; and maintenance requirements. In

this study the correlation between net feed intake and maintenance requirements (DFI/MWt) was very high and positive (r_p=0.92). Therefore, cattle with higher net feed intake also have higher maintenance requirements. This was not surprising since the animals were relatively old when they entered the feed test. Hence, variation in average daily gain was low so most of the variation in daily feed intake reflected variation in maintenance requirement.

The feeding rate of cattle in this experiment was moderately correlated with mid-weight, hot carcass weight and daily feed intake (r_p=0.49, 47 and 0.50 respectively). These results are similar to the findings of Frisch and Vercoe (1969) who measured the feeding rate of three breeds of cattle; Brahmans, Africandes and a cross between Hereford and Shorthorn, by weighing the amount of feed consumed during set intervals. They reported that the feeding rate of animals was highly repeatable and significantly correlated with voluntary feed intake (r_p=0.61; P>0.01) and live-weight gain (r_p=0.63; P<0.01). Furthermore, Frisch and Vercoe (1969) reported that feeding rate was moderately to highly correlated with fasting metabolism (an indicator of maintenance requirements) and daily feed intake. The correlation between estimated maintenance requirements and feeding rate in this study was low but positive.

The early work of Forbes et al. 1972 concluded that rate of eating is negatively related to the mean or whole tract retention time and positively related to voluntary feed intake when considered across a range of diets. The conclusion that feeding rate plays a role in the energetic efficiency of the animal is also similar to the findings of this study where the correlation between net feed intake and feeding rate was moderate and positive (r=0.32). Furthermore, the regression analysis revealed that animals with 10% lower net feed intakes ate 57.5% slower than those with the average net feed intake. Therefore, the rate of feeding could provide a valuable index of rumen metabolism.

Both the number of feed sessions per day and the amount of time feeding significantly influenced net feed intake, whereby the more efficient animals had less eating sessions and spent less time per day feeding. This supports the study of de Haer et al. (1993) who found that the variation in feed intake activity of pigs, described by the number of visits per day and daily eating time, accounted for 44% of the variation in net feed intake (de Haer et al.

1993). The more efficient pigs spent less time eating and visited the feed hopper more widely spread over the day. The relationship between NFI and eating behaviour could also be explained by neuropeptides such as serotonin and dopamine, which could act as possible candidate genes for NFI. Serotonin or 5-hydroxytryptamie (5-HT) is a monoamine neurotransmitter, which is believed to alter food intake in rodents, by altering the quantity of food intake, pattern of eating and appetite for specific nutrients, whilst dopamine D2 receptors are associated with increased meal size and decreased meal frequency (in a review by Clifton 2000).

The ability of ruminants to mechanically reduce food particle size can be a limiting factor for feed intake (Bosch et al. 1992). In addition, the energy required to chew and ruminate can account for a significant proportion of the total energy requirement of the animal. Susenbeth et al. (1998) found that the amount of energy required for eating and ruminating was 30% of the metabolisable energy intake in low-quality roughages and 10% in high quality roughages, which leads to a significant reduction in the proportion of energy available for maintenance and production. It is possible then, that one of the factors contributing to reduced net feed intake and maintenance requirements could be the increased efficiency of an animal to 1) reduce the particle size of feed through chewing and 2) ruminate. Further studies examining eating and ruminating rates, and the energy requirements associated with these processes in cattle, with varying net feed intakes would be need to be done to confirm this.

It has been well documented that when there is competition for feed, as in a feedlot situation animal's form dominance hierarchies at both feed and water troughs. Although this study was not designed to detect differences in feeding or drinking behaviour, feeder usage was monitored daily and never reached above 85% usage per day to ensure that effects of competition for feed were minimal, certain feeding traits have been analysed. Interestingly, there was no difference between the amount of feed consumed during daylight and night hours. Also, animals that consumed more feed during the night were more efficient. This begs the question, "did these animals eat at night because a hierarchy had been formed within the pen or do more efficient animals eat more during the night as an energetic advantage, when the temperature is cool"?

A weighted average of the time the animals entered and spent in the feeders was calculated as a means of determining whether a hierarchy had been formed. It would appear in this study that the animals had formed a dominance hierarchy with in each pen. Those animals that had higher weighted values tended to eat more at night and have lower net feed intakes. There did not appear to be any relationship between the weight values and growth, body weight or breed.

7.5 Conclusion

Since the cost of feed is the single largest cost to most livestock producers, improvements in feed efficiency that do not reduce product quality will increase profitability. This chapter has dealt with modeling net and daily feed intake with various production, behaviour and carcass traits. It would appear that animals that are more efficient (low net feed intake) have lower maintenance requirements, consume less feed per day, eat at a slower rate, require less feeding sessions per day and spend less time each day feeding, with no difference in the temperament traits docility score and flight distance. Cattle that require less feeding sessions per day could be more efficient due to neuropeptides such as serotonin and dopamine or because they spend less time chewing and ruminating which require energy.

There were several differences between the findings of the current study and those performed in cattle selected for divergent net feed intake. The major discrepancy was the indicators of protein turnover. McDonagh et al. (2001) found that steers from the first generation of selection for low net feed intake had the potential to have tougher meat due to lower levels of myofibre disassembly and higher levels of calpastatin. In contrast this study found the peak force of the *longissimus dorsi* of cattle with higher NFI was greater than cattle with lower NFI indicating that cattle with higher NFI could have tougher meat. Further studies quantifying the effect of low NFI on meat tenderness, and fertility (not discussed in this thesis) would be of interest before commercial selection of NFI is adopted.

Chapter 8.

Mapping quantitative trait loci for feed efficiency in cattle

8.1 Introduction

In Chapters 4 and 5, the underlying biology and QTL for net feed intake (NFI) were examined in mice. The results from these chapters were interesting with nine QTL for NFI and a large number of candidate genes located throughout the mouse genome. In these chapters, mice were used as a model species for cattle. Chapters 6 and 7 defined NFI and the associated traits in cattle. The results from these chapters suggested that although there were missing values, the estimate of feed intake was accurate, and consequently, NFI could be adequately mapped in cattle. To reduce the number of candidate genes to be confirmed and to locate the QTL in cattle, a mapping study in the species of interest (cattle) was undertaken.

The cost of feed is the single largest expense for any livestock producer and in most beef cattle enterprises the breeding herd accounts for 65-85% of the total feed requirements (Ferrell and Jenkins 1984, Montano-Bermudez et al. 1990). Of this 65-85%, 65-75% is used for maintenance alone (Figure 8.1). The reason the breeding herd requires such a large proportion of the total energy requirements is because cattle are a large, slowly maturing species with a low annual reproductive rate. Furthermore, only a single product is returned (meat). The breeding cow requires a proportionately higher level of raw 'inputs' to maintain itself than is required to produce the actual 'product', represented by the cow's offspring (Pitchford 2002). Therefore, improvements in the maintenance efficiency of breeding cows should result in increased total meat production for a given amount of feed.

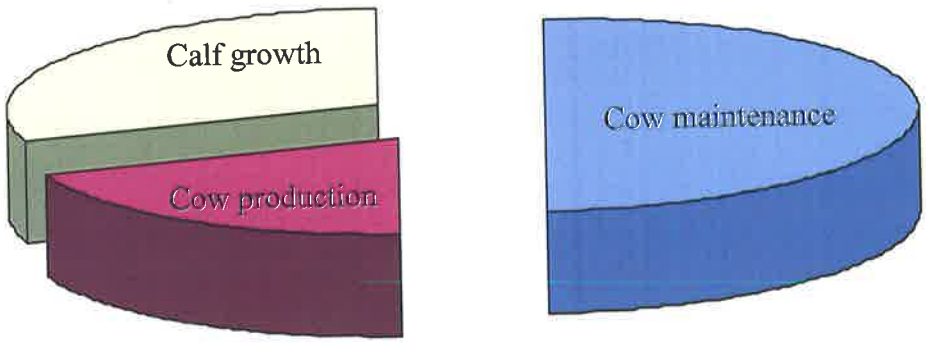


Figure 8.1 Feed requirements in an average production system

Archer et al. (1999) have shown that selection for NFI provides an opportunity to significantly reduce feed costs in livestock breeding programs. The current estimate of the measurement of NFI in Australia is approximately $500 ($300 test plus $200 feed) per animal, which is clearly prohibitively expensive. A cheaper alternative with the potential to significantly reduce generation interval would be to use a DNA test for markers of genes affecting NFI.

The location of the genes influencing NFI remains the current limitation of a DNA based test for NFI. The aim of this chapter was to locate regions in the cattle genome that contain genes affecting NFI and associated traits.

8.2 Materials & Methods

8.2.1 Experimental design and phenotyping

The cattle used in this experiment are described in Chapter 3 on the animal resources and general methods. Briefly, cattle from all six cohorts were placed on feed intake tests. The amount of feed consumed each day was recorded electronically for each animal. Feed intake data was processed by calculating least-squares means for each animal over the test period. Day was included in the model to allow for weather, personnel, time of feeding, and any other factors that would affect the intake of all cattle. Average daily gain was calculated as the regression coefficient for weight against day of test. Most traits were normally distributed with the exception of number of eating sessions and time spent eating. These traits were transformed by taking the natural logarithm. An analysis of variance was performed to evaluate the differences between the fixed effects of cohort, breed and sire, using Proc GLM (SAS 1998).

8.2.2 Genotyping

Approximately 150 (Table B.1, Appendix B) micro-satellite markers were genotyped across all autosomes (sex chromosomes not genotyped) using radioactive PAGE for each animal. Each gel was independently scored twice. There were 3-9 markers typed per chromosome for all 29 autosomes, at approximately 20 cM intervals. All genotyping was done by AgResearch, New Zealand.

Micro- satellite markers were genotyped for 366 calves that survived to weaning (77 1996-drop, 153 1997-drop, and 136 1998-drop). In the half-sib design utilised, sires were all classified as being "AB". Thus, progeny would either inherit an "A" or "B" from the sire and another allele from the dam. Consequently, the genotypes in the progeny were AA, AB, BB, AC, or BC where "C" = any other allele. If progeny were AB, then they were not informative for the analysis because it is not clear which allele they received from the sire (A or B). The *all* function of CRI-MAP (Green et al. 1990) was used to construct the marker linkage maps as described in Chapter 3. The final linkage maps were compared with the MARC97 bovine genome linkage maps for conserved order and distance (Bovine ArkDB; http://texas.thearkdb.org/browser?species=cow; accessed 20th January 2002). The MARC97 marker locations were used for the linkage analysis.

8.2.3 QTL Analysis

Genotype probabilities were calculated using "QTL Express" (Seaton et al. 2002; http://qtl.cap.ed.ac.uk; accessed November 2002) for every 1cM interval on each chromosome. Animals were assigned a value of either 0 (which represents an A allele) or 1 (which represents a B allele) or 0.5 if uninformative. The genotype probability was then calculated between 0.5 and 1, depending on the level of confidence. Phenotypes were regressed against the genotype probabilities for every chromosome. Cohort and breed of dam were included as factors in the model and the regression was nested within sire. Additional models that included two QTL or QTL by breed of dam interactions were also tested for a number of traits.

8.2.4 Threshold values

Experiment-wise threshold values were calculated according to Lander and Kruglyak (1995). Suggestive linkage was defined as $P < 0.05$ for a genome-wide test, where one false positive was expected in 20 genome scans. To satisfy this criterion an F statistic of greater than 7.77 was required for tests including three sires. Significant linkage was defined as $P<0.001$ for a genome-wide test, where one false positive was expected in 1000 genome scans. Significant linkage occurred when three sires were tested and the F statistic was greater than 9.14.

8.3 Results

8.3.1 Phenotypic Analysis

In all cohorts, as expected, just over 10% of animals were "shy feeders" that would not eat sufficient from the electronic feeders and were removed from their pen and fed separately. Therefore, feed intake data was collected on 323 animals (Table 8.1). Unfortunately, the weight records for some animals were not accurate and so NFI was calculated for 319 animals. The maximum value for feeding sessions (32) was very high because some animals had the tendency to come in and out of the feeders many times while eating. Others just calmly ate their fill and then backed out to the pen when finished.

Table 8.1 Summary of phenotypic data

Trait	Number	Mean	CV (%)	Min.	Max.
MWt (kg)	324	625.6	13	382.6	824.3
MMWt ($kg^{0.73}$)	324	109.7	10	76.8	134.5
ADG (kg/day)	324	0.94	61	-1.43	2.35
DFI (kg/day)	323	12.94	17	6.37	18.95
NFI (kg/day)	319	0.05	14*	-5.04	7.23
MR (kg feed/ kg MWt)	319	0.021	16	0.012	0.037
GE (ADG /kg feed)	319	0.074	65	-0.224	0.202
ES (ln(No./day))	314	1.96	30	0.65	3.48
ET (ln(min/day))	314	4.56	7	3.83	5.37
ER (g/sec)	314	2.44	30	1.05	4.71
AS (min/session)	314	16.44	66	2.41	60.87
MS (kgfeed/session)	314	2.17	62	0.37	7.28

*SD divided by mean DFI since mean NFI close to zero by definition.

Abbreviations as described in Table 1.

The differences between cohorts were highly significant for all traits (Table 8.2). The 1996 and 1997 drops (96H, 96S, 97H, and 97S) were tested at similar weights, but the 1998-drop had lighter females (98H) and heavier steers (98S). This was because the 1998-drop steers had been grain fed prior to the commencement of the feed intake test. Consequently, they had very low average daily gains. The 1996-drop was the only group where steers and heifers were measured at the same time. The results from this drop indicated that steers

were 8% heavier, gained weight 17% faster, ate 4% more, had 12% higher gross efficiency, consumed 13% less meals and ate 8% faster than the heifers. In general, feed intake and time spent feeding was higher at Struan (1997 and 1998 drops) than Tullimba (1996). Also, the number of feeding sessions was significantly lower at Struan indicating animals did not have *ad libitum* access to feed. However, careful examination of the intake data revealed that feeder usage was less than the recommended 85%, indicating ample opportunity for animals to eat *ad libitum*.

Table 8.2 Least squares means (±s.d.) and tests of significance for cohorts

Trait	96H	96S	97H	97S	98H	98S	Sig.
NFI	-1.11±0.77^{a}	-1.38±0.96^{a}	-0.49±1.43^{b}	0.20±1.47^{c}	1.92±2.03^{d}	0.14±1.81^{c}	***
DFI	11.4±1.5^{a}	11.9±1.5^{ab}	12.3±1.89^{b}	13.7±2.0^{cd}	14.1±2.48^{c}	13.4±2.13^{d}	***
MR	0.019±0.001^{a}	0.019±0.002^{a}	0.021±0.002^{b}	0.021±0.002^{b}	0.025±0.004^{c}	0.019±0.003^{a}	***
GE	0.087±0.018^{ab}	0.098±0.020^{b}	0.109±0.018^{c}	0.084±0.017^{a}	0.077±0.054^{a}	-0.000±0.037^{d}	***
ADG	1.00±0.23^{a}	1.17±0.27^{bc}	1.31±0.21^{b}	1.13±0.20^{ac}	1.07±0.71^{ac}	0.03±0.35^{d}	***
MWt	594±66^{a}	644±72^{b}	598±67^{a}	658±72^{b}	569±67^{c}	701±69^{d}	***
ES	2.76±1.70^{a}	2.62±1.73^{b}	1.98±0.73^{c}	2.15±0.76^{d}	1.65±0.74^{e}	1.26±0.75^{f}	***
ET	4.36±2.42^{a}	4.32±2.55^{a}	4.66±2.73^{b}	4.51±2.55^{c}	5.01±3.27^{d}	4.31±2.97^{a}	***
ER	2.52±0.67^{a}	2.73±0.68^{b}	2.05±1.0^{c}	2.72±0.76^{b}	1.62±1.16^{d}	3.17±0.34^{e}	***
AS	5.3±0.43^{a}	5.9±1.0^{a}	15.5±1.7^{b}	10.8±0.90^{c}	30.7±2.26^{d}	23.0±2.29^{e}	***
MS	0.77±0.77^{a}	0.91±0.96^{a}	1.75±0.63^{b}	1.62±0.99^{b}	2.80±0.13^{c}	4.01±0.27^{d}	***

Means within a row with different superscripts differ (P<0.05); *** P<0.001.
Abbreviations as described in Table 1.

Significant breed and sire effects indicate the presence of genetic variation in a trait. Breed of dam differences were significant for daily feed intake, maintenance requirements, growth, body weight, eating rate and meal size (Table 8.3). Limousin backcross cattle (¾Limousin ¼Jersey) were 16% heavier, gained 11% more weight, ate 12% more, had 6% larger meal sizes and ate 17% faster than the Jersey backcross animals (¾Jersey ¼Limousin). There were no significant differences in NFI, gross efficiency, number of feeding sessions, the amount of time spent feeding and the average length of feeding between Limousin and Jersey backcross animals (Figure 8.2).

Table 8.3 Breed of dam and sire least squares means (±s.e.)

Trait	Jersey	Limo	Sig.	Ryan	Lou	Tom	Sig.
NFI	-0.10±1.78	-0.14±1.91	n.s.	0.21±1.99^{a}	-0.12±1.51^{ab}	-0.44±1.96^{b}	*
DFI	12.1±2.12	13.5±2.07	***	13.3±2.15^{a}	12.9±1.89^{a}	12.2±2.42^{b}	***
MR	0.021±0.003	0.002±0.003	**	0.002±0.003^{a}	0.002±0.003^{ab}	0.002±0.004^{b}	†
GE	0.075±0.051	0.076±0.045	n.s.	0.073±0.042	0.073±0.047	0.076±0.056	n.s.
ADG	0.90±0.58	1.00±0.57	*	0.95±0.55	0.98±0.59	0.92±0.59	n.s.
MWt	580±68	675±68	***	641±80^{a}	631±81^{a}	611±86^{b}	***
ES	2.08±1.60	2.06±1.68	n.s.	1.90±1.51^{a}	1.94±1.45^{a}	2.04±1.85^{b}	***
ET	4.54±3.42	4.53±3.53	n.s.	4.59±3.58^{a}	4.59±3.48^{a}	4.50±3.28^{b}	***
ER	2.33±0.47	2.73±0.24	***	2.54±0.24^{a}	2.39±0.36^{b}	2.48±0.37^{ab}	†
AS	14.9±2.34	15.5±2.45	n.s.	16.7±2.46^{a}	15.9±2.46^{a}	12.1±2.10^{b}	***
MS	1.83±0.22	2.12±0.36	***	2.26±0.40^{a}	1.93±0.24^{b}	1.75±0.21^{c}	***

Means within a row with different superscripts differ (P<0.05); *** P<0.001; ** P<0.01; *P <0.05; † P <0.10; n.s. not significant. Abbreviations as described in Table 1.

There were significant differences between sires and breeds in mid-weight and feed intake. In contrast to breed there was a significant difference between the sires in NFI and the length of each feeding session. In general, progeny sired by "Tom" were different from the progeny of the other two sires. Tom's progeny were 4% lighter but ate 7% less and had a 4% lower NFI than the average of the other two sires' progeny. Tom's progeny also had 11% more feeding sessions but spent 9% less time eating (Figure 8.3). The sire differences were smaller than breed differences for weight but were similar in size for feed intake and efficiency.

In the previous chapter, phenotypic correlations were calculated between the traits measured, with daily feed intake more closely related to weight (r_p=0.5) than growth (r_p=0.1, Table 7.5). The phenotypic correlation between NFI and daily feed intake was 0.84 indicating that approximately 29% of the variance in intake was associated with variation in size (MMWt) and growth rate (ADG). Maintenance requirements and gross efficiency were calculated to aid the interpretation of results. Maintenance requirements and NFI were highly correlated (r_p=0.93). Gross efficiency was more highly correlated with average daily gain (r_p= 0.94) than intake (r_p= -0.14).

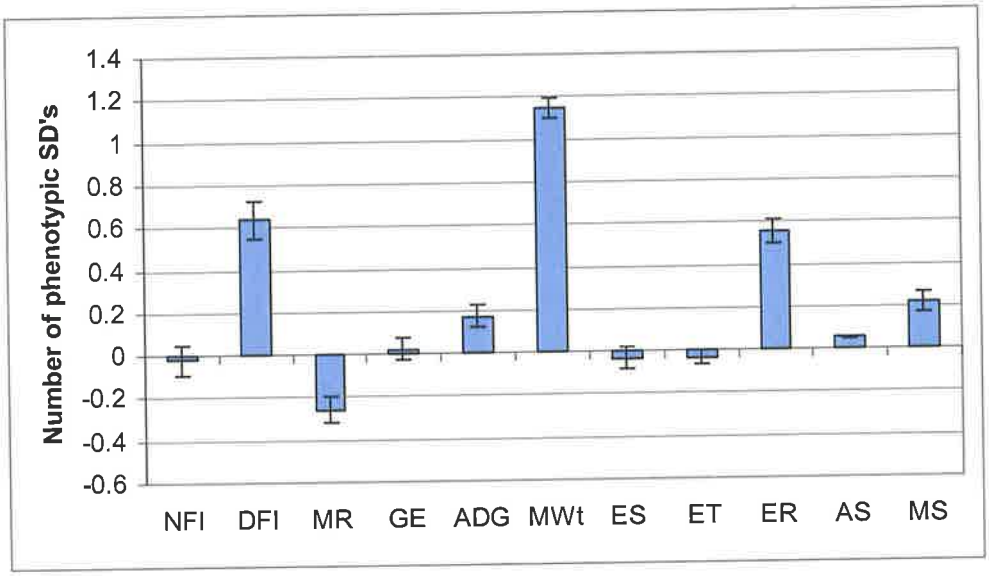


Figure 8.2 **Differences between the breeds, expressed as the number of phenotypic standard deviations of Limousin backcross animals from the Jersey backcross animals**

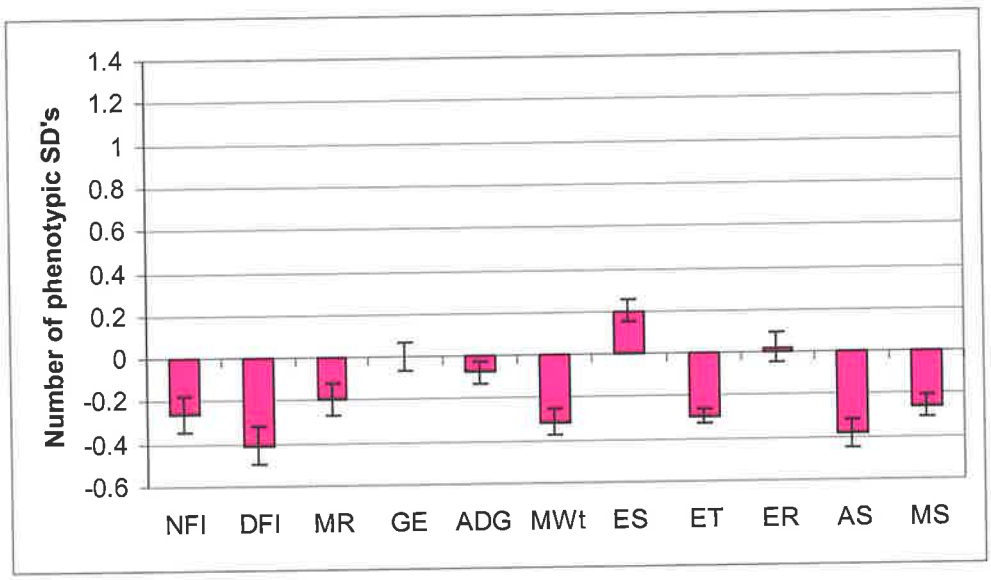


Figure 8.3 **Differences between Tom's progeny and the progeny from the other two sires.**

8.3.2 QTL analysis

8.3.2.1 Feed intake and efficiency QTL

The half-sib interval mapping analysis revealed that most QTL were segregating in one family, with only two QTL for feed efficiency traits segregating in two families. There were no QTL for feed efficiency or body weight traits that segregated in all 3 sire families. Four suggestive QTL for NFI were detected on BTA 1, 8, 9 and 20 (Table 8.4, and Table 8.5). The largest QTL for NFI was located on BTA1 and accounted for 98% of the phenotypic variation observed in NFI in Tom's family. Also located in this region were QTL for all traits measured in this study. However, not all QTL in this region were segregating in the same family.

Maintenance requirement (MR) was calculated as the amount of feed consumed per kilogram of body weight. There were four suggestive QTL for maintenance requirements that were located in same regions as the four suggestive NFI QTL. The sign and size of the additive effects were of the same magnitude and segregating in the same family (Tom) as the four NFI QTL. In contrast, there were five QTL (four suggestive and one significant) segregating for DFI, but only two were located on the same chromosome as the NFI QTL.

In total, there were 10 QTL located across six chromosomes for the eating behaviour traits (number of eating sessions, eating rate, time spent feeding, meal size, and average length of each feeding session). Interestingly, of the 10 QTL segregating for the eating behaviour traits, only the QTL for ES on BTA 8 was segregating near a QTL for NFI. BTA 13 contained QTL for all five eating behavioural traits. ER and ET were closely located near the start of the chromosome, whilst AS, MS, and ES were closely located at the distal end of BTA 13.

8.3.2.2 Growth and body weight QTL

There were more QTL detected for both growth and body weight traits than for intake traits. As for the intake traits, QTL detected for growth and body weight were also segregating in only one or two of the sire families. All of the NFI QTL were segregating in Tom's family, whilst six of the seven QTL for mid-weight were also segregating in Tom's family. The QTL with the largest additive effect on mid-weight was located on BTA 25 (Table 8.5) and accounted for 50% of the phenotypic standard deviation in that trait.

Of the eight QTL detected for growth, six were on the same chromosome and in close proximity to the QTL detected for gross efficiency. The additive effects of these six overlapping QTL were the same sign and segregating in the same families. The largest QTL for average daily gain was segregating in Ryan's progeny on BTA 1, accounting for 51% of the phenotypic standard deviation. Five of the eight growth QTL were segregating in Ryan's progeny.

Table 8.4 The position, LOD score, size of additive effect (phenotypic standard deviations) and sire family of QTL segregating for feed intake, efficiency, growth and body weight for BTA 1-10.

Chr.	Trait	Position[a]	LOD	Ryan	Lou	Tom
1	ADG	87	4.4**	-0.52		
	GE	87	2.5*	-0.41		
	MR	91	3.1*			-0.84
	DFI	92	4.6**			-0.80
	NFI	**92**	**3.2***			**-0.81**
	MWt	96	3.0*		-0.27	-0.37
2	ER	7	2.2*		-0.37	
3	ADG	103	2.5*		-0.49	
	GE	103	2.3*		-0.45	
4	MWt	18	2.0*			-0.34
5	DFI	45	2.3*			-0.70
	MWt	105	2.3*			-0.38
	MS	112	2.1*	0.24	-0.25	
6	DFI	27	2.1*			-0.52
	MWt	41	2.0*			-0.34
7	GE	43	2.5*	0.39		
	ADG	44	3.0*	0.45		
	MS	51	2.1*	-0.28	-0.28	
8	**NFI**	**68**	**2.3***			**-0.66**
	MR	68	3.1*			-0.60
	ES	79	2.4*			0.49
9	**NFI**	**13**	**2.0***	**0.51**		**-0.50**
	MR	18	2.0*	0.45		
	ADG	33	3.1*	0.42	-0.28	
	DFI	53	2.3*	0.54		
	MWt	59	2.1*	0.26	0.28	

* Genome-wide (suggestive) P<0.05 significance ; ** Genome-wide (significant) P<0.001 significance; [a] Position cM. Abbreviations as described in Table 1.

Table 8.5 The position, LOD score, size of additive effect (phenotypic standard deviations) and sire family of QTL segregating for feed intake, efficiency, growth and body weight for BTA 11-29.

Chr.	Trait	Position[a]	LOD	Ryan	Lou	Tom
11	ADG	66	2.8*	-0.42		
13	ER	27	2.1*	0.36		
	ET	34	3.8*	-0.29	-0.33	
	AS	82	2.4*	0.28	-0.32	
	MS	83	3.3*	0.29	0.31	
	ES	85	2.5*	-0.28		
14	MWt	13	3.1*	-0.38		-0.32
15	ADG	75	2.1*			-0.33
	GE	77	2.0*			-0.40
16	DFI	62	2.2*	-0.43		0.48
18	ADG	18	2.2*	-0.47		
	GE	18	2.1*	-0.44		
19	MS	79	2.4*		0.36	
	ADG	95	2.2*		0.44	
	GE	99	2.0*		0.37	
20	**NFI**	**49**	**2.1***			**-0.59**
	MR	49	2.0*			0.39
25	MWt	33	3.1*			-0.51

*Genome-wide (suggestive) P<0.05 significance ; **Genome-wide (significant) P<0.001 significance; [a] Position cM

Abbreviations as described in Table 1.

8.4 Discussion

8.4.1 Feed intake and efficiency QTL

A genome wide scan of all 29 bovine autosomes was undertaken on Jersey-Limousin back cross animals from six cohorts. The scan was performed on three half-sib families, each containing approximately 110 progeny, for feed intake and efficiency traits. Interval mapping, detected four suggestive QTL segregating for NFI. A further four QTL with LOD scores between 1.5 and 1.9 were also detected. The QTL with the largest additive effect was on BTA1. This QTL was segregating in Tom's family only. In the same region, QTL were detected for daily feed intake, maintenance requirements, gross efficiency, mid-weight and average daily gain. Upon close inspection, it would appear that this QTL results in larger animals that eat more. However, it was significant for NFI, but, as with most QTL identified in this study, did not have the same effect in all families. The largest effect for daily feed intake and maintenance requirements was clearly in Tom's progeny. In contrast, the QTL was significant for mid-weight in Ryan's progeny and average daily gain in Lou's progeny. Furthermore, the average daily gain QTL did not map to exactly the same location as the NFI QTL, although the resolution of this study was not sufficient to discount it as the same QTL. In Tom's progeny, this QTL resulted in animals that were 5% lighter, ate 16% less, had 14% lower maintenance requirements and an 11% lower NFI. Consequently, they were slightly lighter but significantly more efficient.

In addition to the QTL located on BTA1, three other suggestive QTL were found. While not as significant, all QTL were segregating in Tom's family, with the QTL on BTA 9 also segregating in Ryan's family. Interestingly, Tom's family had the lowest NFI, and in general, was significantly different from the progeny of the other two sires for the traits measured. The size of the additive effect of these QTL ranged from 50 to 81 percent of the phenotypic standard deviation in NFI (Table 8.4).

8.4.2 Growth and body weight QTL

Interestingly, all of the QTL detected for gross efficiency were in the same location as six of the eight growth trait QTL. These QTL were segregating in the same families and in the same direction. Only one of the daily feed intake QTL (on BTA1) was located near a gross efficiency QTL. It is most likely that these are different genetic loci because the QTL are segregating in different families, although it is possible that they could be different allelic

variations of the same gene. Since gross efficiency is calculated from both average daily gain and daily feed intake, it would appear that improvements in gross efficiency are driven by increasing growth rates rather than decreasing daily feed intake. Improving growth rates, however, does not always improve the overall efficiency of the production system. Genotypes with higher growth rates, and consequently higher gross efficiencies, also tend to have greater mature weights and hence higher feed requirements at maturity.

Seven suggestive QTL for body weight were detected by interval mapping all bovine autosomes in the Limousin-Jersey backcross animals. Six of these QTL were segregating in Tom's family and two in both Lou and Ryan's families. The QTL with the largest additive effects was segregating in Tom's family only on BTA25. This QTL accounted for 50% of the phenotypic standard deviations of mid-weight. Other suggestive QTL with large additive effects for mid-weight were located on BTA 5 and BTA14. Recent mapping studies in beef cattle have reported a number of QTL for carcass and growth traits on these same bovine chromosomes. Davis et al. (1998) reported three QTL for birth weight on BTA 5, 6, and 14. Stone et al (1999) detected and mapped two significant QTL on BTA 1 and 5 and four suggestive QTL on BTA 7, 11, 14, and 18 which affected carcass and growth traits. Casas et al. (2000) also showed QTL were segregating in regions on BTA 5, 6, 7, 14, and 19 for carcass composition and growth similar to those detected herein.

8.4.3 Candidate gene(s) for feed efficiency

Candidate genes from the bovine map were investigated for feed efficiency. Of the four groups of potential candidate genes (Chapter 5), only three were appropriate for this cattle study: 1) growth regulation and body composition, 2) energy regulation, and 3) neuropeptide signalling (Table 8.6). The biorhythm genes may be good candidates for feed efficiency however they have not been mapped in cattle yet.

Table 8.6 Candidate genes for net feed intake

Candidate	BTA	Abbrev.
Growth regulation and body composition		
PBX/Knox-1 related homeodomain containing polypeptide	1	PKNOX1
Pituitary growth factor	1	POU1F1
Somatostatin	1	SST
Cathepsin	8	CSTB
Lipoprotein lipase	8	LPL
Very low density lipoprotein receptor	8	VLDLR
Insulin-like growth factor 2 receptor	9	IGF2R
Growth hormone receptor	20	GHR
Energy regulation		
Phosphofructokinase, liver type	1	PFKL
Aldolase B fructose biphosphate	8	ALDOB
Phosphoribosyl pyrophosphate synthetase 1	9	PRPS1
Hexosammindase B, beta polypeptide	20	HEXB
Neuropeptide signalling		
Solute carrier family 1 member 2 (facilitated glucose transporter)	1	SLC1A2
Cannabinoid receptor	9	CNR1
Opioid receptor mu 1	9	OPRM1
Serotonin receptor 1A	20	HTR1A
Solute carrier family 1 member 3	20	SLC1A3

8.4.3.1 Growth regulation and body composition

IGF-1 is produced by the liver and other tissues and is considered to be the prime effector of growth hormone actions on growth and development (Zampf and Hunziker 1994). There is increasing evidence for the suitability of circulation plasma insulin-like growth factor – 1 (IGF-1) as a genetic predictor of economically important traits in beef cattle (Herd et al. 1995; Davis and Simmen 2000; and Johnston et al. 2001). Included in these traits is NFI (Johnston et al. 2001). Herd et al. (2002) observed in beef cattle that phenotypic variation in levels of IGF-1 was associated with genetic variation in growth, NFI, and rib and rump fat, such that selection against IGF-1 should result in increased

growth rate and improved feed efficiency whilst lowering the subcutaneous fat thickness. Johnston et al. (2001) found that the genetic correlation between NFI and IGF-1 concentration was 0.56 and 0.39 in two groups of beef cattle. Tomas et al. (1998) also observed increased growth rates and feed utilisation efficiency and a reduction in carcass fat in broiler chickens infused with IGF-1. Therefore, IGF-1 has been suggested as a candidate gene to be used as an indirect selection criterion for feed efficiency. However, IGF-1 and its receptor are located on BTA5 and BTA21, respectively, and there were no QTL for NFI or gross efficiency detected on either of those bovine chromosomes in the study herein.

A daily feed intake and a mid-weight QTL were detected on different regions of BTA5 in Tom's progeny. Both QTL were negative in action but do not appear to be the same QTL as they are over 50 cM apart. From this study, it would appear that IGF-1 is not a good candidate for NFI in the population herein. In contrast, there was a QTL for NFI segregating on BTA9, which contains the insulin-like growth factor-2 receptor gene (IGF2R)(Table 8.6). In the study of Tomas et al. (1998), IGF2 was associated with metabolic regulation in broiler chickens. Further study of the IGF2R gene and its association with NFI in cattle is required.

Muscle

There were other candidate genes associated with growth identified in regions containing QTL for NFI. These genes include cathepsin B (CTSB), growth hormone receptor (GHR), PBX/Knox-1 related homeodomain containing peptide (PKNOX1) and pituitary specific transcription factor 1 (POU1F1). GHR and POU1F1 have been discussed as suitable candidates for mice (Chapter 5) because of their actions on growth hormone. PKNOX1 is a regulating protein expressed in many tissues involved in growth and morphogenesis. It is involved in the binding of DNA to myogenic transcription factors. In contrast, another candidate gene CTSB is associated with intracellular degradation and turnover of proteins. In Chapter 4, there were no significant differences between lines of mice selected for divergent NFI in protein synthesis or degradation. However, protein degradation was only crudely estimated. In Angus cattle selected only on yearling weight gain, an associated response in protein synthesis, degradation, and oxygen uptake to feed intake was observed (Parnell et al. 1994). In the same animals, Oddy et al. (1998) found that steers from the low

growth rate line had inherently higher rates of protein synthesis in muscle than the high growth rate line steers. Oddy et al. (1998) suggested that animals with a higher gross efficiency (i.e. from the high growth rate line) had lower rates of protein synthesis in the muscle and used more oxygen in processes other than protein turnover in the muscle. These cattle findings support earlier studies by Oddy et al. (1995) in sheep divergently selected for weaning weight. Since cathepsin B is involved in proteolysis, it is a possible candidate for NFI in cattle.

Fat

Energy ingested as fat that is not needed for the energetic demands of the body is stored as adipose tissue. Lipogenesis is the deposition of fat, which occurs in adipose tissue and in the liver. Fatty acids enter the adipocyte to be oxidised or esterified into phospholipids or triacylglycerol. Lipoprotein lipase (LPL) is an enzyme that influences the stores of triacylglyceride by regulating the flow of free fatty acids into adipocytes (Greenwood 1985). Very low-density lipoproteins (VDLD) transport lipids and activate LPL. Since there was a significant difference between the mouse NFI selection lines in body fat percentage (Chapter 4) and after one generation of selection for NFI in cattle (Richardson et al. 2001), both LPL and VLDLR are candidate genes for feed efficiency.

8.4.3.2 Energy regulation

Interestingly, there were a number of genes coding for enzymes involved in energy homeostasis also located in the chromosomal regions containing cattle NFI QTL. Two of these enzymes, aldolase B fructose biophosphate (ALDOB) and phosphofructokinase liver (PFKL) are involved in the glycolytic pathway in the liver. Glycolysis is the initial pathway in the catabolism of carbohydrates. PFKL carries out the second ATP dependent phosphorylation step, essentially an irreversible process. This is important because it is the primary site for regulation of the flow of carbon through glycolysis. PFKL activity is very sensitive to the energy status of the cell and the levels of citrate and fatty acids (Mathews and Van Holde 1990). ALDOB catalyses the 'splitting of sugar', where fructose –1, 6-biphosphate a 6-carbon sugar is broken into two 3-carbon compounds, dihydroxyacetone phosphate and D-glyceraldehyde-3-phosphate. The two other enzymes, hexosaminidase B (HEXB), and phosphoribosyl pyrophosphate synthetase 1 (PRPS1), are involved in the degradation of glycolipid and nucleotide synthesis, respectively. These four enzymes are

involved in either the production of ATP or they catalyse reactions requiring ATP, making them potential candidates for NFI.

8.4.3.3 Neuropeptide signalling

As described in Chapter 5, neuropeptides contribute to the regulation of energy balance through a variety of different mechanisms. These include both positive and negative feedback mechanisms (Figure 5.8).

Positive regulators

The opiod system is a key mediator of neuropeptide Y-induced feeding and energy regulation in the central nervous system (Kotz et al. 2000). A number of studies (Marks-Kaufman and Kanarek 1990; Cole et al. 2000; and Hagan et al. 2001) have reported that opiods play a role in mediating fat-selective intake. Evidence also attributes opiods with increased intake of an inherently preferred food, regardless of its macronutrient content (Cooper and Turkish 1989 and Gosnell et al. 1990). Cole et al. (2000) found that opiod receptor subtype antagonists selective for mu receptors reduced feed intake and body weight in lean and obese Zucker rats. Consequently, the opiod system along with other bioactive systems in the brain, including dopamine, neuropeptide Y, cholecystokinin, histamine and serotonin have been implicated in the etiology of genetic obesity (Cole et al. 2000) and could also play a role in the efficiency of feed utilisation. Interestingly, the cannabinoid receptor (CNR1) gene has been physically mapped to BTA 9. As with the opiod receptor, the cannabinoid receptor has also been linked to several behavioural and physiological processes including motor activity, thermoregulation, pain reception, and eating behaviour (Hoehe et al. 1995; Devane et al. 1992).

There were two other neurotransmitters that positively regulate energy balance, solute carrier family 1 member a2 and member a3 (SLC1A2 and SLC1A3 respectively). Both SLC1A2 and SLC1A3 are involved in glutamate transport. Glutamate is extensively metabolised to support whole-animal energy and nitrogen homeostasis (Heitmann and Bergman 1981; Wu 1998; Nissim 1999). Glutamate appears to be an important precursor of metabolic energy for ruminants, serving as a source of oxidisable fuel and gluconeogenic carbons (Fahey and Berger 1988). A family of structurally related membrane proteins regulates glutamate transport in the central nervous system and in

peripheral organs. In the central nervous system, glutamate transporters mediate the removal of glutamate released at excitatory synapses and influence the kinetics of the glutamate receptors. Given that glutamate is important to ruminant energy and nitrogen metabolism and that plasma transport capacity is thought to limit glutamate metabolism, both SLC1A2 and SLC1A3 could be considered as candidates for feed efficiency.

Negative regulators

The remaining two candidate genes (Table 8.6), serotonin receptor 1A (HTR1A) and somatostatin (SST) negatively regulate the body's energy balance. Serotonin acts through a large number of receptors to influence a range of physiological activities (discussed in Chapter 5). HTR1A has been shown to influence body temperature, blood pressure, locomotor activity, ACTH secretion, and stimulate appetite, and relieve anxiety (Hannon and Hoyer 2002). SST (discussed in Chapter 5) is a hypothalamic hormone that inhibits growth hormone (Brazeau et al. 1973) and gastric motility (Yamada and Chiba 1989). Growth hormone is involved in the production of hepatic IGF1, and both are involved in the control of postnatal growth (Lupu et al. 2001).

8.5 Conclusion

The aim of this chapter was to locate the regions of the bovine genome containing QTL for NFI and to investigate any possible candidate genes in those regions. Four suggestive QTL for NFI were located on BTA 1, 8, 9, and 20. This study revealed that three of the four NFI QTL were segregating in one family only. As a result, the size of the effect could be large even at "borderline" significance levels. It also implies that the QTL identified were not fixed in the parent "purebred" populations. This is important for future research, as the QTL and candidate gene alleles are not specific for Jersey or Limousin and are more likely to be segregating in other breeds or species.

It must be remembered that the power of this study is not sufficient to ensure definitive identification of all segregating QTL. A secondary screen involving additional animals and markers is required to verify the four suggestive QTL for NFI. Analysis of additional families and breeds is also required to determine the effect and frequency of alleles influencing feed efficiency. Such analyses may reduce the number of potential candidate

genes from the 17 identified herein. Furthermore, exploiting the comparative maps of cattle and mice could also reduce the number of potential NFI QTL (Chapter 9).

Chapter 9.

General discussion and conclusions

9.1 Homology between cattle and mouse studies – how good are mice as a model?

Traditionally, selection programs have been based on phenotypic observations. Unfortunately, this approach is not efficient for all traits as some phenotypes are expressed late in life, in one sex only, are measured after an animal has been slaughtered, or are too expensive to assess. In these cases, using the genotype of the animal instead of the phenotype provides a powerful tool for selection very early in life. This thesis has focused on detecting regions of both mouse and cattle genomes that contain regions segregating for net feed intake (NFI). Mice have been used as a model species for cattle, given the cost of measuring feed intake in cattle is approximately $500 per head. Due to their short generation interval and litter bearing capacity, mice can provide a powerful mapping population in a short period of time.

Comparing regions containing QTL for NFI in mice with those of cattle provides further evidence of loci controlling NFI in that homeologous region. Using both the cattle (BOVMAP, http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/main.pl?BASE= accessed November 2003; and BOVGBASE, http://bos.cvm.tamu.edu/bovgbase.html accessed November 2003) and mouse databases (MGI, http://www.informatics.jax.org/ accessed November 2003), there were two regions in the mouse genome that contained QTL for NFI that appeared to align with QTL detected for NFI in the cattle study (Figure 9.1). There were a further two QTL detected in cattle that had LOD scores of 1.6 that were comparable with mouse QTL (Table 9.1, Appendix C). Although a LOD score of 1.6 was not statistically significant, it is possible that the QTL are segregating in cattle in these regions but the study herein did not have the power to detect them. The power to detect a segregating QTL that explains only a few percent of the phenotypic variance will generally require genotyping a minimum of 500 individuals (Weller 2001). Also, multiple interval mapping, which utilises maximum likelihood, was used to detect QTL in the mouse population compared with interval mapping using the regression technique in cattle. Multiple interval mapping is more precise and powerful than interval mapping in estimating QTL positions and size (Zeng et al. 2000) because it allows multiple QTL in multiple intervals to be mapped simultaneously.

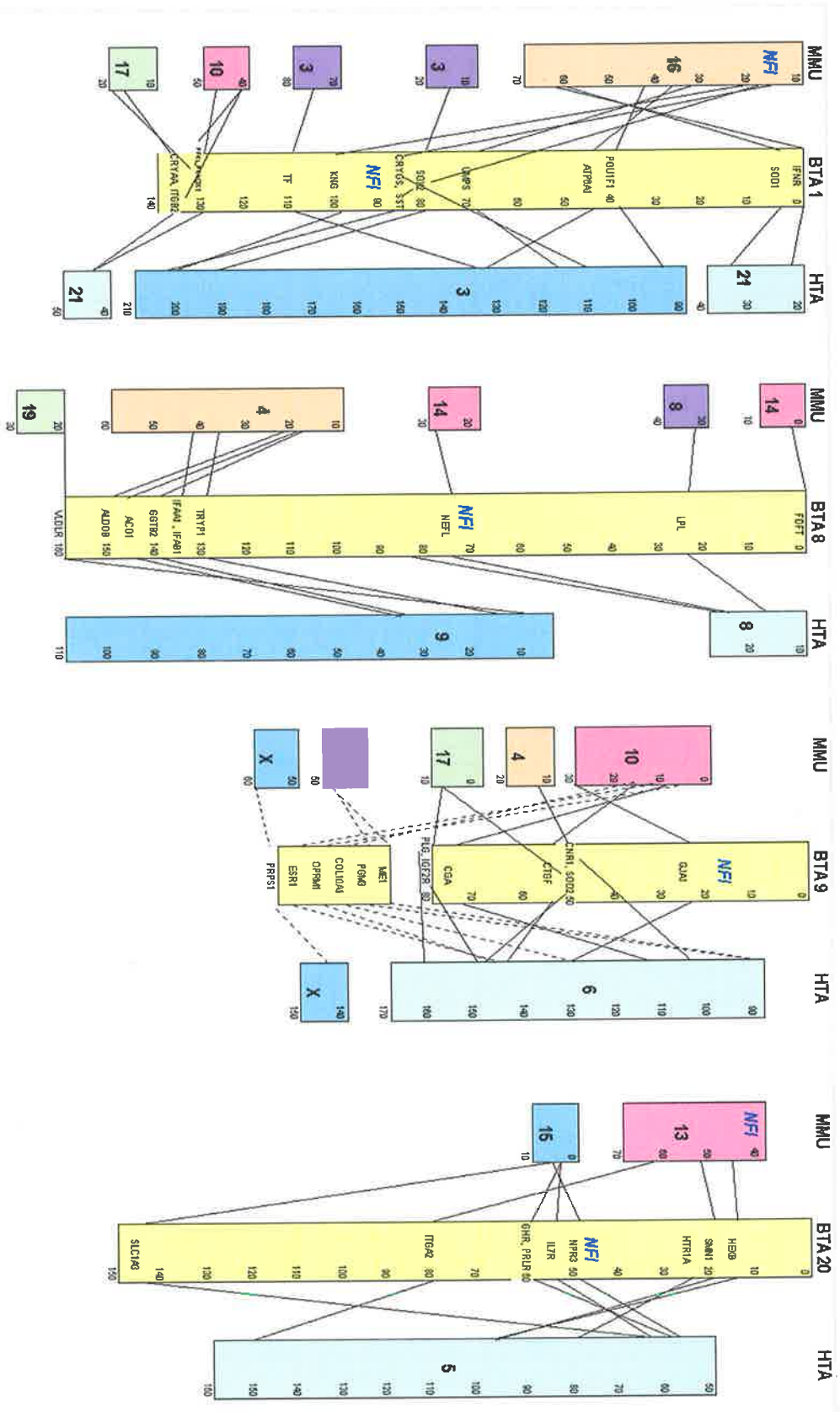


Figure 9.1 **Bovine (BTA 1, 8, 9, 20), human and mouse comparative maps with NFI QTL. Gene position from BOVMAP, MGI, and GDB databases.**

Table 9.1 Size of effect of QTL in comparative regions (based on Figure 9.1 and Appendix C)

Mouse				Cattle		
Chrom.	Position (cM)[a]	Add.[b]	Dom.[b]	Chrom.	Position (cM)[a]	Add.[b]
16	13	-0.18	0.94	1	92	0.98
13	39	0.22	-0.16	20	49	0.57
1	75	-0.84	1.17	16*	64	0.50
5	9	0.60	0.72	6*	27	0.46

* LOD score of 1.6

[a] site of the largest effect (additive or dominant) in phenotypic standard deviations

[b] size of effect in phenotypic standard deviations

It was quite surprising that four potential NFI QTL were in common between cattle and mice given the extreme differences between these two species. The digestive systems of these species are markedly different. Cattle are pre-gastric fermentors containing rumen microbes to digest fibre and break down cellulose, allowing the bulk of the digestion of feed to occur in the first two chambers of the stomach. Conversely, mice are post-gastric fermentors that do not have micro-organisms that break down cellulose. Furthermore, mice are a litter bearing species, while cattle tend to be single bearing animals. In addition, selection for improved (low) NFI in mice resulted in increased body fat percentage (Chapter 4), yet in cattle, leaner animals are produced. Therefore, it is possible that different loci or genes are controlling NFI in these species. Lastly, even if QTL are homeologous between mice and cattle, one of the NFI alleles could be fixed in either the mouse or cattle mapping populations.

9.2 Fine mapping

QTL mapping based on linkage analysis of a genome scan was performed in both mice and cattle for NFI. Four QTL regions appeared to be homeologous between the species. To further investigate these four homeologous chromosomal regions, further mapping was performed in the mouse population. The aim of further genotyping was to fine map these regions by increasing the number of observed meioses. The most efficient way to fine map this population would have been to use an advanced intercross. Unfortunately, the mapping resource was no longer available to produce F_3 or F_4 progeny.

Other methods of fine mapping include population wide linkage disequilibrium and identity by descent, which are both based on observing linkage disequilibrium between QTL and close marker loci. Linkage disequilibrium or allelic association mapping is most commonly applied to binary data. This strategy is limited to a select number of traits where individuals displaying the trait can be traced to a single allele at one locus. This may not be the most appropriate approach for fine mapping the NFI alleles because it is a continuous trait and is influenced by a number of loci. The principal behind identity by descent mapping is to find a region that is conserved in individuals with a common ancestor (Kinghorn and Van der Werf 2000). Identity by decent mapping has mostly been used in humans to map disease traits. However, the problem with this approach is that identity by descent probabilities can be complicated to calculate. Consequently, neither linkage disequilibrium or identity by descent mapping were used to fine map NFI QTL in this study. To increase the number of observed meioses, the progeny of the two sires that had not been genotyped were utilised. Markers (Table A.2, Appendix A) were spaced at approximately 10cM intervals on MMU 1, 5, 13 and 16, in the regions containing QTL. In addition, extra markers were genotyped in the two sire families that had been previously analysed.

Due to the time required to complete multiple interval mapping, composite interval mapping using QTL Cartographer (Basten 2001) was performed. Only two of the five QTL were significant after fine mapping. Following the composite interval mapping, a half-sib analysis was performed using QTL Express (Seaton et al. 2002; http://qtl.cap.ed.ac.uk; accessed November 2003). The half-sib analysis revealed that all five QTL were segregating in the family of sire 1021 only. This suggests that the high and low NFI alleles were not fixed in the mouse NFI selection lines. This is not surprising given the lines had only been mated for eight generations and there is still large variation in intake (CV= 20%) within each line.

Table 9.2 Mouse fine mapping results

	Initial Genome Scan				Fine Mapping			
Chrom.	**Pos.**	**LOD**	**Add.**	**Dom.**	**Pos.**	**LOD**	**Add.**	**Dom.**
1	75	4.6	-0.84	-1.17	70	3.1	-0.61	0.15
5	9	3.1	0.60	0.72				
13	39	3.2	0.22	-0.16				
16	13	3.0	-0.28	0.94	12	2.9	-0.36	0.39
16	43	4.3	-0.47	0.61				

9.3 Candidate genes

Candidate genes for NFI were investigated at 20 cM intervals from the QTL in both mouse and cattle genomes. The location of candidate genes for NFI in the mouse genome that had not yet been mapped in cattle were estimated using comparative maps between humans, cattle and mice. In the regions (Table 9.1) homeologous between cattle and mouse at least 19 candidate genes appear to be located near NFI QTL in mice and cattle (Table 9.3). In Chapter 4, differences were observed between two lines of mice selected for divergent NFI were observed in body composition, activity and gastric emptying, whilst the results from the protein turnover study were inconclusive. There were no differences observed in average daily gain or body weight. In Chapter 7, differences between cattle with high and low NFI were observed in feeding behaviour and body composition. Consequently, it is possible that one or more of the candidate genes involved in biorhythm, growth and body composition regulation, or energy regulation would be suitable candidates for feed efficiency. The neuropeptides appear to be the most likely group of candidates because they influence gastric emptying, body composition, feeding behaviour and activity. Studies of serotonergic mechanisms in the control of meal size and feeding rate suggest important roles for serotonin receptors, whilst dopamine D2 receptors are associated with increased meal size and decreased meal frequency (in a review by Clifton 2000). It should be noted that the synteny between mouse and bovine genomes are imprecise and consequently the candidate genes indicative only, until the bovine sequence is published.

Table 9.3 Candidate genes in mouse and cattle

Gene	Abbrev.	MMU	BTA	BTA_{est}
Biorhythm				
Period homolog 2	*PER2*	*1*	*NM*	*2*
Circadian locomotor output cycles kaput	CLOCK	5	NM	6*
Growth and body composition regulation				
Insulin-like growth factor binding protein 2	*IGFBP2*	*1*	*NM*	*2*
Insulin-like growth factor binding protein 5	*IGFBP5*	*1*	*NM*	*2*
Myogenin	*MYOG*	*1*	*16**	
Calpastatin	CAST	13	7	
Pituitary specific transcription factor 1	*POU1F1*	*16*	*1*	
Energy regulation				
ATPase Ca^{++} transporting plasma membrane 4	*ATP2B4*	*1*	*16**	
ATPase H^{+} transporting, lysosomal V1 subunit-A isoform 1	*ATP6V1A1*	*16*	*1*	
Neurotransmitters				
Serotonin receptor 5B	*HTR5B*	*1*	*NM*	*11*
Cholecystokinin A receptor	CCKAR	5	NM	6*
Dopamine receptor D5	DRD5	5	NM	6
Serotonin receptor 5A	HTR5A	5	NM	6*
Cocaine and amphetamine regulated transcript	CART	13	NM	20
Serotonin receptor 1A	HTR1A	13	20	
Solute carrier family 6 A3 (neurotransmitter to dopamine)	SLC6A3	13	UNK	20
Dopamine receptor 3	*DRD3*	*16*	*NM*	*1*
Serotonin receptor 1F	*HTR1F*	*16*	*NM*	*1*
Somatostatin	*SST*	*16*	*1*	

BTA_{est} = estimated bovine chromosome from human comparative maps; * = LOD score 1.6; NM = Not mapped; UNK = mapped but location unknown.

Italics indicates candidate genes on MMU 1 and 16

9.4 Future work – Marker Assisted Selection

Predicting the impact of genetic markers for NFI through an economic analysis provides a good way to demonstrate the benefits of marker assisted selection to producers. According to Davis and DeNise (1998), calculating the benefit of adopting such a technology requires focus on three main areas: 1) where returns are realised, 2) the source of the returns, and 3) the accuracy of the estimated genetic value. Using the program "Z-plan" (Graser et al. 1994, Nitter et al. 1994), Pitchford (2002) investigated the economic aspects of DNA tests for NFI. Five scenarios were simulated for a two-tiered self-replacing population of 20,000 breeding cows (10,000 breeding unit, 10,000 commercial unit). Genetic improvement was only generated in the breeding unit and transferred to the commercial unit through the use of bulls selected from the breeding unit. Twenty bulls per year were selected for use in the breeding unit as AI sires, and each sire was used for an average of 2.5 years. The breeding objective based on production of 650kg live weight steers fed for the high quality Japanese market:

1) Conventionally measure NFI on all sires in the breeding unit (BASE).

2) Conventionally measure NFI and genotype all sires in the breeding unit with the cost of genotyping at $20 (NFI20).

3) Conventionally measure NFI and genotype all sires in the breeding unit with the cost of genotyping at $80 (NFI80).

4) Genotyping all sires in the breeding unit with the cost of genotyping at $20 (QTL80).

5) Genotyping all sires in the breeding unit with the cost of genotyping at $80 (QTL80).

The selection criteria included weight at various ages (birth, 200d, 400d, 600d and mature cows), fertility traits (days to calving, calving difficulty score and scrotal size) and scan traits (rib and P8 fat depth, eye muscle area, and intra-muscular fat percentage) in addition to the above NFI scenarios. These criteria were chosen to mimic the commercial situation if a producer was to use genotypic information on NFI. Other assumptions were made regarding phenotypic and genetic variance, gene effect, heritability and other costs. These assumptions were based on previous work on cattle selected for and against NFI (Archer et al. 2004), the results from the QTL studies herein (Chapter 8) and current prices for artificial insemination.

Based on the above conditions, the simulations revealed that regardless of whether NFI was measured, QTL information would increase the accuracy of selection, and therefore, improve the profit per cow. It was clear that under the above conditions, measuring NFI in stud bulls is still profitable (Figure 9.2). In addition, all scenarios with genetic markers were more profitable than just measuring NFI. Furthermore, the cost of the DNA test ($20 versus $80) had only a small effect on profit (approximately 10 cents/unit). Subsequently, if the QTL information (from Chapter 8) used in the economic analysis can be verified in other cattle breeds, then marker-assisted selection based on the QTL information could be implemented as part of cattle stud breeding objectives.

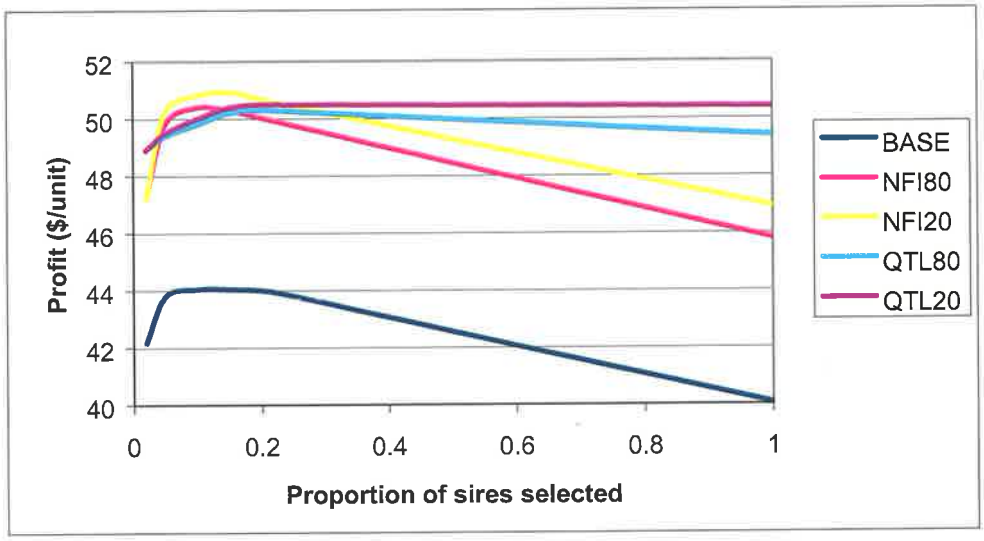


Figure 9.2 Economic value of QTL for NFI (taken from Pitchford 2002)

9.5 Conclusion

The main aims of this thesis were to identify regions of the mouse and cattle genome that contain loci segregating for feed efficiency and to gain a clearer understanding of the underlying biology of the trait. Using passive infrared sensors, large differences in daily activity were observed between the unique mouse NFI selection lines. Selection for low post-weaning NFI results in mice with lower feed intake, daily activity and rate of gastric emptying, with little or no change in body weight or growth. In Limousin-Jersey cross cattle, there was no difference in the temperament traits of flight distance and docility score between cattle with higher and lower NFI. However, there were differences in feeding behaviour between the two groups of cattle. Cattle that were more efficient had lower maintenance requirements, ate less and more slowly, had fewer visits to the feed hopper, and spent less time feeding each day.

Clearly, there were a number of loci segregating in both species. Mice appeared to provide a reasonable model for cattle in that four QTL appeared to be homeologous between the species. There were 28 potential candidate genes identified in the mouse study and 17 in the cattle study. Fine mapping of the mouse population and comparative mapping between the two species reduced this number to 19 possible candidate genes. Of these 19 candidate genes, the 11 neuropeptides are of particular interest because they have the ability to influence feeding behaviour, activity, body composition and gastric emptying. However, further studies are required to verify these genes as candidates for NFI.

The implication from this thesis is that a commercial DNA test for feed efficiency in beef cattle could be developed, provided that the QTL regions identified in this thesis are confirmed in other cattle population. This would lead to improved profitability for Australia's beef industry.

Appendix A

Table A.1 Microsatellite markers used for initial genome-wide scan, map positions and the percentage of informative individuals (mouse study)

Marker	cM	% Inf. Prog.	Marker	cM	% Inf. Prog.
D1Mit231	8.7	80	D10Mit80	2.2	50
D1Mit70	15.3	53	D10Mit38	20.8	93
D1Mit22	32.8	31	D10Mit42	41.5	43
D1Mit84	56.8	30	D10Mit14	69.9	55
D1Mit14	82.0	50	D11Mit62	2.2	30
D1Mit17	110.1	85	D11Mit208	29.5	99
D2Mit83	21.9	43	D11Mit212	48.1	50
D2Mit66	48.1	50	D11Mit168	75.4	75
D2Mit59	72.1	90	D12Mit38	1.1	25
D2Mit230	97.3	60	D12Mit114	23.0	68
D3Mit164	2.2	93	D12Mit167	47.0	38
D3Mit184	20.8	75	D12Mit150	56.8	60
D3Mit147	59.0	63	D13Mit57	2.2	85
D3Mit19	66.7	97	D13Mit147	37.2	90
D4Mit101	5.5	58	D13Mit151	50.3	33
D4Mit111	25.1	12	D14Mit126	6.6	48
D4Mit175	47.0	81	D14Mit115	47.0	26
D4Mit226	74.3	50	D15Mit53	7.7	39
D5Mit125	7.7	58	D15Mit63	28.4	25
D5Mit156	37.2	66	D15Mit159	49.2	20
D5Mit25	45.9	25	D16Mit9	4.4	18
D5Mit97	64.5	25	D16Mit103	21.9	93
D5it287	82.0	75	D16Mit189	40.4	43
D6Mit86	0.0	19	D16Mit86	51.4	73
D6Mit186	20.8	18	D17Mit57	1.1	59
D6Mit149	40.4	62	D17Mit66	19.7	77
D6Mit15	66.7	77	D17Mit152	32.8	55
D7Mit178	0.0	44	D17Mit129	49.2	88
D7Mit158	18.6	68	D18Mit 74	18.3	11
D7Mit281	38.3	82	D18Mit184	26.2	60
D7Mit105	49.2	30	D18Mit4	57.2	67
D7Mit46	64.5	6	D19Mit42	2.2	7
D8Mit124	4.4	92	D19Mit63	24.0	80
D8Mit177	31.7	6	D19Mit36	40.4	42
D8Mit112	55.7	45	D19Mit6	57.9	86
D9Mit126	4.4	18	DXMit55	1.1	85
D9Mit71	24.0	79	DXMit210	24.0	92
D9Mit35	47.0	44	DXMit5	54.6	80
D9Mit19	68.9	82	DXMit249	70.8	80

% Inf. Prog. – Percentage of informative progeny

Table A.2 Microsatellite markers used for fine mapping, map positions and the sire families genotyped (mouse study)

Chrom.	Marker	Pos. cM	Infor.	S1002	S1011	S1021	S1027
1	D1Mit231	8.7	80			✓	✓
	D1Mit70	15.3	53			✓	✓
	D1Mit22	32.8	31			✓	✓
	D1Mit84	56.8	30			✓	✓
	D1Mit26	64.5		✓	✓	✓	✓
	D1Mit256	76.5			✓		
	D1Mit14	82.0	50			✓	✓
	D1Mit143	84.2		✓			
	D1Mit36	91.8					✓
	D1Mit206	94.0		✓	✓		
	D1Mit17	110.1	85	✓	✓	✓	✓
5	D5Mit125	7.7	58	✓	✓	✓	✓
	D5Mit387	10.9		✓	✓	✓	
	D5Mit255	25.1		✓	✓	✓	
	D5Mit156	37.2	66			✓	✓
	D5Mit25	45.9	25	✓	✓	✓	✓
	D5Mit97	64.5	25	✓	✓	✓	✓
	D5it287	82.0	75			✓	✓
13	D13Mit57	2.2	85	✓	✓	✓	✓
	D13Mit242	15		✓	✓	✓	
	D13Mit147	37.2	90	✓	✓	✓	✓
	D13Mit76	42.6		✓	✓	✓	
	D13Mit151	50.3	33	✓	✓	✓	✓
16	D16Mit9	4.4	18			✓	✓
	D16Mit56	12		✓	✓	✓	
	D16Mit103	21.9	93			✓	✓
	D16Mit38	26.2		✓	✓		
	D16Mit103	33.9				✓	✓
	D16Mit189	40.4	43	✓	✓	✓	✓
	D16Mit86	51.4	73	✓	✓	✓	✓

Appendix B

Table B.1 Microsatellite markers on BTA 1-4 map positions and the percentage of informative individuals (cattle study)

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
1	BMS2321	0.0	79	✓		✓
	BMS711	7.3	81		✓	✓
	BMS4017	20.8	84	✓	✓	✓
	INRA49	53.5	77	✓	✓	✓
	CSSM032	74.2	86	✓	✓	✓
	BMS1789	86.9	84	✓		✓
	BM1824	94.6	88	✓	✓	✓
	BMS599	111.8	90	✓	✓	✓
	BMS2263	121.1	88	✓	✓	✓
2	ILST26	0.0	72	✓	✓	
	TEXAN2	15.0	75			✓
	OARHH30	17.9	77	✓	✓	
	TGLA377	19.7	76			✓
	ILSTS30	27.8	74	✓		✓
	RM356	44.4	75	✓		✓
	BMS2626	62.2	87	✓	✓	✓
	BM644	80.9	72			✓
	BMS356	92.8	91	✓	✓	
	BM2113	98.7	92	✓	✓	✓
3	INRA006	0.0	81	✓	✓	✓
	BMS963	16.1	89	✓	✓	✓
	BMS482	16.6	88	✓		
	BL41	25.7	86	✓	✓	✓
	MCM58	29.3	82			✓
	BM4129	32.8	78	✓		
	HUJ246	48.4	66	✓		✓
	BMS2145	74.4	83		✓	✓
	BMS835	79.6	89	✓	✓	✓
	BMS896	95.4	75		✓	✓
	BMC4214	103.5	84	✓	✓	✓
4	BMS1788	0.0	88	✓	✓	✓
	BMS1172	18.9	92	✓		✓
	BMS1237	22.2	95	✓	✓	✓
	MAF70	33.3	84	✓		
	MAF50	39.0	92		✓	✓
	RM067	39.8	93	✓	✓	
	BMS495	47.1	91	✓		
	INRA72	50.9	95	✓	✓	✓
	BMS779	55.5	90		✓	✓
	ILST062	59.9	87	✓	✓	
	OARCP26	63.6	79	✓		
	BMS648	79.0	88	✓	✓	✓
	TGLA159	88.9	87	✓	✓	✓

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
5	BMS1095	0.0	60	✓		
	BMS610	12.8	82	✓	✓	✓
	AGLA293	31.2	68	✓		
	OARFCB05	34.0	71	✓		✓
	MAF23	62.0	63		✓	
	CSSM22	68.6	65	✓		
	BMS1248	87.0	91	✓	✓	✓
	BMS772	105.1	89	✓	✓	✓
	BM2830	120.2	91	✓	✓	✓
6	INRA133	0.0	89	✓	✓	✓
	BM1329	27.3	89	✓	✓	✓
	BM143	41.2	93	✓	✓	✓
	BMS483	55.8	74	✓		
	BM4621	64.1	82	✓	✓	
	BM415	68.1	8 1	✓		✓
	BM8124	86.0	65	✓		✓
	BM2320	112.5	61	✓	✓	
7	BM7160	0.0	79	✓	✓	✓
	RM006	22.1	85	✓	✓	✓
	TGLA303	38.5	89	✓	✓	✓
	BM741	50.4	69		✓	
	BM6117	61.0	65	✓		
	BMS2258	75.0	80	✓		✓
	INRA192	82.4	85	✓	✓	✓
	BMS1331	91.1	70	✓		
	BM9065	102.4	56			✓
	BMS522	120.7	46			✓
	BMS1247	132.0	55	✓		✓
8	RM321	0.0	84	✓		✓
	RM372	3.3	86		✓	✓
	BMS1591	12.3	78		✓	✓
	BM4006	31.3	71		✓	
	TGLA13	35.6	77		✓	
	BMS1341	35.6	77			✓
	BMS2072	43.0	86	✓	✓	✓
	BM711	67.8	92	✓	✓	✓
	CSSM147	94.7	63	✓		
	BMS836	98.4	64	✓		✓
9	BM757	0.0	67	✓		
	ETH225	7.5	81	✓	✓	✓
	BM2504	24.6	74	✓	✓	✓
	RM216	31.4	94	✓	✓	✓
	BMS817	37.4	95	✓	✓	✓
	BMS1148	45.2	92	✓	✓	✓
	BMS1290	58.4	92	✓	✓	✓
	BM4208	84.0	90	✓	✓	✓
	BMS1967	101.9	90	✓	✓	✓

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
10	CSSM38	0.0	94	✓	✓	✓
	BMS528	12.0	91	✓	✓	✓
	BMS861	30.3	87	✓	✓	✓
	BM875	39.5	81			✓
	BM888	43.4	86	✓	✓	✓
	BMS1620	64.7	87	✓	✓	✓
	TGLA272	79.8	80	✓	✓	
	BMS2614	91.4	75	✓		✓
11	BM827	0.0	55	✓		
	BMS2131	9.5	68			✓
	BMS2325	11.7	68			✓
	BM304	24.4	95	✓	✓	✓
	RM096	31.3	91	✓		✓
	INRA111	43.0	87			✓
	BMS1716	47.7	94	✓	✓	✓
	BMS1822	61.2	93	✓		✓
	RM150	65.5	92	✓	✓	✓
	BMS1048	75.8	84		✓	✓
	BMS989	85.5	91	✓	✓	✓
	RM363	90.9	94	✓	✓	✓
	BMS2315	103.3	90	✓	✓	✓
	HEL13	114.5	65			✓
12	BMS410	0.0	87	✓	✓	✓
	BM6108	15.8	78	✓	✓	
	AGLA226	37.5	70		✓	✓
	BM6404	56.0	92	✓	✓	✓
	BMS975	61.2	87		✓	✓
	RM113	78.3	83	✓	✓	✓
	BMS1316	98.7	72		✓	✓
13	TGLA23	0.0	92	✓	✓	✓
	BMS1742	15.0	89	✓	✓	✓
	ILSTS59	34.0	96	✓	✓	✓
	HUJ616	43.8	94	✓	✓	✓
	BMS1669	49.5	81		✓	
	RM327	62.1	84	✓	✓	
	BL1071	68.6	90	✓	✓	✓
	AGLA232	79.5	86	✓	✓	
	BMS2319	85.3	86	✓	✓	✓
14	BMS1678	0.0	87	✓	✓	✓
	ILSTS011	4.4	81			✓
	RM011	21.5	87		✓	✓
	ILSTS008	29.0	89	✓		✓
	BM302	30.7	89	✓		✓
	BMS740	38.0	84			✓
	BMS108	44.6	87	✓	✓	
	BM4513	56.3	94	✓	✓	✓
	BL1036	72.5	61		✓	

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
15	BR3510	0.0	77	✓	✓	
	BMS2533	4.2	80	✓		✓
	MAF65	21.4	67			✓
	HEL1	26.7	79	✓	✓	
	JAB4	27.6	77			✓
	POTCHA	58.6	93		✓	✓
	BM4325	58.9	93	✓	✓	✓
	BMS812	67.8	96	✓	✓	✓
	TGLA75	71.8	89		✓	
	BMS820	80.6	91	✓	✓	✓
	BM848	80.6	91	✓		
	BMS429	93.0	74	✓		✓
16	BMS357	0.0	79	✓	✓	✓
	HUJ614	4.4	69		✓	
	BM1311	23.4	66		✓	✓
	BMS1907	39.1	72		✓	
	CSSM28	49.4	89	✓	✓	✓
	BM719	70.9	92	✓	✓	✓
	BM3509	77.4	97	✓	✓	✓
	INRA13	80.8	89		✓	
	HUJ625	82.4	87	✓	✓	
17	RM156	0.0	88	✓	✓	✓
	BMS2220	16.2	84	✓	✓	
	BMS941	30.1	92	✓	✓	✓
	OARFCB48	41.8	88	✓	✓	✓
	ILSTS23	54.3	78	✓		✓
	BM8125	73.9	81	✓		✓
	BL50	79.9	79		✓	
	BM1862	86.3	81	✓	✓	
	BM1233	98.6	82	✓	✓	✓
18	BMS1355	0.0	75	✓	✓	✓
	TEXAN10	18.2	86	✓	✓	✓
	INRA121	29.0	84		✓	✓
	BM8151	38.7	86	✓		✓
	INRA63	45.9	92	✓	✓	
	BM7109	46.8	93	✓	✓	✓
	ILSTS2	53.1	83	✓		
	TGLA227	78.6	90	✓	✓	✓
19	BM9202	0.0	56	✓		✓
	HEL10	15.9	62	✓		✓
	BMS2142	44.7	75		✓	✓
	BP20	46.5	75			✓
	BM17132	58.6	78	✓		✓
	ETH3	81.5	88	✓	✓	✓
	RM388	85.9	81			✓
	BMS601	99.5	86	✓	✓	✓

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
20	RM106	0.0	74		✓	
	BM1225	5.3	86	✓	✓	✓
	TGLA304	17.3	77		✓	✓
	TGLA126	28.5	76	✓		✓
	BM4107	49 .7	93	✓	✓	✓
	BMS1120	51.1	94	✓	✓	✓
	BMS703	52.2	92		✓	
	BM5004	61.6	91	✓	✓	✓
	BMS521	72.3	75		✓	✓
21	BM8115	0.0	85	✓	✓	✓
	RM151	11.7	85		✓	
	BM3413	15.0	89	✓	✓	✓
	AGLA233	20.4	86		✓	✓
	BM103	30.5	90	✓	✓	✓
	BMC4228	39.8	82	✓		✓
	ILSTS016	50.0	78		✓	
	TGLA122	67.3	87	✓	✓	✓
	BMS743	81.5	75		✓	✓
22	INRA26	0.0	86	✓	✓	✓
	BM1558	14.4	81	✓	✓	✓
	BM1303	24.0	80		✓	
	AGLA13	26.5	80	✓	✓	
	BMS390	43.3	76	✓		
	BM2613	48.4	85	✓	✓	✓
	BMS875	58.2	70	✓		
	HMH1R	74.1	58		✓	
	BM4102	76.5	56			✓
23	INRA132	0.0	39	✓		
	CYP21	36.0	89	✓	✓	✓
	BM1818	50.9	72			✓
	BP34	57.5	75	✓		
	BM1905	64.3	85		✓	✓
	BM1443	67.2	86	✓	✓	
24	BM7151	0.0	90	✓	✓	✓
	CSSM31	19.2	85	✓	✓	✓
	BMS1743	34.7	85	✓	✓	✓
	INRA90	47.2	62		✓	
25	BM4005	0.0	84	✓	✓	✓
	BP28	10.3	79			✓
	BM737	15.5	84	✓	✓	✓
	BMS1353	33.0	74		✓	✓
26	BMS651	0.0	88	✓	✓	✓
	BM1314	22.3	80	✓	✓	✓
	BM6041	46.7	75		✓	
	MAF92	53.7	62	✓	✓	
	ILSTS091	67.4	44	✓		

Chrom.	Marker	Pos. cM	% Inf. Prog.	Ryan	Lou	Tom
27	BMS2168	0.0	81	✓	✓	✓
	BM6526	9.1	74		✓	✓
	CSSM43	34.1	90	✓	✓	✓
	INRA134	45.5	73			✓
	BM203	64.1	92	✓	✓	✓
28	BMC6020	0.0	88	✓	✓	✓
	BL25	15.3	85	✓	✓	✓
	BM6466	33.3	87		✓	✓
	BMS1714	39.6	90	✓	✓	✓
	BMC2208	49.9	74	✓		✓
29	BMS1857	0.0	92	✓	✓	✓
	BMS764	8.8	91	✓	✓	✓
	OARVH110	23.0	83		✓	✓
	OARHH22	40.6	87	✓	✓	✓
	BMC1206	57.0	77		✓	✓
	BMS1948	60.1	75		✓	✓

Appendix C

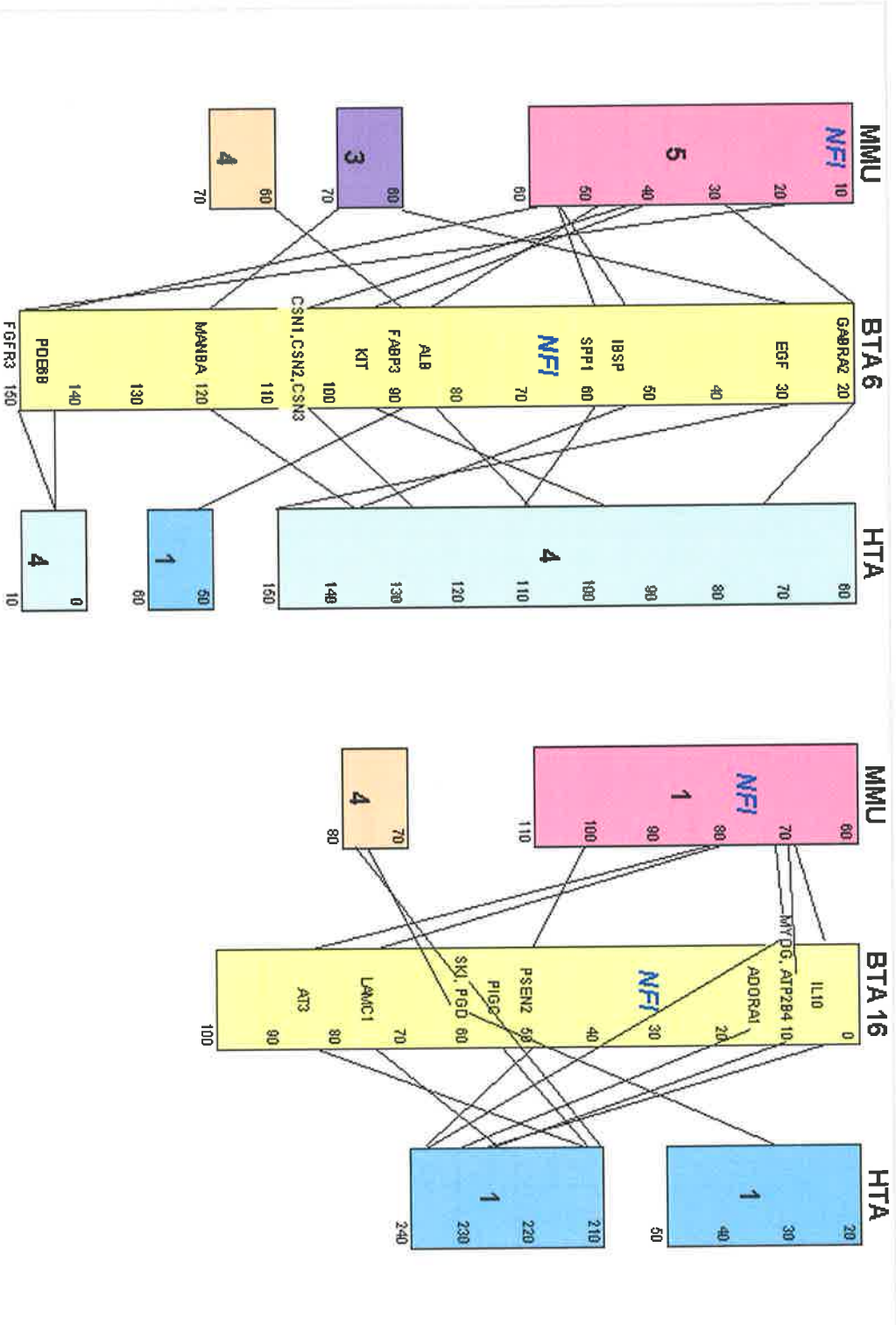


Figure C.1 Bovine (BTA 6 and 16), human and mouse comparative maps with NFI QTL. Gene positions from BOVMAP, GDB and MGI databases.

Publications

Refereed Publications

Chapter 6. The effect of incomplete data on the estimate of feed intake in beef cattle

Fenton, M.L., W.S. Pitchford, P.F. Arthur, J.A. Archer, R.M. Herd, and C.D.K. Bottema (2004) "The effect of missing data on the estimate of average daily feed intake in beef cattle". Australian Journal of Experimental Agriculture (in press).

Conference Proceedings

Chapter 4. Studies on the metabolic basis for feed efficiency

Symonds, E.L., **Fenton, M.L.,** Tivey, D., Omari, T.I., and Butler, R.N. (2003) "Gastric emptying is accelerated in a mouse model of high food intake". Journal of Gastroenterology and Hepatology, S11.

Chapter 5. Mapping QTL for feed efficiency in mice

Fenton, M.L., C.D.K. Bottema and W.S. Pitchford (2000) "Mapping feed intake genes in mice". 27th International Society of Animal Genetics , pg 37

Fenton, M.L., C.D.K. Bottema, and W.S. Pitchford (2001) "Mapping quantitative trait loci for feed efficiency in mice – a preliminary analysis". 14th Australian Association of Animal Breeding and Genetics, 179-182.

Bottema, D.K.D., **M.L. Fenton**, R. Afolayan, and W.S Pitchford (2002) "Comparative QTL mapping in mice for livestock production traits". 49th GSA, pg16.

Chapter 8. Mapping QTL for feed efficiency in cattle

Pitchford, W.S.P., **M.L. Fenton**, A.J. Kister, and C.D.K. Bottema. (2002) "QTL for feed intake and associated traits". 7th World Congress of Genetics Applied to Livestock Production, CD-ROM communication n° 10-15.

References

References

Albrecht, U., Z. S. Sun, G. Eichele and C. C. Lee (1997). "A differential response of two putative mammalian circadian regulators, mper1 and mper2 , to light." Cell 91(7): 1055-1064.

Alexander, R. C., R. Wright and W. Freed (1996). "Quantitative trait loci contributing to phencyclidine-induced and amphetamine-induced locomotor behavior in inbred mice." Neuropsychopharamacology 15(5): 484-490.

Allan, M. F., Nielsen, M.K. and Pomp, D.P. (2000). "Gene expression in hypothalamus and brown tissue of mice divergently selected for heat loss." Physiological Genomics 3: 149 - 156.

Allen, M. S. (1996). "Physical contraints on voluntary intake of forages by ruminants." Journal Animal Science 74: 3063-3075.

Anderson, V. N. and G. K. Smith (1987). "Effects of feeding and light cycles on activity rhythms of maternally isolated rat pups." Physiology and Behavior 39(2): 169-181.

Andersson, L., I. Edsfors-Lilja, H. Ellegren, M. Johansson and L. Marklund (1994). "Mapping trait loci by crossbreeding genetically divergent populations of domestic animals." Animal Biotechnology 2: 255-231.

Anunciado, R. V. P., T. Ohno, M. Mori, A. Ishikawa, S. Tanaka, F. Horio, M. Nishimura and T. Namikawa (2000). "Distribution of body weight, blood insulin and lipid levels in the SMXA recombinant inbred strains and the QTL analysis." Experimental Animals 49(3): 217-224.

ARC (1980). The nutrient requirements of ruminant livestock. Commonwealth Agriculture Bureaux. Slough, UK.

Archer, J. A. (1996). Genetic variation in the efficiency of feed utilisation by animals. Animal Science. Adelaide, Australia, University of Adelaide.

Archer, J. A., P. F. Arthur, R. M. Herd, P. F. Parnell and W. S. Pitchford (1997). "Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle." Journal of Animal Science 75(8): 2024-2032.

Archer, J. A. and S. A. Barwick (1999). Economic analysis of net feed intake in industry breeding schemes. Proceedings of the 13th Conference of the Association for the Advancement of Animal Breeding and Genetics, Mandurah, Western Australia,, AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 13:337-340.

Archer, J. A. and L. Bergh (2000). "Duration of performance tests for growth rate, feed intake and feed efficiency in four biological types of beef cattle." Livestock Production Science 65(1-2): 47-55.

Archer, J. A. and W. S. Pitchford (1996). "Phenotypic variation in residual food intake of mice at different ages and its relationship with efficiency of growth, maintenance and body composition." Animal Science 63(1): 149-157.

Archer, J. A., E. C. Richardson, R. M. Herd and P. F. Arthur (1999). "Potential for selection to improve efficiency of feed use in beef cattle: A review." Australian Journal of Agricultural Research 50(2): 147-161.

Archer, J. A., S. A. Barwick and H.-U. Graser (2004). "Economic evaluation o fbeef cattle breeding schemes incorporating performance testing of young bulls for feed intake." Australian Journal of Experimental Agriculture 44 (4-5): 393-404.

ArkDB, B. (2002). "Bovine ArkDB - http://texas.thearkdb.org/browser?species=cow."

Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson and P. F. Parnell (2001a). "Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle." Journal of Animal Science 79: 2805-2811.

Arthur, P. F., R. M. Herd, J. Wright, G. Xu, K. Dibley, E. C. Richardson, D. P. Poppi, D. J. Farrell and K. F. Lowe (1996). "Net feed conversion efficiency and its relationship with other traits in beef cattle." Animal Production in Australia. Proceedings of the Australian Society of Animal Production 21: 107-110.

Arthur, P. F., G. Renand and D. Krauss (2001b). "Genetic parameters for growth and feed efficiency in weaner versus yearling Charolais bulls." Australian Journal of Agricultural Research 52(4): 471-476.

Arthur, P. F., G. Renand and D. Krauss (2001c). "Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls." Livestock Production Science 68(2-3): 131-139.

Asakawa, A., A. Inui, N. Ueno, M. Fujimiya, M. A. Fujino and M. Kasuga (1999). "Mouse pancreatic polypeptide modulates food intake, while not influencing anxiety in mice." Peptides 20(12): 1445-1448.

Banks, R. (1994). Lambplan: Genetic evaluation for the Australian Lamb Industry. 5th World Congress of Genetics Applied to Livestock Production., University of Guelph, Ontario, Canada.

Banks, W. A., A. J. Kastin, W. Huang, J. B. Jaspan and L. M. Maness (1996). "Leptin enters the brain by a saturable system independent of insulin." Peptides 17(2): 305-311.

Barendse, W., S. M. Armitage, et al. (1994). "A genetic linkage map of the bovine genome." Nature Genetics 6(3): 227-235.

Barendse, W., D. Vaiman, et al. (1997). "A medium-density genetic linkage map of the bovine genome." Mammalian Genome 8(1): 21-28.

Barlow, R. (1978). " Biological ramifications of selection for pre-weaning growth in cattle - a review." Animal Breeding Abstracts 46: 469-491.

Barwick, S. A., H. U. Graser and J. A. Archer (1999). Recording new beef performance measures - effects on the accuracy of selection for profitability. Proceedings of the 13th Conference of the Association for the Advancement of Animal Breeding and Genetics, Mandurah, Western Australia, AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 13:203-206

Barwick, S. A., A. L. Henzell and H. U. Graser (1994). Developments in the construction and use of selection indexes for genetic evaluation of beef cattle in Australia. 5th World Congress of Genetics Applied to Livestock Production.

Basten, C. S., B. S. Weir and Z.-B. Zeng (2001). QTL Cartographer. Raleigh, N.C., Department of Statistics, North Carolina State University.

Bateman, D. N. and T. A. Whittingham (1982). "Measurement of gastric emptying by real-time ultrasonography." Gut 23: 524-427.

Baumann, H., K. K. Morella, D. W. White, M. Dembski, P. S. Bailon, H. Kim, C. F. Lai and L. A. Tartalia (1996). "The full-length leptin receptor had signalling capabilities of interleukin 6-type cytokine receptors." Proceedings of the National Academy of Sciences of the United States of America. 93(16): 8374-8378.

Baxter, R. C. (2000). "Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities." American Journal of Physiology 278: E967-E976.

Bernier, J. F. and C. C. Calvert (1987). "Effect of a major gene for growth on protein synthesis in mice." Journal Animal Science 65: 982-995.

Bernier, J. F., C. C. Calvert and R. L. Baldwin (1987). "Energetics of protein synthesis in mice with a major gene for growth." Journal of Nutrition 117(12): 2036-2045.

Biondini, P. E., T. M. Sutherland and L. H. Haverland (1968). "Body composition of mice selected for rapid growth rate." Journal of Animal Science 27: 5-12.

Bishop, S. C. and W. G. Hill (1985). "Effects of selection on growth, body composition, and food intake in mice. III. Correlated responses: growth, body composition, food intake and efficiency and catabolism." Genetical Research 46(1): 57-74.

Bishop, S. C., S. King, W. G. Hill and A. Robertson (1984). Selection for components of growth and food intake in mice. 35th Annual Meeting of the EAAP,, The Hague, Netherlands,, The Hague.

Bjorkeman, D. J., J. G. Moore, P. D. Klein and D. Y. Graham (1991). "13C-Bicarbonate breath test as a measure of gastric emptying." American Journal of Gastroenterology 86: 821-823.

Blaxter, K. L. and F. W. Wainman (1966). "The fasting metabolism of cattle." British Journal of Nutrition 20: 103-111.

Bolonid, L., M. Bortolotti, V. Santi, T. Calleti, S. Gaiani and G. Labo (1985). "Measurement of gastric emptying time by real-time ultrasonography." Gastroenterology 89: 752-759.

Bordas, A. and F. Minvielle (1997). "Effects of temperature on egg laying hens from divergent lines selected on residual feed consumption." Genetics, Selection, Evolution 29(3): 279-290.

Bordas, A., M. Tixier-Boichard and P. Merat (1992). "Direct and correlated responses to divergent selection for residual food intake in Rhode Island red laying hens." British Poultry Science 33: 741-754.

Bosch, M. W., S. C. W. Lammers-Wienhoven, G. A. Bangma, H. Boer and P. W. m. van Adrichem (1992). "Influence of stage of maturity of grass silages on digestion processes in dairy cows. 2. Rumen contents, passage rates, distrivbution of rumen and faecal particles and mastication activity." Livestock Production Science 32: 265-281.

Bovine Genome Database (2002). "Bovine Genome Database - http://bos.cvm.tamu.edu/bovgbase.html."

Bovmap Database (2002). Bovmap Database - http://locu.jouy.inra.fr/cgi-bin/lgbc/mapping/common/main.pl?BASE. 2002.

Bracher, V., R. Steiger and S. Huser (1995). "Preliminary results of combined xylose absorption hydrogen excretion test in horses with chronic intestinal disease." Archiv fur Tierheikunde 137: 295-305.

Braden, B., S. Adams and L. Duan (1995). "The [13C]acetate breath test accurately reflects gastric emptying of liquids and semisolid test meals." Gastroenterology 108: 1048-1055.

Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemin (1973). "Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone." Science 173: 77-79.

Brelin, B. and E. Brannang (1982). "Phenotypic and genetic variation in feed efficiency of growing cattle and their relationship with growth rate, carcass traits, and metabolic efficiency." Swedish Journal of Agricultural Research 12: 29-34.

Brockmann, G. A., C. S. Haley, U. Renne, S. A. Knott and M. Schwerin (1998). "Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth." Genetics 150(1): 369-381.

Brockmann, G. A., J. Kratzsch, C. S. Haley, U. Renne, M. Schwerin and S. Karle (2000). "Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F2 variance of growth and obesity in DU6i x DBA/2 mice." Genome Research 10(12): 1941-1957.

Brody, S. (1945). Bioenergetics and Growth. New York, Reinhold Publishing Corporation.

Brown, M. A., R. R. Frahm and R. R. Johnson (1977). "Body composition of mice selected for preweaning and postweaning growth." Journal Animal Science 45(1): 18-23.

Bunger, L., M. G. Macleod, C. A. Wallace and W. G. Hill (1998). Direct and correlated effects of selection for food intake corrected for body weight in the adult mouse. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia,, World Congress on Genetics Applied to Livestock Production.; Armidale; Australia.

Butler, R. N. (1996). "On the breath." Today's Life Science July: 36-40.

Buttazzoni, L. G. and I. L. Mao (1989). "Genetic parameters of estimated net feed efficiencies for milk production, maintenance and body weight change in dairy cows." Journal of Dairy Science 72: 671-677.

Cameron, N. D. (1998). Across species comparison in selection for feed efficiency. Proceedings of the 6th world congress of genetics applied to livestock production., Armidale, Australia.

Cameron, N. D., G. R. Nute, S. N. Brown, M. Enser and J. D. Wood (1999). "Meat quality of Large White pig genotypes selected for components of efficient lean growth rate." Animal Science 68(1): 115-127.

Carlson, J. R. (1969). Growth regulators. Animal growth and nutrition. Philadelphia, Lea and Febiger.

Casas, E., S. D. Shackelford, J. W. Keele, R. T. Stone, S. M. Kappes and M. Koohmaraie (2000). "Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin." Journal of Animal Science 78(3): 560-569.

Castle, W. E. (1921). "An improved method of estimating the number of genetic factors concerned in cases of blending inheritance." Proceedings of the National Academy of Science 81: 6904-6907.

CGO (1996). "Comparative genome organization, First international workshop." Mammalian Genome 7: 717-734.

Chan, C. P., R. J. Hansen and J. S. Stern (1985). "Protein turnover in insulin-treated, allocan-diabetic lean and obese Zucker rats." Journal of Nutrition 115(8): 959-969.

Cheek, D. B., A. B. Holt, D. E. Hill and J. L. Talbert (1971). "Skeletal muscle cell mass and growth: the concept of the deoxyribonucleic acid unit." Pediatric Research 5: 312-328.

Cheverud, J. M., E. J. Routman, F. A. M. Duarte, B. v. Swinderen, K. Cothran, C. Perel and B. Van Swinderen (1996). "Quantitative trait loci for murine growth." Genetics 142(4): 1305-1319.

Churchill, G. A. and R. W. Doerge (1994). "Empirical threshold values for quantitative trait mapping." Genetics 138: 963-971.

Clifton, P. G. (2000). "Meal patterning in rodents: psychopharmacological and neuroanatomical studies." Neuroscience and Biobehavioral Reviews 24(2): 213-222.

Cole, J. L., A. Ross and J. Bodnar (2000). "Dietary history affects the potency of chronic opiod receptor subtype antagonist effects upon body weight in rats." Nutritional Neuroscience 1: 405-417.

Coleman, D. L. (1973). "Effects of parabiosis of obese with diabetes and normal mice." Diabetologia 9(4): 294-298.

Conover, K. L., H. P. Weingarten and S. M. Collins (1987). "A procedure for within trial repeated measurement of gastric emptying in the rat." Physiology & Behavior 39: 303-308.

Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohannesian, C. C. Marco, L. J. McKee, T. L. Bauer and J. F. Caro (1996). "Serum immunoreactive-leptin concentrations in normal-weight and obese humans." New England Journal of Medicine 334(5): 292-295.

Cooper, S. J. and S. Turkish (1989). "Effects of naltrexone on food preferance and concurrent behavioral responses in food-deprived rats." Pharmacology Biochemistry and Behavior 33: 17-20.

Cusin, I., A. Sainsbury, P. Doyle, F. Rohner-Jeanrenaud and A. Jeanrenaud (1995). "The Ob gene and insulin." Diabetes 44: 1467-1470.

Dado, R. G. and M. S. Allen (1994). "Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows." Journal of Dairy Science 77(1): 132-144.

Danicke, S., R. Nieto, G. E. Lobley, M. F. Fuller, D. S. Brown, E. Milne, A. G. Calder, S. Chen, I. Grant and W. Bottcher (1999). "Responses in the absorptive phase in muscle and liver protein synthesis rates of growing rats." Archives of Animal Nutrition 52(1): 41-52.

Darvasi, A. (1997). "The effect of selective genotyping on QTL mapping accuracy." Mammalian Genome 8(1): 67-68.

Darvasi, A. and M. Soller (1992). "Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus." Theoretical and Applied Genetics 85(2-3): 353-359.

Darvasi, A. and M. Soller (1994). "Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus." Genetics 138(4): 1365-1373.

Darvasi, A. and M. Soller (1997). "A simple method to calculate resolving power and confidence interval of QTL map location." Behavior Genetics 27(2): 125-132.

Dauncey, M. J. (1986). "Activity-induced thermogenesis in lean and genetically obese (ob/ob) mice." Experientia 42(5): 547-549.

Dauncey, M. J. and D. Brown (1987). "Role of activity-induced thermogenesis in twenty-four hour energy expenditure of lean and genetically obese (OB/OB) mice." Quarterly Journal of Experimental Physiology 72: 549-559.

Davis, G. P. and S. K. DeNise (1998). "The impact of genetic markers on selection." Journal Animal Science 76: 2331-2339.

Davis, M. E. and R. C. M. Simmen (2000). "Genetic parameter estimates for serum insulin-like growth factor-1 concentration and carcass traits in Angus beef cattle." Journal of Animal Science 78(9): 2305-2313.

de Gortari, M. J., B. A. Freking, R. P. Cuthbertson, S. M. Kappes, J. W. Keele, R. T. Stone, K. A. Leymaster, K. G. Dodds, A. M. Crawford and C. W. Beattie (1998). "A second-generation linkage map of the sheep genome." Mammalian Genome 9: 204-209.

de Haer, L. C. M. (1992). "Feed intake behaviour affects performance." Pigs 8(4): 23.

de Haer, L. C. M., P. Luiting and H. L. M. Aarts (1993). "Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing.'" Livestock Production Science 36: 233-253.

de Vries, A. G. and E. Kanis (1994). "A growth model to estimate economic values for food intake capacity in pigs." Animal Production 55(2): 241-246.

Demarest, K. (2001). "Further characterization and high-resolution mapping of quantitative trait loci for ethanol-induced locomotor activity." Behavior Genetics 31(79-91).

Demchyshyn, L. L., F. McConkey and H. B. Niznik (2000). "Dopamine D5 receptor agonist high affinity and constitutive activity profile conferred by carboxyl-terminal tail sequence." Journal of Biological Chemistry 275(31): 23446.

Devane, W. A., L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Etinger and R. Mechoulam (1992). "Isolation and structure of a brain constituent that binds to the cannabinoid receptor." Science 258(1946-1949).

Dib, C. and S. Faure (1996). "A comprehensive genetic map of the human genome based on 5264 microsatellites." Nature 380(6570): 152-154.

Dickerson, G. E. (1970). "Efficiency of animal production - molding the biological components." Journal of Animal Science 30: 849-859.

Dickson, D. P., G. R. Barr, L. P. Johnson and D. A. Wieckert (1970). "Social dominance and temperament of Holstein cows." Journal of Dairy Science 53: 904-909.

Dietrich, W., H. Katz, S. E. Lincoln, H. S. Shin, J. Friedman, N. C. Dracopoli and E. S. Lander (1992). "A genetic map of the mouse suitable for typing intraspecific crosses." Genetics 131(2): 423-447.

Dietrich, W. F., J. Miller, et al. (1996). "A comprehensive genetic map of the mouse genome." Nature London 380(6570): 149-152.

Diggle, P. (1990). Time Series: A Biostatistical Introduction. Oxford, Claredon Press.

Ding, S. T., A. P. Schinckel, T. E. Weber and H. J. Mersmann (2000). "Expression of porcine transcription factors and genes related to fatty acid metabolism in different tissues and genetic populations." Journal of Animal Science 78(8): 2127-2134.

Doerge, R. W. and G. A. Churchill (1996). "Permutation tests for multiple loci affecting a quantitative character." Genetics 142: 285-294.

Dourish, C. T., W. Rycroft and S. D. Iversen (1989). "Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors." Science Washington 245(4925): 1509-1511.

Dove, H. and R. W. Mayes (1991). "The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review." Aust. J. Agric. Res. 42(6): 913-952.

Dransfield, E. (1999). Meat tenderness - the u-calpain hypothesis. 45th International Congress of Meat Science and Technology.

Dryden, S., H. Frankish, Q. Wang and G. Williams (1994). "Neuropeptide Y and energy balance: one way ahead for treatment of obesity?" Eur. J. Clin. Invest. 246: 293-308.

Eckstein, F., T. Pavicic, S. Nedbal, U. Wehr, E. M. Lochmuller, E. Wolf and A. Hoeflich (2001). Insulin-like growth factor (IGF)-binding protein-2 overexpression regulates bone growth in vivo and suggest growth hormone action via local IGF. Orthopaedic Research Society, San Francisco, California.

Eggen, A. and R. Fries (1995). "An integrated cytogenetic and meiotic map of the bovine genome." Animal Genetics 26(4): 215-236.

Eisen, E. J., B. J. Lang and J. E. Legales (1969). "Comparison of growth functions within and between lines of mice selected for large and small body weight." Theoretical and Applied Genetics 39: 251-260.

Eissen, J. J., A. G. de Haan and E. Kanis (1999). "Effect of missing data on the estimate of average daily feed intake of growing pigs." Journal of Animal Science 177: 1372-1378.

Elo, K., S. Wesolowski, M. K. Nielsen, L. D. Van Vleck and D. Pomp (2002). Fine mapping of genes regulating heat loss in mice. Proceedings of the 7th world congress on genetics applied to livestock production, Montpelier, France.

Emery, R. S. (1969). Lipids and adipose tissue. Animal growth and nutrition. Philadelphia, Lea and Febiger: 236-254.

Erickson, J. C., C. E. Clegg and R. D. Palmiter (1996). "Sensitivity to leptin ans susceptibility to seizures in mice lacking neuropeptide Y." Nature 381: 4115-4118.

Essen, P., M. A. Mcnurlan, J. Wernerman, E. Milne, E. Vinnars and P. J. Garlick (1992). "Short-term starvation decreases skeletal muscle protein synthesis rate in man." Clinical Physiology 12: 287-299.

Essen-Gustavsson, B. and S. Fjelkner-Modig (1985). "Skeletal muscle characteristics in different breeds of pigs in relation to sensory properties of meat." Meat Science 13(1): 33-47.

Essen-Gustavsson, B., K. Karlstrom and K. Lundstrom (1992). "Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality." Meat Science 31(1): 1-11.

Essen-Gustavsson, B. and A. Lindholm (1983). Influence of exercise on muscle metabolic characteristics in pigs and on backfat thickness at slaughter. Proceedings of the Fifth International Conference on Production Disease in Farm Animals., Uppsala; Sweden, Swedish University of Agricultural Sciences.

Ewen, K. R., M. Bahlo, S. A. Treloar, D. F. Levinson, B. Mowry, J. W. Barlow and S. J. Foote (2000). "Identification and analysis of error types in high-throughput genotyping." American Journal of Human Genetics 67: 727-736.

Exton, S. (2001). Testing beef cattle for net feed efficiency - standards manual. NSW Agriculture, Trangie Agricultural Centre, NSW.

Fahey, G. C. and L. L. Berger (1988). Digestive physiology and nutrition. The ruminant animal. D. C. Church. Englewood, NJ, Prentice-Hall: 269-297.

Fan, L. Q., D. R. C. Bailey and N. H. Shannon (1995). "Genetic parameter estimation of postweaning gain, feed intake and feed efficiency for Hereford and Angus bulls fed two different diets." Journal of Animal Science 73(2): 365-372.

Fenton, M. L. (1997). The effect of pre-weaning nutrient supply on post-weaning growth and efficiency. Honours Thesis, University of Adelaide, Adelaide, Australia: 119.

Ferrell, C. L. and T. G. Jenkins (1984). "Energy utilisation by mature, non-pregnant, non-lactating cows of different types." Journal Animal Science 58: 234-243.

Ferrell, C. L. and T. G. Jenkins (1985). "Cow type and the nutritional environment: nutritional aspecets." Journal of Animal Science 61: 725-711.

Ferretti, L., B. G. D. Urquhart, et al. (1997). "Cosmid-derived markers anchoring the bovine genetic map to the physical map." Mammalian Genome 8(1): 29-36.

Figlewicz, D. P., M. W. Shwartz, R. J. Seeley, M. Chavez, D. G. Baskin, S. C. Woods and D. Porte (1996). "Endocrine regulation of food intake and body weight." Journal of Laboratory and Clinical Medicine 127: 328-332.

Fisher, A. D., C. A. Morris, L. R. Matthews and S. W. Peterson (2000). "Cattle behaviour: comparison of measures of temperament in beef cattle." Proceedings of the New Zealand Society of Animal Production 60: 214-217.

Flint, J., R. Corley, J. C. DeFries, D. W. Fulker, J. A. Gray, S. Miller and A. C. Collins (1995). "A simple genetic basis for a complex physiological trait in laboratory mice." Science 269: 1432-1435.

Forbes, J. M., J. A. Wright and A. Bannister (1972). "A note on the rate of eating in sheep." Animal Production 15: 211-214.

Fordyce, G. and M. E. Goddard (1984). "Maternal influence on the temperament of Bos indicus cross cows." Proceedings of the Australian Society of Animal Production 15: 345-348.

Fordyce, G., J. R. Wythes, W. R. Shorthose, D. W. Underwood and R. K. Shepherd (1988). "Cattle temperaments in extensive beef herds in northern Queensland. 2. Effect of temperament on carcass and meat quality." Australian Journal of Experimental Agriculture 28(6): 683-693.

Foster, W. H., D. J. Kilpatrick and I. H. Heaney (1983). " Genetic variation in the efficiency of energy utilisation by the fattening pig." Animal Production 37: 387-393.

Fraser, D. (1995). Behavioral concepts important for environmental design. Animal Behavior and the Design of Livestock and Poultry Systems. Indianapolis, Natural Resources, Agriculture, and Engineering Services Publ.: 30-44.

Frederich, R. C., B. Lollmann, A. Hamann, A. Napolitano Rosen, B. B. Kahn, B. B. Lowell and J. S. Flier (1995). "Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity." Journal of Clinical Investigation 96(3): 1658-1663.

Fries, R. (1993). "Mapping the bovine genome: methodological aspects and strategy." Animal Genetics 24(2): 111-116.

Frisch, J. E. and J. E. Vercoe (1969). "Liveweight gain, food intake and eating rate in Brahman, Africander and Shorthorn x Hereford cattle." Australian Journal of Agricultural Research 20: 1189-1195.

Frisch, J. E. and J. E. Vercoe (1984). "An analysis of growth of different cattle genotypes reared in defferent environments." Journal of Agricultural Science 103: 137-153.

Fujii, J., K. Otsu, F. Zorzato, S. de Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien and D. H. MacLennan (1991). "Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia." Science 253(5018): 448-451.

Gabarrou, J. F., P. A. Geraert, M. Picard and A. Bordas (1997). "Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake." Journal of Nutrition 127(12): 2371-2376.

Garlick, P. J. (1980). Protein turnover in the whole animal animal and specific tissues. Comprehensive Biochemistry, Elsevier Scientific Publishing. 19 B Protein Metabolism: 77-135.

Garlick, P. J., D. J. Millward and W. P. T. James (1973). "The diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats." Biochemical Journal 136(4): 935-945.

Garlick, P. J., D. J. Millward, W. P. T. James and J. C. Waterlow (1975). "The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat." Biochimica et Biophysica Acta 414(1): 71-84.

Garlid, K. D., Jaburek, M., and Jezek, P. (1998). "The mechanism of proton transport mediated by mitochondrial uncoupling proteins." FEBS Letters 438: 10-14.

Geiser, F., G. Kortner and I. Schmidt (1998). "Leptin increases energy expenditure of a marsupial by inhibition of daily torpor." American Journal of Physiology Reg. Int. Comp. Biol(44): R1627-R1632.

Gelernter, J., D. Vandenbergh, S. D. Kruger, D. L. Pauls, R. Kurlan, A. J. Pakstis, K. K. Kidd and G. Uhl (1995). "The dopamine transporter protein gene (SLC6A3): primary linkage and mapping studies in Tourette syndrome." Genomics 30(3): 459-463.

Gellin, J., S. Brown, J. A. Marshall Graves, M. Rothschild, l. Schook, J. Womack and M. Yerle (2000). "Comparative gene mapping workshop: progress in agriculturally important animals." Mammalian Genome 11: 140-144.

Genstat (1989). Genstat 5 Reference Manual. Oxford, Claredon Press.

Georges, M. and L. Andersson (1996). "Livestock genomics comes of age." Genome Research 6(10): 907-921.

Gerald, C., M. W. Walker, L. Criscione, E. L. Gustafson, C. Batzl-Hartmann, K. E. Smith, P. Vaysse, M. M. Durkin, T. M. Laz, D. L. Linemeyer, A. O. Schaffhasuer, S. Whitebread, K. G. Hofbauer, R. I. Taber, T. A. Branchek and R. L. Weinshank (1996). "A receptor subtype involved in neuropeptide-Y-induced food intake." Nature 382(6587): 168-171.

Gershenfeld, H. K., P. E. Neumann, C. Mathis, J. N. Crawley, X. Li, S. M. Paul and X. Li (1997). "Mapping quantitative trait loci for open-field behavior in mice." Behavior Genetics 27(3): 201-210.

Ghoos, F., B. D. Maes and Geypens B. (1993). "Measurements of gastric emptying rate of solids by means of a carbon-labeld octanoic acid breath test." Gastroenterology 104: 1640-1647.

Gilja , O. H., P. R. Detmer and J. M. Jong (1997). "Intragastric distribution and gastric emptying assessed by three-dimensional ultrasonosgraphy." Gastroenterology 113: 38-49.

Gill, K., A. Boyle, K. Lake and N. Desaulniers (2000). "Alcohol-induced locomotor activation in C57BL/6J. A/J, and AXB/BXA recombinant inbred mice: strain distribution patterns and quantitative trait loci analysis." Psychopharmacology 150: 412-421.

Gimelfarb, A. and R. Lande (1994a). "Simulation of marker-assisted selection in hybrid populations." Genetic Research 63: 39-47.

Gimelfarb, A. and R. Lande (1994b). "Simulation of marker-assisted for non-additive traits." Genetic Research 64: 127-136.

Gimelfarb, A. and R. Lande (1995). "Marker-assisted selection and marker -QTL associations in hybrid populations." Theoretical and Applied Genetics 91: 522-528.

Gosnell, B. A., D. D. Krahn and M. J. Majchrzak (1990). "The effects of morphine on diet selection are dependent upon baseline diet preferences." American Journal of Physiology 279(R86-R92).

Grant, R. J. and J. L. Albright (2001). "Effect of animal grouping on feeding behavior and intake of dairy cattle." Journal of Dairy Science 84(Elect. Supplement): E156-E163.

Graser, H. U., G. Nitter and S. A. Barwick (1994). "Evaluation of advanced industry breeding schemes for Australian beef cattle. II. Selection on combinations of growth, reproduction and carcase criteria." Australian Journal of Agricultural Research 45(8): 1657-1669.

Green, P., K. Falls and S. Crooks (1990). CRI-MAP documentation.

Greenwood, M. R. (1985). "The relationship of enzyme activity of feeding behavior in rats: lipoprotein lipase as the metabolic gatekeeper." International Journal of Obesity 9: 67-70.

Griffith, G. H., G. M. Owen, S. Kirkman and R. Shields (1966). "Measurement of rate of gastric emptying using chromium-51." Lancet i: 1244-1245.

Grobet, L., L. J. R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset and M. Georges (1997). "A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle." Nature Genetics 17(1): 71-74.

Groenen, M. A. M., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, M. Soller, H. Takahashi, A. Vignal and A. Ponce de Leon (2000). "A consensus linkage map of the chicken genome." Genome Research 10(1): 137-147.

Groenen, M. A. M., R. Crooijmans, A. Veenendaal, H. H. Cheng, M. Siwek, J. J. v. d. Poel and J. J. Van der Poel (1998). "A comprehensive microsatellite linkage map of the chicken genome." Genomics San Diego 49(2): 265-274.

Grundemar, L. and R. Hakanson (1994). "Neuropeptide Y effector systems: Perspectives for drug development." Trends in Pharmacological Science 15(5): 153-159.

Gunsett, F. C. (1984). "Linear index selection to improve traits defined as ratios." Journal Animal Science 59: 1185-1193.

Hafez, E. S. E. (1969). Prenatal Growth. Introduction to animal growth. Philadelphia, Lea & Febiger: 1-17.

Hagan, M. M., P. A. Rushing, S. C. Benoit, S. C. Woods and R. J. Seeley (2001). "Opioid receptor involvement in the effect of AgRP-(83-132) on food intake and food selection." American Journal of Physiology 280(3(2)): R814-R821.

Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley and J. M. Friedman (1995). "Weight-reducing effects of the plasma protein encoded by the obese gene." Science Washington 269(5223): 543-546.

Haley, C. S. and S. A. Knott (1992). "A simple regression method for mapping quantitative trait loci in line crosses using flanking markers." Heredity 69: 312-324.

Haley, C. S., S. A. Knott, C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy and E. B. Burnside (1994). Interval mapping. Proceedings, 5th World Congress on Genetics Applied to Livestock Production., Guelph; Canada, International Committee for World Congresses on Genetics Applied to Livestock Production;.

Hannon, J. and D. Hoyer (2002). "Serotonin receptors and systems: endless diversity?" Acta Biologica Szegediensis 46(1-2): 1-12.

Hansen, L., B. Hartmann, T. Bisgaard, H. Mineo, P. N. Jorgense and J. J. Holst (2000). "Somatostatin restrains the secretion of glucagon-like peptide-1 and -2 from isolated perfused porcine ileum." American Journal of Physiology 280: E1010-E1018.

Harper, G. S. (1999). "Trends in skeletal muscle biology and the understanding of toughness in beef." Aust. J. Agric. Res. 50: 1105-1129.

Harris, P. V. and W. R. Shorthose (1988). Meat texture. Developments in Meat Science. R. Lawrie. UK, London, Elsevier: 245 - 296.

Hastings, I. M., S. M. Moruppa, L. Bunger and W. G. Hill (1997). "Effects of selection on food intake in the adult mouse." Journal of Animal Breeding and Genetics 114(6): 419-434.

Heitmann, R. N. and E. N. Bergman (1981). "Glutamate interconversions and glucogenicity in the sheep." American Journal of Physiology 241(461-472).

Helge, J. W., A. M. Fraser, A. D. Kriketos, A. B. Jenkins, G. D. Calvert, K. J. Ayre and L. H. Storlien (1999). "Interrelationships between muscle fibre type, substrate oxidation and body fat." International Journal of Obesity 23(9): 986-991.

Henken, A. K., W. van der Hel, H. Brandsma and M. W. A. Verstegen (1991). "Differences in energy metabolism and protein rentetnion of limit-fed growing pigs of several breeds." Journal Animal Science 69(4): 1443-1453.

Henriksson, J. (1995). "Muscle fuel selection: effect of exercise and training." Proc. Nutr. Soc. 54: 125-138.

Henshall, J. M. and M. E. Goddard (1999). "Multiple-trait mapping of quantitative trait loci after selective genotyping using logistic regression." Genetics 151(2): 885-894.

Herd, R. M., J. A. Archer and P. F. Arthur (1997). "The effect of divergent selection for yearling growth rate on growth at pasture and final size of angus steers." Proceedings of the 12th Conference of the Association for the Advancement of Animal Breeding and Genetics, AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 12: 323-327.

Herd, R. M., P. F. Arthur, J. A. Archer and D. J. Johnston (2002). IFG1 is associated with genetic variation in key production traits in young angus cattle. Proceedings Australian Society of Animal Production. Adelaide, Australia.

Herd, R. M., P. F. Arthur, K. Zirkler, C. A. Quinn and V. H. Oddy (1995). Heritability of IGF-1 in beef cattle. Proceedings of the 11th Conference of the Association for the Advancement of Animal Breeding and Genetics, Roseworthy, South Australia. AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 11: 694-698.

Herd, R. M. and S. C. Bishop (2000). "Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle." Livestock Production Science 63(2): 111-119.

Herd, R. M., E. C. Richardson, R. S. Hegarty, R. Woodgate, J. A. Archer and P. F. Arthur (1998). "Pasture intake by high versus low net feed efficient angus cows." Proceedings Australian Society of Animal Production 22: 137-140.

Hermesch, S., B. G. Luxford and H.-U. Graser (2000). "Genetic parameters for lean meat yield, meat quality, reproduction and feed efficiency traits for Australian pigs. 2. Genetic relationships between production, carcass and meat quality traits." Livestock Production Science 65: 249-259.

Hitzemann, R., K. Demarest, J. Koyner, L. Cipp, N. Patel, E. Rasmussen and J. McCaughran, Jr. (2000). "Effect of genetic cross on the detection of quantitative trait loci and a novel approach to mapping QTLs." Pharmacology Biochemistry and Behavior 67: 767-772.

Hoehe, M., B. Otterud, W. T. Hsieh, M. M. Martinez, D. Stauffer, J. Holik, W. H. Berrettini, W. F. Byerley, E. S. Gershon, J. M. Lalouel and M. Leppert (1995). "Genetic mapping of adrenergic receptor genes in humans." Journal-of-Molecular-Medicine-Berlin. 73: 299-306.

Hood, R. L. and R. A. Pym (1982). " Correlated responses for lipogenesis and adipose tissue cellularity in chickens selected for body weight gain, food consumption and food conversion efficiency." Poultry Science 61(1): 122-127.

Horowitz, M., P. J. Collins, B. E. Chatterton, P. E. Harding, J. M. Watts and D. J. C. Shearman (1984). "Gastric emptying after gastroplasty for morbid obesity." British Journal of Surgery 71(6): 435-437.

Horvat, S. and J. F. Medrano (1995). "Interval mapping of high growth (hg), a major locus that increases weight gain in mice." Genetics 139(4): 1737-1748.

Horvat, S. and J. F. Medrano (1996). "Fine genetic mapping of the region surrounding the high growth (hg) locus in mouse chromosome 10: targeting random amplified polymorphic DNA (RAPD) markers." Mammalian Genome 7(4): 312-314.

Hosiptal, F., L. Moreau, F. Lacoudre, A. Charcosset and A. Gallais (1997). "More on the efficiency of marker-assisted selection." Theoretical and Applied Genetics 95: 1181-1189.

Howarth, R. E. and R. L. Baldwin (1971). "Synthesis and accumulation of protein and nucleic acid in rat gastrocnemius muscles during normal growth, restricted growth and recovery from restricted growth." Journal of Nutrition 101: 477-484.

Hughes, T. (2003). Direct and correlated responses to seven generations of divergent selection for post-weaning net feed intake in mice. Department of Animal Science. Adelaide, South Australia, University of Adelaide.

Inui, A. (2000). "Transgenic study of energy homeostasis equation: implications and confounding influences." FASEB Journal 14(14): 2158-2170.

Ishikawa, A., Y. Matsuda and T. Namikawa (2000). "Detection of quantitative trait loci for body weight at 10 weeks from Philippine wild mice." Mammalian Genome 11(10): 824-830.

Jansen, R. C. (1993). "Interval mapping of multiple quantitative trait loci." Genetics 135: 205-211.

Johnson, D. L., R. C. Jansen, J. A. M. v. Arendonk and J. A. M. van Arendonk (1999a). "Mapping quantitative trait loci in a selectively genotyped outbred population using a mixture model approach." Genetical Research 73(1): 75-83.

Johnson, Z. B., J. J. Chewning and R. A. Nugent, III (1999b). "Genetic parameters for production traits and measures of residual feed intake in Large White swine." Journal of Animal Science 77(7): 1679-1685.

Johnson, Z. B., J. J. Chewning and R. A. Nugent, III (1999c). "Genetic parameters for production traits and measures of residual feed intake in Large White swine." Journal of Animal Science 77(7): 1679-1685.

Johnston, D. J., R. M. Herd, A. Reverter and V. H. Oddy (2001). Heritability of IGF-1 in beef cattle and its association with grwoth and carcase traits. Proceedings of the 14th Conference of the Association for the Advancement of Animal Breeding and Genetics. Queenstown, New Zealand. AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 13:163-166

Jones, C. D. (2001). "Extension of the Castle-Wright effective factor estimator to sex linkage and haplodiploidy." Journal of Heredity 92(3): 274-276.

Jungst, S. B., L. L. Christian and D. L. Kuhlers (1981). "Response to selection for feed efficiency in individually fed Yorkshire boars." Journal Animal Science 53: 323-331.

Kanis, E. and W. J. Koops (1990). "Daily gain, food intake and food efficiency in pigs during the growing period." Animal Production 353-364.

Kao, C. H. and Z. B. Zeng (1997). "General formulae for obtaining the MLEs and the asymptotic variance-covariance matrix in mapping quantitative trait loci." Biometrics 53: 653-665.

Kao, C. H., Z. B. Zeng and R. Teasdale, D. (1999). "Multiple interval mapping for quantitative trait loci." Genetics 152: 1203-1216.

Karlsson, A. H., R. E. Klont and X. Fernandez (2000). Skeletal muscle fibres as factors for pork quality. Proc. of the EAAP, Zurich, Switzerland.

Katle, J. (1991). "Selection for efficiency of food utilisation in laying hens: causal factors for variation in resiudal food consumption." British Poultry Science 32: 955-969.

Katle, J., H. B. Bensten and B. O. Braastad (1984). Correlated traits with residual feed consumption. Proceedings of XVII Worlds Poultry Congress, Helski.

Katle, J. and N. Kolstad (1991). "Selection for efficiency of food ustilisation in laying hens: direct response in residual food consumption and correlated responses in weight gain, egg production and body weight." British Poultry Science 32: 939-953.

Kato, M. V., Y. Ikawa, Y. Hayashizaki and H. Shibata (1998). "Paternal imprinting of mouse serotonin receptor 2A gene Htr2 in embryonic eye: A conserved imprinitng regulation on the RB/RB locus." Genomics 47: 146-148.

Katsuura, G., A. Asakawa and A. Inui (2002). "Roles of pancreatic polypeptide in regulation of food intake." Peptides 23(2): 323-329.

Keightley, P. D. and W. G. Hill (1992). "Quantitative genetic variation in body size of mice from new mutations." Genetics 131: 693-700.

Keightley, P. D., K. H. Morris, A. Ishikawa, V. M. Falconer and F. Oliver (1998). "Test of candidate gene-quantitative trait locus association applied to fatness in mice." Heredity 81(6): 630-637.

Kennedy, G. C. (1953). "The role of depot fat in the hypothalamic regulation of food intake in the rat." Proc. R. Soc. Lond. 140: 578-592.

Kinghorn, B. and J. H. J. Van der Werf (2000). Identifying and incorporating genetic markers and major genes in animal breeding programs. Positional cloning, candidate genes, and comparative mapping. Belo Horizonte, Brazil: 115-118.

Knap, P. W. and H. A. M. van der Steen (1994). Testing of breeding pigs in group housing. Proceeding of the 45th Annual Meeting of the European Association of Animal Production., Edinburgh, U.K.

Knott, S. A., L. Marklund, C. S. Haley, K. Andersson, W. Davies, H. Ellegren, M. Fredholm, I. Hansson, B. Hoyheim, K. Lundstrom, M. Moller and L. Andersson (1998). "Multiple marker mapping of quantitative trait loci in a cross between outbred wild boar and large white pigs." Genetics 149(2): 1069-1080.

Knott, S. A., D. B. Neale, M. M. Sewell and C. S. Haley (1997). "Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine." Theoretical and Applied Genetics 94(6-7): 810-820.

Koch, R. M., L. V. Cundiff and K. E. Greogory (1982). "Heritabilities and genetic, environmental and phenotypic correlations of carcass traits in a population of diverse biological types and their implications in selection programs." Journal Animal Science 55(6): 1319-1332.

Koch, R. M., L. A. Swiger, D. Chambers and K. E. Gregory (1963). "Efficiency of feed use in beef cattle." Journal of Animal Science 22: 486-494.

Kolaczynski, J. W., J. P. Ohannesian, R. V. Considine, C. C. Marco and J. F. Caro (1996). "Response of leptin to short-term and prolonged overfeeding in humans." Journal of Clinical Endocrinology and Metabolism 81(11): 4162-4165.

Koritshconer, N. P., M. Alvarez-Dolado, S. M. Kurz, M. F. Heikenwalder, C. Hacker, F. Vogel, A. Munoz and M. Zenke (2001). "Thyroid hormone regulates the obesity gene tub." EMBO reports 2(6): 499.

Korver, S. (1988). "Genetic aspects of feed intake and feed efficiency indairy cattle: a review." Livestock Production Science 20: 1-13.

Korver, S., E. A. M. v. Eekelen, H. Vos, G. J. Nieuwhof, J. A. M. v. Arendonk, E. A. M. Van Eekelen and J. A. M. Van Arendonk (1991). "Genetic parameters for feed intake and feed efficiency in growing dairy heifers." Livestock Production Science 29(1): 49-59.

Kotz, C. M., G. M.J., L. M.J. and B. C. J. (2000). "Regional effect of naltrexone in the nucleus of the solitary tract in blockade of NPY-induced feeding." American Journal of Physiology 278: R499-R503.

Koyner, J., K. Demarest, J. McCaughran, Jr., L. Cipp and R. Hitzemann (2000). "Identification and time dependence of quantitative trait loci for basal locomotor activity in the BXD recombinant inbred series and a B6D2 F2 intercross." Behavior Genetics 30(3): 159-170.

Lambert, P. D., J. P. H. Wilding, A. A. M. AlDokhayel, C. Bohuon, E. Comoy, S. G. Gilbey and S. R. Bloom (1993). "A role for neuropeptide-Y, dynorphin, and noradrenaline in the central control of food intake after food deprivation." Endocrinology 133(1): 29-32.

Lande, R. and R. Thompson (1990). "Efficiency of marker-assisted selection in the improvement of quantitiative loci." Genetics 124: 743-756.

Lander, E. S. and D. Botstein (1989). "Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps." Genetics 121(1): 185-199.

Lander, E. S. and L. Krugylak (1995). "Genetic dissection of complex traits." Nature Genetics 11: 241-247.

Langhans, W., M. Duss and E. Scharrer (1987). "Decreased feeding and supraphysiological plasma levels of glucagon after glucagon injections in rats." Physiology & Behavior 41(1): 31-36.

Larsson, H., S. Elmstahl, G. Berglund and B. Ahren (1998). "Evidence for leptin regulation of food intake in humans." Journal of Clinical Endocrinology and Metabolism 83(12): 4382-4385.

Le Lay, S., S. Krief, C. Farnier, I. Lefrere, X. Le Lieprvre, R. Bazin, P. Ferre and I. Dugail (2001). "Cholesterol, a cell size-dependent signal that regulates glucose metabolism and gene expression in adipocytes." Journal of Biological Chemistry 276(20): 16904-16910.

le Neindre, P., G. Trillat, J. Sapa, F. Menissier, J. N. Bonnet and J. M. Chupin (1995). "Individual differences in docility in Limousin cattle." Journal of Animal Science 73(8): 2249-2253.

Le Roy, I., F. Perez-Diaz, A. Cherfouh and P. L. Roubertoux (1999). "Preweanling sensorial motor development in laboratory mice: quantitative trait loci mapping." Dev. Psychobiol. 34(2): 139-158.

Leamy, L. J., E. J. Routman and J. M. Cheverud (1998). "Quantitative trait loci for fluctuating assymetry of discrete characters in mice." Heredity 80(4): 509-518.

Lebherz, H. G. and W. J. Rutter (1969). "Distribution of fructose diphoshpate aldolase variants in biological systems." Biochemistry 8: 109-121.

Levin, B. E. and V. H. Routh (1996). "Role of the brain in energy balance and obesity." American Journal of Physiology 271(3): 2, R491-R500.

Liu, B.-H. (1998). Statistical genomics: linkage, mapping, and QTL analysis. Boca Raton, CRC Press.

Liuttula, M. (1989). "Selection experiment for feed efficiency in egg laying hens." Nordisk Jordbrugsforskning 71: 428-436.

Lobley, G. E. (1993). Protein Metabolism and Turnover. Quantitative Aspects of Ruminant Digestion and Metabolism. J. M. F. Forbes, J., CAB International: 313-339.

Luiting, P. (1990). "Genetic variation of energy partitioning in laying hens: causes of variation in residual feed consumption." Poultry Science 46: 133-152.

Luiting, P. (1994). Correlated metabolic responses in a divergent selection experiment on residual feed intake in White Leghorns. Proceedings 9th European poultry conference, Glasgow, UK, WPSA (United Kingdom Branch); Andover; UK.

Luiting, P., J. W. Schrama, W. V.D Hel and E. M. Urff (1991). "Metabolic differences between White Leghorns selected for high and low residual food consumption." British Poultry Science 32(4): 763-782.

Luiting, P. and E. M. Urff (1991a). "Optimization of a model to estimate residual feed consumption in the laying hen." Livestock Production Science 27(4): 321-338.

Luiting, P. and E. M. Urff (1991b). "Residual feed consumption in laying hens. 1. Quantification of phenotypic variation and repeatabilities." Poultry Science 70: 1655-1662.

Lupu, F., J.D. Terwilliger, K. Lee, G. V. Segre, and A. Efstratiadis (2001). "Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth." Developmental Biology 141-162.

Lynch, M. and B. Walsh (1998). Genetics and analysis of quantitative traits. Sunderland, Massachusetts, Sinauer Associates.

Maes, B., F. Ghoos, B. Geypens, G. Mys, M. Hiele, P. Rutgeerts and e. al. (1994). "Combined carbon-13-glycine/carbon-14-octanoic acid breath test to monitor gastric emptying rates of liquids and solids." Journal of Nuclear Medicine 35: 824-831.

Mao, J. N. C., J. Burnside, M.-C. Postel-Vinay, J. D. Pesek, J. R. Chambers and L. A. Cogburn (1998). "Ontogeny of growth homone receptor gene expression in tissue of growth-selected strains of broiler chickes." Journal of Endocrinology 156: 67-75.

Marks-Kaufman, R. and R. B. Kanarek (1990). "Diet selection following a chronic morphine and naloxone regimen." Pharmacology Biochemistry and Behavior 35: 665-669.

Maruyama, K., M. L. Sunde and P. W. Swick (1978). "Growth and muscle protein turnover in the chick." Biochemical Journal 176: 573-582.

Mathews, C. K. and K. E. Van Holde (1990). Biochemistry. Redwood, California, USA, Benjamin Cummings.

Matthews, L. R., J. F. Carragher and J. L. Slater (1997). Effects of flightiness, sociability and previous handling experience on the behaviour of cattle yards. Proceedings of the 31st International Congress of the ISEA, Prague, Czech Republic.

Mayer, E. (1994). The physiology of gastric storage and emptying. Physiology of the gastrointestinal tract, third edition. L. Johnson. New York, Raven Press: 929-976.

McCarthy, J. C. (1982). The laboratory mouse as a model for animal breeding: A review of selection for increased body weight and litter size. 2nd world congress of genetics applied to livestock prodcution.

McDaneld, T. G., M. K. Nielsen and J. L. Miner (2002). "Uncoupling proteins and energy expenditure in mice divergently selected for heat loss." Journal of Animal Science 80: 602-608.

McDonagh, M. B., R. M. Herd, E. C. Richardson, V. H. Oddy, J. A. Archer and P. F. Arthur (2001). "Meat quality and the calpain system of feedlot steers following a single generation of divergent selection for residual feed intake." Australian Journal of Experimental Agriculture 41(7): 1013-1021.

McIeary, L. M., V. R. Carruthers and P. G. Neil (1997). "Use of breath test to determine the fate of swallowed fluids in cattle." American Journal of Veterinary Research 58: 1314-1319.

McNurlan, M. A., A. M. Tomkins and P. J. Garlick (1979). "The effect of starvation on the rate of protein synthesis in rat liver and small intestine." Biochemical Journal 178: 373-379.

Mercer, J. G., C. B. Lawrence and T. Atkinson (1996). "Hypothalamic NPY and CRF gene expression in the food-deprived Syrian hamster." Physiology and Behavior 60(1): 121-127.

Meyer, K. (1993). DF REML Users Notes.

Michaud, E. J., R. L. Mynatt, R. J. Miltenberger, M. L. Klebig, J. E. Wilkinson, M. B. Zemel, W. O. Wilkison and R. P. Woychik (1997). "Role of the agouti gene in obesity." Journal of Endocrinology 155(2): 207-209.

Miller, B. G., J. K. Kirkwood, K. Howard, A. Tuddenham and A. J. F. Webster (1981). "A self-compensating, closed-circuit respiration calorimeter for small mammals and birds." Laboratory Animals 15(4): 313-317.

Miserez, A. R., G. Cao, L. C. Probst and H. H. Hobbs (1997). "Structure of the human gene encoding sterol regulatory element binding protein 2 (SREBP2)." Genomics 40: 31-40.

Montano-Bermudez, M., M. K. Nielsen and G. H. Deutscher (1990). "Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk." Journal Animal Science 68: 2279-2288.

Moody, D. E., D. Pomp and M. K. Nielsen (1998). Isolation of quantitative trait loci (QTL) controlling energy balance in mice. Proceedings of the 6th World Congress on Genetics Applied to Livestock Production., Armidale; Australia.

Moody, D. E., D. Pomp, M. K. Nielsen, L. D. v. Vleck and L. D. van Vleck (1999). "Identification of quantitative trait loci influencing traits related to energy balance in selection and inbred lines of mice." Genetics 152(2): 699-711.

Moore, D. (2001). Chapter 2. Preparation and analysis of DNA. Current protocols in molecular biology. V. B. Chanda. United States of America, John Wiley & Sons. 1.

Moreau, L., A. Charcosset, F. Hosiptal and A. Gallais (1998). "Marker-assisted selection efficiency in populations of finite size." Genetics 148(1353-1365).

Morisson, M., A. Bordas, J. M. Petit, C. Jayat Vignoles, R. Julien and F. Minvielle (1997). "Associated effects of divergent selection for residual feed consumption of reproduction, sperm characteristics, and mitochondria for spermatozoa." Poultry Science 76(3): 425-431.

Morley, J. E. (1990). "Appetite regulation by gut peptides." Annu. Rev. Nutr. 10: 383-395.

Morley, J. E., E. N. Hernandez and J. F. Flood (1987). "Neuropeptide Y increases food intake in mice." American Journal of Physiology Reg. Int. Comp. Phys(22): R516-R522.

Moruppa, S. M. (1990). "Energy expenditure and locomotor activity in mice selected for food intake adjusted for body weight." Theoretical and Applied Genetics 79(1): 131-136.

Mount, L. E. and J. V. Willmont (1967). "The relation between spontaneous activity, metabolic rate and 24 hour cycle in mice at different environmental temperatures." Journal of Physiology 190: 371-380.

Mouse Genome Informatics (2002). "http://www.informatics.jax.org/."

Mousel, M. R., W. W. Stroup and M. K. Nielsen (2001). "Locomotor activity, core body temperature, and circadian rhythms in mice selected for high or low heat loss." Journal of Animal Science 79(4): 861-868.

Mrode, R. A. and B. W. Kennedy (1993). "Genetic variation in measures of food efficiency in pigs and their genetic relationships with growth rate and backfat." Animal Production 56: 225-232.

Mrode, R. A., C. Smith and R. Thompson (1990). "Selection for rate and efficiency of lean gain in Hereford cattle: 2. Evaluation of correlated responses." Animal Production 51(1): 35-46.

Muranty, H. and B. Goffinet (1997). "Selective genotyping for location and estimation of the effect of a quantitative trait locus." Biometrics 53(2): 629-643.

Murata, Y., G.-H. Sun-Wada, T. Yoshimizu and A. Yamamoto (2002). "Differential localization of the vacuolar H+ pump with G subunit Isoforms (G1 and G2) in mouse neurons." Journal of Biological Chemistry 277(39): 36296-36303.

Mutt, V. (1988). "Secretin and cholecystokinin." Gastrointestinal hormones(251-320; Advances in Metabolic Disorders): Vol. 11.

Nagase, H., S. Inoue, K. Tanaka, Y. Takamura and A. Niijima (1993). "Hepatic glucose-sensitive unit regulation of glucose-induced insulin secretion in rats." Physiology and Behavior 53(1): 139-143.

Nakatani, T., N. Tsuboyama Kasaoka, M. Takahashi, S. Miura and O. Ezaki (2002). "Mechanism for peroxisome proliferator-activated receptor-alpha activator-induced up-regulation of UCP2 mRNA in rodent hepatocytes." Journal of Biological Chemistry 277(11): 9562-9569.

Nielsen, M. K., B. A. Freking, L. D. Jones, S. M. Nelson, T. L. Vorderstrasse and B. A. Hussey (1997b). "Divergent selection for heat loss in mice: II. Correlated responses in feed intake, body mass, body composition, and number born through fifteen generations." Journal of Animal Science 75(6): 1469-1476.

Nielsen, M. K., L. D. Jones, B. A. Freking and J. A. DeShazer (1997a). "Divergent selection for heat loss in mice: I Selection applied and difrect response through fifteen generations." Journal Animal Science 75: 1461-1468.

Nissim, I. (1999). "Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes." American Journal of Physiology 277: F439-F497.

Nitter, G., H. U. Graser and S. A. Barwick (1994). "Evaluation of advanced industry breeding schemes for Australian beef cattle. I. Method of evaluation and analysis for an example population structure." Australian Journal of Agricultural Research 45(8): 1641-1656.

Noben-Trauth, K., J. K. Naggert, M. A. North and P. M. Nishina (1996). "A candidate gene for the mouse mutation tubby." Nature 380: 534-538.

Noblet, J. and J. M. Perez (1993). "Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis." Journal of Animal Science 71(12): 3389-3398.

Obrien, S.J.,, Womack J. E., L. A. Lyons, K. J. Moore, N. A. Jenkins and N. G. Copeland (1993). "Anchored reference loci for comparative genome mapping in mammals." Nature Genetics 3: 103-112.

Oddy, V. H. (1999). "Protein metabolism and nutrition in farm animals: an overview." Proc. VIIIth Int. Symp. Protein Met. Nutr.: 7-23.

Oddy, V. H., G. S. Harper, P. L. Greenwood and M. B. McDonagh (2001). "Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef." Australian Journal of Experimental Agriculture 41(7): 921-942.

Oddy, V. H., R. M. Herd, M. B. McDonagh, R. Woodgate, C. A. Quinn and K. Zirkler (1998). "Effect of divergent selection for yearling growth rate on protein metabolism in hind-limb muscle and whole body of Angus cattle." Livestock Production Science 56(3): 225-231.

Oddy, V. H., P. A. Speck, H. M. Warren and P. C. Wynn (1995). "Protein metabolism in lambs from lines divergently selected for weaning weight." Journal of Agricultural Science 124(1): 129-137.

Olofsson, J. (1999). "Competition fo total mixed diets fed fo ad libitum intake using one of four cows per feeding station." Journal of Dairy Science 82: 69-79.

Olofsson, J. (2000). "Feed availability and its effects on intake, production and behaviour in dairy cows." Acta Universitatis Agriculturae Sueciae Agraria(221): 45 p.

Osborne, T. F. (2001). "CREating a SCAP-less liver keeps SREBPs pinned in the ER membrane and prevents increased lipid synthesis in response to low cholesterol and high insulin." Genes and Development 15: 1873-1878.

Oster, H., A. Yasui and G. T. Van Der Horst (2002). "Disruption of mCry2 restores circadian rhythmicity in mPer2 mutant mice." Genes and Development 16(20): 2633-2638.

Parks, J. R. (1982). A theory of feeding and growth in animals. Verlag, Berlin, Springer.

Parnell, P. F., R. M. Herd, P. F. Arthur and J. Wright (1995). "Breeding for improved net feed conversion efficiency in beef cattle." Proceedings of the 11th Conference of the Association for the Advancement of Animal Breeding and Genetics. 11: 384-388.

Parnell, P. F., R. M. Herd, D. Perry and B. Bootle (1994). The Trangie experiment - responses in growth rate , size maternal ability, reproductive performance, carcase composition, feed requirements and herd profitiability. Proceedings Australian Society of Animal Production.

Penner, A. and F. Hollander (1940). "The use of phenol red as a dilution indicator in gastric analysis." American Journal of Physiology 7: 202-205.

Perry, D., A. J. Ball, J. M. Thompson and V. H. Oddy (1997). The relationship between residual feed intake and body components in animals selected for divergent growth rate. Proceedings of the 12th Conference of the Association for the Advancement of Animal Breeding and Genetics, Dubbo, NSW, Australia, AAABG Distribution Service c/- A.G.B.U.; Armidale; Australia. 12: 238-241.

Persuad, P., G. Simm and W. G. Hill (1991). "Genetic and phenotypic parameters for yield, food intake and efficiency of dairy cows fed ad libitum. 1. Estimates for 'total' lactation measures and their relationship with live-weight traits." Animal Production 52: 435-44.

Pethick, D. W. and J. B. Rowe (1996). "The effect of nutrition and exercise in carcass parameters and the level of glycogen in skeletal muscle of Merino sheep." Australian Journal of Agricultural Research 47(4): 525-537.

Pitchford, W. S. (2002). Mapping net feed efficiency genes. Roseworthy, South Australia, University of Adelaide.

Pree, J. and E. Rutschke (1983). "The circadian rhythm of the dopamine content of the brain of Periplaneta americana L." Zoologische Jahrbucher Abteilung fur Algemeine Zoologie und Physiologie der Tiere 87(4): 455-462.

Pringle, T. D., R. L. West, S. E. Williams and D. D. Johnson (1995). Calpain/calpastatin and double muscling., UGA Animal and Dairy Science Annual Report: 61-65.

Pritchard, L. E., A. V. Turnbull and A. White (2002). "Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signalling and obesity." Journal of Endocrinology 172: 411-421.

Pullar, J. D., Webster, A.J.F. (1977). "The energy cost of fat and protein deposition in the rat." British Journal of Nutrition 12: 355 - 363.

QTLExpress (2001). QTL Express - http://qtl.cap.ed.ac.uk. 2001 - 2003.

Raimbault, S., S. Dridi, F. Denjean, J. Lachuer, E. Couplan, F. Bouillaud, A. Bordas, C. Duchamp, M. Taouis and D. Ricquier (2001). "An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds." Biochemical Journal 353(3): 441-444.

Rance, K. A., W. G. Hill and P. D. Keightley (1997). "Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F2 population." Genetical Research 70(2): 117-124.

Reeds, P. J. (1991). "The energy cost of protein deposition." European Association of Animal Production 58: 473-478.

Reeds, P. J., A. Cadenhead, M. F. Fuller, G. E. Lobley and J. D. McDonald (1980). "Protein turnover in growing pigs. Effects of age and food intake." British Journal of Nutrition 43(3): 445-455.

Richardson, E. C., R. M. Herd, P. F. Arhtur, J. Wright, G. Xu, K. Dibley and V. H. Oddy (1996). "Possible physiological indicators for net feed conversion efficiency in beef cattle." Proceedings Australian Society of Animal Production. 21: 103-106.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer and P. F. Arthur (2001). "Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake." Australian Journal of Experimental Agriculture 41(7): 1065-1072.

Richardson, E. C., R. J. Kilgour, J. A. Archer and R. M. Herd (1999). "Pedometers measure differences in activity in b ulls selected for high and low net feed efficiency." Proc. Aust. Soc. Study Animal Behavior 26: 16.

Robinson, D. W. (1971). "Cellular basis for changes in body composition." Journal Animal Science 33: 146.

Robinson, D. W. and J. L. Lambourne (1970). "The influence of growth rate and retardation on the nucleic acid and nucleic acid concentration in skeletal muscles and whole body composition of the mouse." Growth 34: 235-255.

Rohrer, G. A., L. J. Alexander, Z. Hu, T. P. L. Smith, J. W. Keele, C. W. Beattie and Z. L. Hu (1996). "A comprehensive map of the porcine genome." Genome Research 6(5): 371-391.

Ronin, Y. I., A. B. Korol and J. I. Weller (1998). "Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis." Theoretical and Applied Genetics 97(7): 1169-1178.

Rudski, J. M., M. Grace, M. A. Kuskowski, C. J. Billington and A. S. Levine (1996). "Behavioral effects of naloxone on neuropeptide Y-induced feeding." Pharmacology Biochemistry and Behavior 54: 771-777.

Sakurai, T., A. Amemiya, et al. (1998). "Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior." Cell Cambridge 92(4): 573-585.

Samec, S., J. Seydoux and A. G. Dulloo (1998). "Role of UCP homologues in skeletal muscles and brown adipose tissue: mediators of thermogenesis or regulators of lipids as fuel substrate?" FASEB Journal 12(9): 715-724.

Samson, A., K. L. Hamilton and A. G. Butt (2000). "Effect of somatostatin on electrogenic ion transport in the duodenum and colon of the mouse, Mus domesticus." Comparative Biochemistry and Physiology. A, Molecular and Integrative Physiology 125(4): 459-468.

SAS (1998). SAS/STAT User's Guide Version 8. Cary, N.C., SAS Inst. Inc.

Sax, K. (1923). "The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgarus." Genetics 8: 552-560.

Schmidt, T. M., R. N. Boisvert and L. W. Tauer (2001). "Measuring the financial risks of New York dairy producers." Journal of Dairy Science 84: 411-420.

Schneider, M. R., E. Wolf, A. Hoeflich and H. Lahm (2002). "IGF-binding protein-5: flexible player in the IGF system and effector on its own." Journal of Endocrinology 172: 423-440.

Schoonjans, K., B. Staels and J. Auwerx (1996). "The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation." Biochimica Et Biophysica Acta 1302: 93-109.

Searle, T. W., N. M. Graham, D. A. Griffiths and D. E. Maragan (1988). " Longitudinal studies of body composition during growth in male, female and castrate male sheep of two breeds with different wool growing capabilities." J. Agric. Sci. 110: 239-247.

Seaton, G., C. Haley, Knott S., M. Kearsey and V. P. (2002). "QTL Express: mapping quantitative trait loci in simple and complex pedigrees." Bioinformatics 18: 339-340.

Selman, C., S. Lumsden, L. Bunger, W. G. Hill and J. R. Speakman (2001). "Resting metabolic rate and morphology in mice (Mus musculus) selected for high and low food intake." Journal of Experimental Biology 204(4): 777-784.

Seman, D. H., J. A. Stuedemann and J. E. Anderson (1997). "Spectral analysis of bovine grazing behavior on Neotyphodium coenophialum infested tall fescue." Applied Animal Behaviour Science 54(2-3): 73-87.

Shimomura, K., S. S. Low-Zeddies, D. P. King, T. D. L. Steeves, A. Whiteley, J. Kushla, P. D. Zemenides, A. Lin, H. Vitaterna, G. A. Churchill and J. S. Takahashi (2001). "Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice." Genome Research 11(6): 959-980.

Shor-Posner, G., J. A. Grinker, C. Marinscu, O. Brown and S. F. Leibowitz (1986). "Hypothalamic serotonin in the control of meal patterns and macronutrient selection." Brain Res. Bull. 17: 663-671.

Shull, G. E. (2000). "Gene knockout studies of Ca2+ transporting ATPases." European Journal of Biochemistry 267(5284-5290).

Siegel, J. A., J. L. Urbain and L. P. Adler (1988). "Biphasic nature of gastric emptying." Gut 29: 85-89.

Smith, K. E. (1998). "Cloned human and rat galani n GLAR3 receptors. Pharmacology and activation of G-protein inwardly rectifying K+ channels." Journal of Biological Chemistry 273: 23321-23326.

Stanley, B. G., S. E. Kyrkouli, S. Lampert and S. F. Leibowitz (1986). "Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity." Peptides 7(6): 1189-1192.

Stanley, B. G., W. J. Thomas, P. D. Lambert, J. P. H. Wilding, A. A. M. Al Dokhayel, C. Bohuon, E. Comoy, S. G. Gilbey, S. R. Bloom and V. Mutt (1993). "Feeding responses to perifornical hypothalamic injection of neuropeptide Y in relation to circadian rhythms of eating behaviour. A role for neuropeptide-Y, dynorphin, and noradrenaline in the central control of food intake after food deprivation ." Peptides 14(3): 475-481.

Steffens, A. B., J. van der Gugten, J. Godeke, P. G. M. Luiten and J. H. Strubble (1986). "Meal-induced increases in parasympathetic and sympathetic activity elicit simultaneous rises in plasma insulin and free fatty acids." Physiology & Behavior 37(1): 119-122.

Stephens, S. (1991). Biological aspects of feeding and growth in mice. Animal Science. Armidale, Australia, University of New England.

Stephens, T. W., M. Basinski, P. K. Bristow, J. M. Blue-Valleskey, S. G. Burgett, L. Craft, J. Hale, J. Hoffman, H. M. Hsiung, A. Kriaciunas, W. MacKellar, P. R. Rosteck Jnr., G. Shoner, D. Smith, F. C. Tinsley, X. H. Zhang. and M. Heinman (1995). "The role of neuropeptide Y in the antiobesity action of the obese gene product." Nature 377: 530-532.

Stone, R. T., J. W. Keele, S. D. Shackelford, S. M. Kappes and M. Koohmaraie (1999). "A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits." Journal of Animal Science 77(6): 1379-1384.

Susenbeth, A., R. Mayer, B. Koehler and O. Neumann (1998). "Energy requirements for eating in cattle." Journal of Animal Science 76(10): 2701-2705.

Symonds, E. L., R. N. Butler and T. I. Omari (2000). "Assessment of gastric emptying in the mouse using the [13C]-Octanoic breath test." Clinical and Experimental Pharmacology and Physiology 27: 671-675.

Symonds, E. L., R. N. Butler and T. I. Omari (2002). "Noninvasive breath tests can detect alternations in gastric emptying in the mouse." European Journal of Clinical Investigation 32: in press.

Symonds, E. L., T. I. Omari and R. N. Butler (2001). "A novel technique for monitoring breath, applied to assessment of gastric emptying in the mouse." Journal of Gastroenterology Hepatology(V13 (Supplement)): A111.

Talbot, C. J., A. Nicod, S. S. Cherny, D. W. Fulker, A. C. Collins and J. Flint (1999). "High-resolution mapping of quantitative trait loci in outbred mice." Nature Genetics 21(3): 305-308.

Tartaglia, L. A., Dembskim, M., Weng,X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., and Deeds, J. (1995). "Identification and expression cloning of a leptin receptor." Cell 83: 1263-1271.

Taylor, B. A. and S. J. Phillips (1996). "Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling." Genomics San Diego 34(3): 389-398.

Taylor, R. C., K. Scmidt-Nielsen and J. L. Raab (1970). "Scaling of energetic cost of running to body size in mammals." American Journal of Physiology 219(4): 1104-1107.

Taylor, S. C. S. (1980a). "Genetic size scaling rules and animal growth." Animal Production 30: 161-165.

Taylor, S. C. S. (1980b). "Genetically standardised growth equations." Animal Production 30: 167-175.

Taylor, S. C. S. (1982). Theory of growth and feed efficiency in relation to maturity in body weight. 2nd World Congress on Genetics Applied to Livestock.

Thompson, J. M. and R. Barlow (1986). The relationship between feeding and growth parameters and biological efficiency in cattle and sheep. 3rd World Congress on Genetics Applied to Livestock Production., University of Nebraska, Lincoln, USA.

Timon, V. M. and E. J. Eisen (1969). "Comparison of growth curves of mice selected and unselected for postweaning gain." Theoretical and Applied Genetics 39: 345-351.

Timon, V. M., E. J. Eisen and J. M. Leatherwood (1970). "Comparasions of ad libitum and restricted feeding in mice selected and unselected for post-weaning gain. II. Carcase composition and energetic efficiency." Genetics 65: 145-155.

Tivey, D. and R. Butler (1999). "Breath analysis - a key to understanding intestinal function." Recent Advances in Animal Nutrition 12: 45-52.

Tixier-Boichard, M., A. Bordas, G. Renand and J. P. Bidanel (2002). Residual food consumption as a tool to unravel genetic components of food intake. Proceedings of the 7th world congress on genetics applied to livestock production, Montpellier, France.

Tomas, F. M., L. M. Jones and R. A. Pym (1988). "Rates of muscle protein breakdown in chickens selected for increased growth rate, food consumption, or efficiency of food utilisation as assessed by N -methylhistidine excretion." British Poultry Science 29: 359-370.

Tomas, F. M. and R. A. Pym (1995). "Muscle protein turnover and growth." Arch. Geflugelk. Sonderheft 1: 23-26.

Tomas, F. M., R. A. Pym and R. J. Johnson (1991). "Muscle protein turnover in chickens selected for increased growth rate, food consumption or efficiency of food utilisation: effects of genotype and relationship to plasma IGF-I and growth hormone." British Poultry Science 32(2): 363-376.

Tomas, F. M., R. A. Pym, J. P. McMurtry and G. L. Francis (1998). "Insulin-like growth factor (IGF) -I but not IGF-II promote lean growth and feed efficiency in broiler chickens." General and Comparative Endocrinology 110(3): 262-275.

Tuggle, C. K. and A. Trenkle (1996). "Control of growth hormone synthesis." Domestic Animal Endocrinology 13: 1-33.

Tulloh, N. M. (1961). "Behaviour of cattle in yards: II . A study of temperament." Animal Behaviour. 9: 25-30.

van Kaam, J., M. A. M. Groenen, H. Bovenhuis, A. Veenendaal, A. L. J. Vereijken and J. A. M. van Arendonk (1999). "Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency." Poultry Science 78(1): 15-23.

Veerkamp, J. H. and S. Brotherstone (1997). "Genetic correlations between linear type traits, food intake, live weight and condition score in Holestein Friesian dairy cattle." Animal Science 64: 385-392.

Vitaterna, M. H., D. P. King, A. M. Chang, J. M. Kornhauser, P. L. Lowrey, J. D. McDonald, W. F. Dove, L. H. Pinto, F. W. Turek and J. S. Takahashi (1994). "Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior." Science 264: 719-725.

Voisinet, B. D., T. Grandin, J. D. Tatum, O. C. SF and J. J. Struthers (1997). "Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments." Journal of Animal Science 75(4): 892-896.

von Felde, A., R. Roehe, H. Looft, E. Kalm and A. Von Felde (1996). "Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars." Livestock Production Science 47(1): 11-22.

Webb, A. J. and J. W. B. King (1983). "Selection for improved food conversion ratio on ad libitum group feeding in pigs." Animal Production 37: 375-385.

Weber, E. and H. Ehrlein (1998). "Reserve capacities of small intestine for absorption of energy." American Journal of Physiology 2 75: R300-R307.

Weber, K., R. Eisman, L. Morey, A. Patty, J. Sparks, M. Tausek and Z. Zeng, B. (1999). "An analysis of polygenes affecting wing shape on chromosome three in *Drosophila melanogaster*." Genetics 153: 773-786.

Webster, A. J. F. (1980). "The energetic efficiency of growth." Livestock Production Science 7(3): 243-252.

Weller, J. I. (2001). Quantitative trait loci analysis in animals, CABI Publishing.

Weller, J. I. and M. Ron (1994). Detection and mapping quantitative trait loci in segregating populations: theory and experimental results. Proceedings, 5th World Congress on Genetics Applied to Livestock Production., Guelph, Ontario, Canada, International Committee for World Congresses on Genetics Applied to Livestock Production; Guelph; Canada.

Whipple, G., M. Koohmaraie, M. E. Dikeman and J. D. Crouse (1990). "Predicting beef longissimus tenderness from various biochemical and histological muscle traits." Journal of Animal Science 68(12): 4193-4199.

Wilding, J., P. Widdowson and G. Williams (1997). "Neurobiology." British Medical Bulletin 53(2): 286-306.

Winick, M. and A. Noble (1966). "Cellular response in rats during malnutrition at various ages." Journal of Nutrition 89: 300-306.

Winkelman, D. C. and R. B. Hodgetts (1992). "RFLPs for somatotropic genes identify quantitative trait loci for growth in mice." Genetics 131(4): 929-937.

Wittaker, J. C., R. N. Curnow, C. S. Haley and R. Thompson (1995). "Using maker-maps in marker-assisted selection." Genetic Research 66: 255-265.

Womack, J. E. and S. R. Kata (1995). "Bovine genome mapping: evolutionary inference and the power of comparative genomics." Current Opinion in Genetics & Development 5: 725-733.

Wright, S. (1968). Evolution and the genetics of populations. Chicago, University of Chicago Press.

Wu, G. (1998). "Intestinal mucosal amino acid catabolism." Journal of Nutrition 128: 1249-1252.

Wulf, D. M., J. D. Tatum, R. D. Green, J. B. Morgan, B. L. Golden and G. C. Smith (1996). "Genetic influences on beef longissimus palatability in Charolais and Limousin sired steers and heifers." Journal Animal Science 74: 2394-2405.

Xu, S. Z. (1998a). "Further investigation on the regression method of mapping quantitative trait loci." Heredity 80(3): 364-373.

Xu, S. Z. (1998b). "Iteratively reweighted least squares mapping of quantitative trait loci." Behavior Genetics 28(5): 341-355.

Xu, S. Z. and C. Vogl (2000). "Maximum likelihood analysis of quantitative trait loci under selective genotyping." Heredity 84(5): 525-537.

Yamada, H., T. Okumura, W. Motomura, Y. Kobayashi and Y. Kohgo (2000). "Inhibition of food intake by central injection of anti-orexin antibody in fasted rats." Biochemical and Biophysical Research Communications 267(2): 527-531.

Yamada, T. and T. Chiba (1989). Somatostatin. Handbook of Physiology. G. Makhlouf. Bethesda, M.D., American Physiology Society: 431-454.

Yang, H. (2002). "Central and peripheral regulation of gastric acid secretion by peptide YY." Peptides 23(2): 349-358.

Yeo, G. S., I. S. Farooqi, B. G. Challis, R. S. Jackson and S. O'Rahilly (2000). "The role of melanocortin signalling in the control of body weight : evidence from human and murine models." Quarterly Journal of Medicine 93(7-14).

York, D. A. (1990). "Metabolic regulation of food intake." Nutrition Reviews 48(2): 64-70.

Yüksel, E. (1979). "Genetic aspects of the efficiency of food utilisation in some farm and laboratory animals." Anim. Breed. Abs. 47(9): 499-504.

Zampf, J. a. and E. B. Hunziker (1994). The somatomedin hypothesis revisited: differential effects of growth hormone and IGF-1 on skeletal growth in the rat in vivo. The Insulin-like growth factors and their regulatory proteins. R. C. Blaxter and R. G. Rosenfeld. Amsterdam, Elsevier: 381-391.

Zeng, Z., B. (1993). "Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci." Proc. Natl. Acad. Sci. USA 90: 10972-10976.

Zeng, Z. B. (1992). "Correcting the bias of Wright's estimates of the number of genes affecting a quantitative character: a further improved method." Genetics 131(4): 987-1001.

Zeng, Z. B., J. J. Liu, L. F. Stam, C. H. Kao, J. M. Mercer and C. C. Laurie (2000). "Genetic architecture of a morphological shape difference between two Drosophila species." Genetics 154(1): 299-310.

Zeng, Z.-B., C. H. Kao and C. S. Basten (1999). "Estimating the genetic architecture of quantitative traits." Genetic Research Cambridge 74(279-289).

Zentek, J. (1992). "Microbial gas formation in the intestine of monogastrics. Part 2. Pulmonary excretion of hydrogen and methane." Ubersichten zur Tierernahrung 20(1): 91-121.

Zhang, W. and C. Smith (1992). "Computer simulation of marker-assisted selection utilizing linkage disequlibrium." Theoretical and Applied Genetics 83: 813-820.

Zhang, W. and C. Smith (1993). "Simulation of marker-assisted selection utilizing linkage disequilibrium: the effects of several additional factors." Theoretical and Applied Genetics 86: 492-496.