DIRECT AND CORRELATED RESPONSES TO SEVEN GENERATIONS OF DIVERGENT SELECTION FOR POST-WEANING NET FEED INTAKE IN MICE

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Contents

Declaration

This work contains no material which has been accepted for the award of any other 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 has been made in the text.

SIGNED: ... Date: 10/04/03.....

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Publications

Publications produced during the period of candidature.

Refereed Papers

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Chapter 1.

Abstract

Seven generations of divergent selection on a phenotypic index of post-weaning net feed intake (intake net of that required for growth and maintenance of body weight) in mice produced a realised heritability estimate of 0.27±0.7. Post-weaning, the high net feed intake line ate 20% more per day than the low net feed intake line (4.46±0.03 vs. 3.73±0.06 g/day), and produced 25% more waste (1.23±0.01 vs. 0.98±0.02 g/day). Despite small negative genetic correlations of net feed intake with daily gain and body weight, correlated responses in these economically important traits were negligible post-weaning. However, more efficient animals tended to be fatter during the early post-weaning phase (17.8±0.6 vs. 14.4±0.3 percent body fat).

The results for intake and growth traits observed at maturity were substantively similar to those observed post-weaning. The divergence between high and low lines in daily intake and daily waste production was 22% (4.62±0.07 vs. 3.77±0.11 g/day) and 28% (1.33±0.03 vs. 1.04±0.04 g/day) respectively. The low net feed intake line was 6% lighter than the high net feed intake line at maturity (29.1±0.8 vs. 30.9±0.5 g). Consequently, the low line was significantly better at maintaining body weight at maturity.

Selection for net feed intake did not alter metabolic parameters substantially. The lines had a similar basal heat production, maintenance heat production and heat increment of feeding. There was some evidence that the lines differed substantially in their level of activity, but the results were inconclusive.

Post-weaning net feed intake was positively correlated with reproductive rate for first parity litters – the low net feed intake line had smaller litters at first parity than the high net feed intake line (9.1±0.3 vs. 10.1±0.3 pups/litter), although the effect was not maintained in later parities. The significant divergence in intake at maturity between the lines disappeared (in females) during pregnancy and early lactation. The lines re-diverged during late lactation.

Overall, the results in mice present a largely positive argument for the use of net feed intake as a selection tool to improve overall production efficiency. Such usage would depend upon a range of factors, both species- and environment-specific. Some factors of note would include: diet, age at selection, age at maturity, reproductive rate (single- versus multi-parous species), ratios of animals (progeny:parents, mature:growing, sires:dams, etc…), and the length of time spent in productive versus non-productive states.

Chapter 2.

Literature Review

"We can perhaps begin to see how growth, food intake, efficiency and body composition may be connected in a way that suggests that the whole complex has to be understood before the different parts can be adequately explained"

Roberts, 1979.

Part A. Livestock production efficiency

A major objective of modern livestock breeding programs is to increase the overall efficiency of production. Irrespective of how efficiency is defined, improvement of raw nutrient utilisation will form an important component of the breeding program objective. In most agriculturally important species, and particularly those on high concentrate diets, feed accounts for a large proportion of the total costs. This is more readily quantified in intensive production systems, e.g. 80% in milk production (Veerkamp and Emmans, 1995) and 60-70% in poultry production (Luiting, 1991), although it has also been estimated in extensive production environments. Dickerson (1978) estimated that over 50% of total feed intake was used solely for the body maintenance of adult and slaughter animals in beef enterprises. Holmes (1977) estimated that only 5.2% of the metabolisable energy fed in beef production systems is recovered as edible energy output.

The topic of feed efficiency is not a new area of interest. In a review by Morris and Wilton (1976), an excess of 100 publications were cited addressing this or closely

related issues. Included in the citations was a paper by Kleiber (1936) entitled "Problems involved in breeding for efficiency in feed utilisation." Brody (1945) addressed the question of how the relationship between biological and economic efficiency may be affected by body size. Fifteen years ago, Fairfull and Chambers (1984) stated that for poultry, "...direct selection for feed efficiency seems to be an idea whose time has arrived". In pig breeding programs, feed efficiency, or its inverse food conversion ratio, has already been incorporated in selection objectives and selection criteria (de Vries and Kanis, 1992) for a number of years.

In sheep, beef cattle and dairy cattle breeding programs, selection objectives and criteria have generally focussed on outputs (Banks, 1994; Barwick *et al.*, 1994; Persaud *et al.*, 1991). Measurement of feed intake in progeny testing schemes in these species has not been practical nor economical. However, the introduction of nucleus breeding schemes has enabled recording of food intake, with the potential for measures of feed efficiency to be incorporated as selection criteria.

Fairfull and Chambers (1984) stated that "...there is little question that selection including feed efficiency would be more effective than selection without it. There are, however, a number of questions still to be resolved, which were elegantly summarised by Luiting (1991), who concluded that "...after considering the role of feed efficiency in the breeding goal, the questions to be solved will be: 1) what extra genetic improvement in feed efficiency may be expected from direct versus indirect selection; 2) which criterion should be used for direct selection; and 3) what are the costs of direct selection?".

Measurement of efficiency

A number of methods for measuring and expressing efficiency exist, and the method of choice often has important implications for making comparisons between studies (e.g. Gibson, 1986). There is no measure of efficiency that can be universally recommended for all situations, as different measures reflect different biological and mathematical aspects of growth and intake. The ideal measure from an whole-industry perspective should identify individuals with the greatest efficiency over a production lifecycle. This could be defined as the ratio of the total feed required for production of saleable product, including the costs associated with obtaining and maintaining a breeding nucleus from which the production is based, to the total production output. This will vary based on the definition of the production system.

Lifecycle production efficiency is a complex biological trait that is the summation of many other traits of importance and is not easily measured on individuals. It is useful instead to break it down into component traits. This is essentially the approach taken by Thompson and Barlow (1986), who recommended maintenance efficiency as a potential means to improve the efficiency of production in species with low reproductive rates, such as beef cattle.

A range of measures of feed utilisation efficiency can be found throughout the literature and have been reviewed extensively elsewhere (Archer *et al.*, 1999; Arthur *et al.*, 1998). The principle measures are briefly defined and described below:

(i) Feed conversion ratio (FCR)

$$FCR = \frac{Feed}{Gain}$$

Feed conversion ratio is a measure of the amount of feed dry matter eaten per unit of bodyweight gain or other production component. Since feed is the numerator, feed conversion ratio should be minimised. Common values for young, growing ruminants are 4-6, whereas pigs and poultry aim for values less than 2.

(ii) Gross efficiency (GE)

$$GE = \frac{Gain}{Feed}$$

Gross efficiency is simply the reciprocal of feed conversion ratio, and as feed is the denominator, should be maximised.

(iii) Maintenance requirement (MR)

$$MR = \frac{Feed}{Weight}$$

When animals are not growing due to lack of feed or maturity, they still require feed to maintain body weight. This requirement may be calculated in a similar manner to FCR where the feed required is expressed on a per live-weight basis.

(iv) Maintenance efficiency (ME)

$$ME = \frac{Weight}{Feed}$$

As with growing animals, there is also another way that feed requirements for maintenance may be reported. The reciprocal of maintenance requirement is maintenance efficiency. This would commonly be used where the animal is of primary interest whereas maintenance requirement would be used for developing feed supply programs. Gross efficiency is a function of both efficiency of weight gain and, maintenance efficiency.

One of the problems with both measures of maintenance is that they depend on the physiological state of the animal even though maintenance is measured on dry animals that are not changing in body weight or composition. Maintenance requirement is difficult to measure on growing animals and mature commercial cows should not be at maintenance, rather they should be pregnant, lactating, both or culled (Pitchford pers. comm.) In some poor environments or during drought this could be extended to gaining condition. Furthermore, maintenance requirements are not constant, but depend on the physiological state and previous level of nutrition (Brody 1945).

(v) Residual (net) feed intake (RFI)

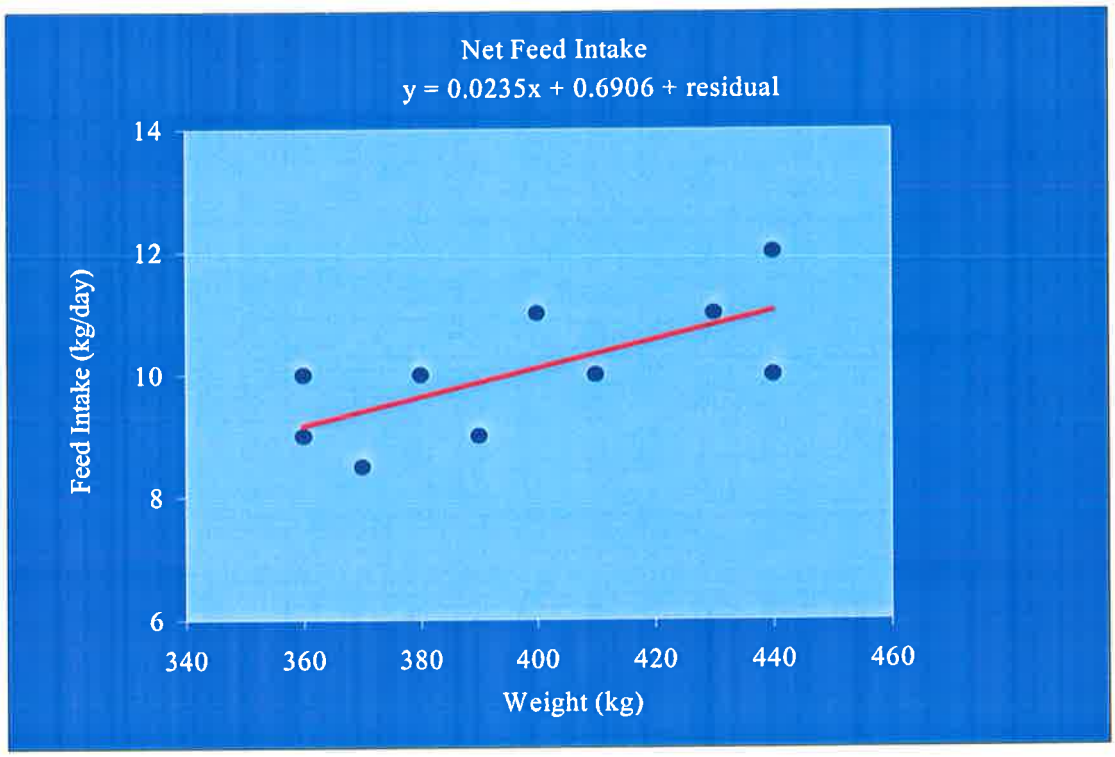
$$RFI = FI - \beta_1(Weight) - \beta_2(Average\ Daily\ Gain) - \ldots - \beta_i(Energy\ Sink_i)$$

Koch *et al.* (1963) examined a number of indices for calculating feed efficiency and suggested that feed intake could be adjusted for body weight and weight gain (or any other production trait or energy sink identified e.g. milk yield, egg production), effectively partitioning feed intake into two components: 1) the feed intake expected for the given level of production; and 2) a residual portion. The residual portion can then be used to identify those animals which deviate from their expected level of feed intake, and they can be classified as high efficiency (negative residual feed intake) or low efficiency (positive residual feed intake). Residual feed intake is also known by a more general moniker, net feed intake (NFI), a term which will be used throughout this text.

The following conceptual data (Figure 2.1) illustrates the calculation of a simple net feed intake incorporating a weight term only. It would be most appropriate for modelling feed efficiency in for example mature, non-lactating dairy cows. As expected, intake (FI) increases linearly with body weight (Wt). However, there is variation around the line of best fit. The equation for the line of best fit models intake using intake when weight is zero (intercept = 0.7 kg/day), a slope (maintenance requirement = 0.02) and some residual variation (net feed intake). Although heavier animals eat more, a number are eating much less or more than predicted from the total population (points shown above and below the line). The same is true of smaller animals. This deviation from expectation is the measure of interest as it is the variation in intake that is effectively independent (phenotypically) of production traits.

The units are kg feed per day, the values are likely to be normally distributed, and by definition the mean is zero.

Figure 2.1. Demonstration of net feed intake using fictitious data for dry cows



Net feed intake is by definition phenotypically independent of the production traits used to calculate expected feed intake. However, Kennedy *et al.* (1993) showed that net feed intake may not be genetically independent of production, and that, to obtain a measure of efficiency which is genetically independent of production, genotypic net feed intake could be calculated using genetic covariances rather than phenotypic covariances.

Net feed (conversion) efficiency (NFE)

Again referring to Figure 2.1, animals with negative net feed intake eat less than expected and are therefore the most efficient. Since a negative number is generally associated with poor performance in selection programs, the sign of net feed intake is often reversed and the new trait termed net feed conversion efficiency, or net feed efficiency. The units for net feed efficiency are -kg/day, which is somewhat strange when compared to most measures of efficiency which are a function of intake as well as gain.

Current trends

The most widely used measure of efficiency in the literature is that of gross efficiency or its inverse, feed conversion ratio. The period of growth over which gross efficiency is measured may be defined on a time constant basis (growth and feed measured between two set points in time), a weight constant basis (feed required for growth from weight *a* to weight *b*) or a maturity constant basis (feed and weight gain measured from stage of maturity *a* to *b*, where maturity may be defined as current weight as a proportion of mature weight).

It is well documented that gross efficiency is both phenotypically and genetically correlated with growth rate, a fact which has been exploited by the pig and poultry industries where gains from improvement in efficiency have primarily come from changes in the growth rate of progeny.

Brelin and Brannang (1982) summarised four studies on cattle which reported a genetic correlation between growth rate and feed conversion ratio defined on a weight

constant basis ranging from -0.61 to -0.95. Heritability estimates for feed conversion ratio from these studies ranged from 0.36 ± 0.07 to 0.45 ± 0.05.

For dairy cattle, gross efficiency is the ratio of energy content in milk over the total energy input from feed. It is relatively easy to calculate, but requires data on milk yield and food intake and their compositions. Heritability estimates on gross efficiency range from 0.36 to 0.86, phenotypic correlation with milk yield from 0.60 to 0.95, and genetic correlation with milk from 0.88 to 0.95 (e.g. Hooven *et al.*, 1972; Freeman, 1975; Blake and Custodio, 1984). Veerkamp and Emmans (1995) stated that selection for yield in dairy cattle will automatically improve gross efficiency because of the higher coefficient of variation for yield compared to intake.

While selection for gross efficiency, whether by direct or indirect selection, may improve the efficiency of the animals producing saleable product, it will not necessarily improve the efficiency of the entire production system. To use beef cattle as an example, genotypes with high growth rates and hence high gross efficiency while growing tend also to have high mature weights and hence higher feed requirements at maturity. Gross efficiency is largely a function of maturity patterns (Salmon *et al.*, 1990) and if an increase in feed requirements at maturity offsets the gains in growth efficiency there may be no change in biological efficiency, especially in maternal breeds (e.g. Holmes, 1973; Anderson, 1978; Dickerson, 1978; Fitzhugh, 1978; Barlow, 1984).

The impact of an increase in feed required to maintain adults on the overall efficiency of an entire production system will depend on both the system itself and the species being farmed. For species with high reproductive rates (e.g. pigs, poultry), an increase

in gross efficiency may provide an increase in the efficiency of the entire system as the increase in maintenance requirement for the breeding herd is relatively small. However, in production systems where the maintenance costs of the breeding herd are high relative to production output (e.g. beef, sheep) an increase in the maintenance requirement of adult animals may offset the gains which are made by increasing the gross efficiency of the growing animals. Gross efficiency is only truly relevant for primi-parous species in situations where the herd consists only of growing animals, such as in a feed-lot. It is also useful for making comparisons where uniform genotypes are used. However, gross efficiency is inadequate for making comparisons between the efficiency of genotypes in the context of an entire production system where reproductive rates are low and the maintenance cost of the breeding herd is significant.

From a purely theoretical viewpoint, there are also issues when using gross efficiency or feed conversion ratio in a selection program. Gunsett (1984) noted that direct selection on feed conversion ratio may not be the best way to improve efficiency, because: 1) the statistical properties of ratios are poor and selection response can be erratic; 2) the use of a ratio as a selection criterion results in different responses in the component traits, which in addition cannot be predicted accurately; and 3) ratios may produce fallacious indications of economic efficiency.

The case for using net feed intake

Koch *et al.*'s (1963) initial model for net feed intake can be simply defined as the difference between the actual feed intake observed and the feed intake predicted from a model. The model can be formulated to include adjustments for any factors which

may affect feed intake, such as weight maintained, changes in bodyweight and other production traits. Because net feed intake is essentially the error term in the statistical model used to predict feed intake, the phenotypic correlation of net feed intake with any factor included in the model is zero, and hence net feed intake as a measure of feed efficiency is phenotypically independent of the level of production. In this way net feed intake differs from gross efficiency, which tends to be highly correlated with the level of production.

Some authors (e.g. Brelin and Brannang 1982; Korver 1988) have suggested that net feed intake represents inherent variation in the basic processes of efficiency of nutrient absorption, the rate of basal metabolism and the energetic efficiencies of the processes of growth and maintenance. If this is the case then there may be a strong relationship between net feed intake of growing animals and other aspects of efficiency (e.g. the efficiency of maintenance at maturity), as net feed intake would represent variation in the intrinsic efficiency of individuals. For this reason, net feed intake has been identified as a measure of efficiency suitable for improving maintenance and production efficiency.

Components of net feed intake

Net feed intake reflects variation in feed intake which is not explained by a model, and so the results obtained will depend upon the model which is used. The source of this variation is of interest, as any additional factors which explain some of the residual variation may improve our understanding of the efficiency complex. The unexplained variation may arise from a number of different sources. These include measurement errors and random deviations from the model as well as individual variation in the

coefficients of the model and other variation not explained by the model. Hence, it is unlikely that all of the residual variation observed is due to differences between individuals in their efficiency of utilising feed. However, the question of interest when variation in feed utilisation efficiency is being examined is how much of the residual variation reflects real differences in efficiency between individuals, and what is the cause of these differences?

In genetic terminology, net feed intake may be considered to consist of genetic effects, environmental effects and random error. The potential of net feed intake as a measure on which selection for efficiency can be based will be determined by the heritability of net feed intake and its genetic correlation with maintenance or production efficiency. As net feed intake is dependent on the model of feed intake used in the calculation, it follows that the heritability of net feed intake must also depend on the model used. However, a search of the literature reveals that most authors estimating the heritability of net feed intake have used a similar model to calculate net feed intake which essentially adjusts for body weight (or metabolic body weight) maintained and any identifiable energy sink associated with production, such as changes in body weight, level of milk production or egg production, during the period in which feed intake was measured, and so it is possible to make meaningful comparisons between different studies.

Heritability of net feed intake (additive genetic variation)

Information currently available indicates that there is phenotypic and genetic variation in measures of feed efficiency of growing cattle. Koots *et al.* (1994) reviewed estimates of heritability for many traits in beef cattle, including feed intake, feed

conversion ratio and gross efficiency. The weighted mean heritability (%) of 23 estimates of feed intake was 34±3, 28 estimates of feed conversion ratio was 32±2, and 9 estimates of gross efficiency was 37±5. Brelin and Brannang (1982) and Korver *et al.* (1991) obtained low genetic correlations between net feed intake and production, suggesting that net intake represented real genetic variation in the relationship between feed intake and production. However, Jensen *et al.* (1992) obtained a negative genetic correlation between net feed intake and average daily gain, and so it is not known whether or not the genetic variation in net intake reported represents variation in feed efficiency or genetic variation in production traits not accounted for when net feed intake is calculated. Overall, the evidence indicates that both phenotypic and genetic variation exist in feed efficiency of growing cattle.

There is less certainty on the existence of genetic variation in efficiency of lactating cattle. Heritability estimates for net feed intake of lactating dairy cattle include 0.19±0.12 (van Arendonk *et al.* 1991), 0.14 (Kennedy *et al.* 1993) and 0.30-0.38 (Veerkamp *et al.* 1995), while Ngwerume and Mao (1992) and Svendsen *et al.* (1993) found no genetic variation in net feed intake. There are very few estimates of heritability for net feed intake of lactating cows calculated from genetic regression. Kennedy *et al.* (1993) found no genetic variation in net feed intake calculated from genotypic regression in lactating dairy cattle. Veerkamp *et al.* (1995) found the heritability of net feed intake in lactating dairy cows decreased to 0.05 when calculated by genotypic regression, and was attributed to a downward bias associated with small data sets. It was concluded that net feed intake has a heritable component and suggested that genetic variation in efficiency of lactating cows exists. It should be noted that all studies were on dairy cattle which have been intensively selected for

milk production. The intense selection might have decreased the variation in efficiency of milk production. A more detailed review of genetic variation in lactating cows has been written by Veerkamp and Emmans (1995).

Morris (1972, cited by Luiting and Urff 1991a) suggested that differences in partial energetic efficiencies between laying hens are expressed more clearly at a sub-optimum energy consumption. To test this, Luiting and Urff (1991a) fed hens *ad libitum* with either a commercial diet or a low energy diet (11.7 and 10.0MJME.kg^{-1} respectively). They found that the magnitude of residual intake was smaller, and less appeared systematic or related to maintenance requirement per kg$^{0.75}$, with a low energy diet than with a commercial diet. Furthermore, less environmental variation, and therefore higher heritabilities and genetic correlations, existed in the low energy diet when compared with the commercial (high energy) diet.

There are 18 estimates of heritability of net feed intake in 7 species/types shown in Table 2.1. The unweighted mean heritability (%) of the 18 estimates is 24±12. This clearly demonstrates that net feed intake is moderately heritable (similar to growth) and can be improved with selection.

Table 2.1. Heritability estimates for net feed intake.

Species	State	N	h^2	Reference
Beef	Growing	1324	28±11	Koch *et al.* (1963)
Beef	Growing	966	41±7	Arthur *et al.* (1997)
Dual	Growing	235	27±23	Brelin & Brannang (1982)
Dairy	Lactating ♀	360	19±12	Van Arendonk *et al.* (1991)
Dairy	Growing ♀	417	22±11	Korver *et al.* (1991)
Dairy	Growing ♂	650	8±5 to 36±17	Jensen *et al.* (1992)
Dairy	Lactating ♀	247	16	Ngwerume & Mao (1992)
Dairy	Lactating ♀	353	0	Svendsen *et al.* (1993)
Dairy	Lactating ♀	204	5	Veerkamp *et al.* (1995)
Pigs	Growing ♂	7562	30, 33, 38	Mrode & Kennedy (1993)
Pigs	Growing ♂	3188	18±3	von Felde *et al.* (1996)
Poultry	Laying ♀	704	42 to 62	Luiting & Urff (1991a)
Poultry	Laying ♀	Realised	12, 21, 28	Bordas *et al.* (1992)
Mice	Growing	1628	27±6	Archer (1996)
Mice	Mature	Realised	16, 23, 27	Hastings *et al.* (1997)
Mice	Growing	Realised	28±0.3	Nielsen *et al.* (1997a)
Mice	Growing	Realised	27±2, 26±3	Hughes *et al.* (1998)
Mice	Growing	Realised	11±1.7, 18±3.4	Sharp *et al.* (1984)
Tribolium	Growing	Realised	32±6	Campo & Turrado (1998)

The majority of results are from statistical estimates of the proportion of additive genetic variation. Realised estimates are from selection experiments for net feed intake and are calculated from a linear regression of selection response on selection differential (pressure).

Variation in partial efficiencies

To obtain an exact measure of the various contributions to net feed intake of an animal would require the use of calorimetric chambers, which is difficult to implement in practice. Of practical interest is a net efficiency measure which allows the study of variability in net feed intake under farm conditions.

The consequences of selecting for milk are well known as evident by observing high producing cows. However, Blake and Custodio (1984) stated that they saw no indication that efficiencies of nutrient utilisation have been influenced by selection for milk yield. They suggested that energy intake traits thus merit consideration in breeding programs for dairy cattle.

Veerkamp and Emmans (1995) concluded that for dairy cattle, stronger evidence needs to be collected before any true genetic variation in partial efficiencies can be assumed, and that the most important sources of genetic variation in "gross" energetic efficiency are likely to be yield, the capacity for feed intake, the extent to which body tissue is mobilised and any differences in partitioning the energy between these components.

Luiting (1990) reviewed variation in metabolisable energy intake in poultry. When comparing both between and within strains, she also found that genetic differences in ability to metabolize gross feed energy were of limited magnitude; the coefficient of variation was 1-3%. The author concluded that variation in net feed intake between strains was mainly caused by maintenance requirements.

Heterosis for net feed intake (non-additive genetic variation)

Luiting (1991) reviewed heterosis in laying hens for feed consumed per egg mass. Estimates ranged from negligible (-1%) to a small amount of heterosis (-14%). The author suggested that variation in fasting heat production, which comprises physical activity, metabolic rate and maintenance of body temperature, is the main component of variation in net feed intake. Luiting stated that because all the figures reviewed applied to the same strains, heterosis seemed not systematically present. The

negligible heterosis estimates in poultry are in line with that expected from simulation (Pitchford, 1991) and mouse studies (Hughes and Pitchford, 1994). Both expected small positive improvements from heterosis because of increased size of the animals.

Correlated responses to selection for net feed intake

Poultry

When reviewing previous literature, Luiting (1990) came to the conclusion that variations in maintenance requirements can probably be explained by variations in feather cover and physical activity, and to a lesser extent by variations in basal metabolic rate, area of nude skin, body temperature and body composition.

Luiting *et al.* (1991) reported that of the differences in heat production between high and low hens, 37-51% was left unexplained. This amount was the same as activity related heat production and must be related to a large extent to basal metabolic rate and to thermal regulation not related to plumage quality scoring, cloacal temperature and shank surface.

Luiting and Urff (1991b) found that the genetic correlation estimates between net feed intake and daily feed intake seemed to be positive, whereas no clear values could be obtained for the ones with metabolic body weight, daily egg mass and body weight gain. Unfortunately this is likely to be due to working with too few animals for estimation of genetic correlations.

Selection for low net feed intake would probably lead to less active animals, presumably especially with regard to stereotypic behaviour patterns (Luiting 1991). This may be regarded as a development towards lower stress susceptibility, but it is

also possible that these animals have less behavioural possibilities to cope with the stress imposed on them by the intensive husbandry system.

Bordas *et al.* (1992) also reported correlated responses to divergent selection for net feed intake in hens. They found significant increases in feed efficiency without losses in egg production. The low net feed intake line also had decreased shank length, wattle length and rectal and comb temperature, suggesting a lowering of heat production or dissipation.

Pigs

Only two pig studies have selected directly on feed conversion ratio (Jungst *et al.*, 1981; Webb and King, 1983), with pigs penned individually or in groups. In the pig studies, responses to several generations of selection were insignificant. Mrode and Kennedy (1993) examined genetic variation in measures of feed efficiency and their relationships with growth rate and back fat of pigs. Heritability of daily feed intake was 0.45, and heritability of measures of net feed intake ranged from 0.30-0.38. About half of the variation in daily feed intake was residual. Genetic correlations with net feed intake and growth rate were small and positive (0.18 to 0.34). However, the correlation between net feed intake and back fat was low when net feed intake was adjusted for back fat (0.15), average when adjusted for growth rate (0.34), but was high when adjusted for lean growth rate (0.61). Thus, the genetic correlations with net feed intake were a function of the traits in the model to calculate net feed intake. This indicates that a significant portion of variation in net feed intake is a function of body composition.

Dairy cattle

The best realistic expectation is for a cow to have the ability to partition a greater than average proportion of energy intake to produce milk. Because of the difficulty in collecting data on large numbers of dairy cows, there are very few estimates of genetic correlations between net feed intake and other traits.

Beef cattle

In beef cattle, there is only one study with selection on feed conversion ratio, the inverse of gross efficiency (Bishop *et al.*, 1991). In this study, selection on males was repeated in the "parental" generation and individual food intake of progeny from selected bulls was not measured. The replicated responses in half-sib food conversion ratio were small, possibly due to the adjustment of feed conversion ratio for perceived variation in maintenance requirements, such that selection differentials (pressure) were reduced.

Brelin and Brannang (1982) and Korver *et al.* (1991) obtained low genetic correlations between net feed intake and production, suggesting that residual intake represented real genetic variation in the relationship between feed intake and production. However, Jensen *et al.* (1992) obtained a negative genetic correlation between net feed intake and average daily gain, and so it is not known whether or not the genetic variation in residual intake reported represents variation in feed efficiency or genetic variation in production traits not accounted for when residual feed intake is calculated.

Implications

Clearly, net feed intake is moderately heritable and response to selection will be similar to that achieved when selecting for growth rate or milk yield. However, there may be correlated responses in other traits that may be favourable or detrimental.

In pigs, selection for (low net feed intake) increased efficiency resulted in small decreases in growth. By definition this should not have been the case phenotypically. However, there was a genetic correlation which is further evidence for the importance of selecting on a genetic index rather than a phenotypic index, as suggested by Kennedy *et al.* (1993).

One concern when testing beef cattle is that selection is likely to be on young male animals but possibly of greater importance to improvement in production system efficiency is to lower the maintenance requirement of the cows (Parnell *et al.* 1994). The genetic correlation between efficiency of growing cattle and lactating heifers reported for dairy cattle (Nieuwhof *et al.* 1992), suggests that this relationship is favourable and consequently improvement in post-weaning efficiency and efficiency of the breeding herd might be made simultaneously.

Of concern to beef cattle producers are the genetic correlations with carcass composition, especially fatness. Mrode and Kennedy (1993) showed clearly that the genetic correlation between net feed intake and fatness (also shown by Jensen *et al.*, 1992) depended on how much variation in fatness was accounted for by the multiple regression equation used to estimate net feed intake. There is some evidence, although certainly not conclusive, that selection for increased efficiency using net feed intake as a criteria may reduce the ability of the animal to conserve excess energy in

energy dense tissues such as internal fat. This may be associated with a reduced capacity to cope with nutritional stress associated with pregnancy and lactation (Cowan *et al.* 1980).

Correlations between net feed intake and tolerance to stress are variable. Luiting (1991) showed that selected hens were likely to be less active. The author concluded that the lower activity may be regarded as a development towards less stress susceptibility, but it is also possible that these animals have less behavioural possibilities to cope with the stress imposed on them by the intensive husbandry system. Also, Luiting *et al.* (1994) cited examples of poorer meat quality in pigs selected for increased efficiency.

Effects of selection on reproductive rate are not clear. However, there is reasonable evidence of decreased fatness in high efficiency cattle and pigs. It is possible that this could result in cows less able to maintain body condition during lactation resulting in longer post-partum anoestrus periods. While there could be increased post-partum anoestrus, high efficiency cows are likely to be more drought tolerant because of lower maintenance requirements.

Gaps in our knowledge

The following areas require further work to ensure optimal methods of utilising genetic variation in net feed intake:

1) Genetic correlations between efficiency and traits of economic importance (e.g. carcass weight and composition, marbling, tenderness) have scarcely been estimated in populations of satisfactory size and structure;

2) Heterogeneity between sexes for net feed intake may exist;

3) Genotype by environment interactions have hardly been evaluated;

4) Information on heterosis of net feed intake is rudimentary;

5) Whether genetic variation over the entire production system truly exists;

6) Modelling to test if relationships between intake and production (growth, milk, eggs) are sufficiently linear and stable to enable use in a linear combination to calculate breeding values for efficiency;

7) Variation in QTLs within breeds as well as between breeds.

Part B. The biological mechanisms underlying variation in net feed intake: the energy balance

What then are the true underlying biological determinants of the observed variation in the statitistical concept that is net feed intake? Parks (1982) described an animal as "a mobile, self-feeding, low pressure, quasi-constant, low temperature macro-assembly of micro-catalytic chemical reactions, which transforms the matter and energy of the input chemicals (food) into energy to be dissipated to the environment as heat and work, stored as live weight and packaged as products such as eggs, milk or young". As such, animals can be considered in terms of an energy balance which subscribes to the laws of thermodynamics. Newton's first law of thermodynamics, the law of conservation of energy, asserts that the total amount of energy in an isolated system remains constant. Hess' law of constant heat summation asserts that heat released by a chain of reactions is independent of the chemical pathways, and dependent only on the end products. In effect these laws ensure that the heat evolved in the enormously complex cycle of biochemical reactions that occur in the body is exactly the same as that which is measured when the same food is converted into the same end-products by simple combustion.

All biological processes including growth, work and reproduction use energy, and, in animals, the source of this energy is food. The energy content of the food is metabolised in the body into other energy forms, only some of which are useful for growth and production. Much of the 'wasted' energy is given off from the body in the form of heat. The mechanisms which govern or produce these energy transformations within an individual are governed both by the animal's external environment and by its genetic makeup. In considering the thermodynamic laws, and assuming a constant

or readily quantifiable environmental effect, animal husbandry is concerned with manipulating animal genetics and environmental factors so as to maximise the amount of input energy converted to saleable product, and minimise that which is lost as waste energy.

Feed Costs

The major organic nutrients (carbohydrates, fats and proteins) are required by animals as materials for the construction of body tissues, for the synthesis of products such as milk, eggs and wool, and as a source of energy for work done by the animal. A unifying feature of these diverse functions is that they all involve a transfer of energy (McDonald *et al.*, 1988). This applies both when chemical energy is converted into mechanical or heat energy, such as nutrient oxidation, and when chemical energy is converted from one form to another, as for example when body fat is synthesised from food carbohydrate.

Energy supply

The quantity of chemical energy present in food can be measured by converting it into heat energy, and determining the heat produced. This conversion is carried out by oxidising the food by burning it; the quantity of heat resulting from the complete oxidation of unit weight of a food is known as the 'gross' energy or heat of combustion of that food. Gross energy is measured in an apparatus known as a bomb calorimeter, which in its simplest form consists of a strong metal chamber (the bomb) resting in an insulated tank of water. The food sample is placed in the bomb, and oxygen admitted under pressure. The temperature of the water is recorded, and the sample is then ignited electrically. Heat produced by the oxidation is absorbed by the bomb and the

surrounding water, and when equilibrium is reached the temperature of the water is taken again. The quantity of heat produced is then calculated from the rise in temperature and the weights and specific heats of the water and the bomb (McClean and Tobin 1987; McDonald *et al.* 1988). Some typical gross energy values are shown in Tables 2.2–2.4 (reproduced from Animal and Human Calorimetry; McClean and Tobin 1987). From these tables, it is apparent that fats contain about two and a half times as much energy as carbohydrates, the difference reflecting the larger ratio of carbon plus hydrogen to oxygen in fats (i.e. fats are in a lower state of oxidation and are therefore capable of yielding more energy when oxidised).

Table 2.2. Calorific factors for carbohydrate (starch) and similar compounds

Material Reference	Q_K Heat of combustion (kJ/g)	a_K Oxygen consumption (l/g)	r_K Respiratory quotient	q_K $= Q_K/a_K$ (kJ/l)
Starch				
Magnus-Levy (1907)	17.2	0.829	1.00	21.1
Lusk (1928)	17.51	0.8288	1.00	21.13
Abramson (1943)	17.6	0.829	1.00	21.18
Kleiber (1961)	16.7	(0.800)	1.00	20.9
Brouwer (1965)	17.6	0.829	1.00	21.2
Elliot & Davison (1975)	-	-	-	21.11
	Q	a	r	q
Methane (per l)	39.36	2.00	0.50	19.68
Ethanol	29.77	1.462	0.667	20.36
Glucose	15.64	0.746	1.00	20.95

Table 2.3. Calorific factors for fat

Reference	Q_F Heat of combustion (kJ/g)	a_F Oxygen consumption (l/g)	r_F Respiratory quotient	$q_F = Q_F/a_F$ (kJ/l)
Magnus-Levy (1907)	38.9	2.019	0.71	19.6
Lusk (1928)	39.60	2.0193	0.707	19.62
Cathcart & Cuthbertson (1931)				
Liver and muscle fat	38.4	1.937	0.718	19.82
Adipose tissue	39.8	2.001	0.711	19.88
Abramson (1943)				
Animal fat	39.8	2.013	0.711	19.82
Human fat	39.9	1.992	0.713	20.1
Brouwer (1965)	39.8	2.013	0.711	19.8
Dargol'tz (1973)				
Avian fat	38.9	2.03	0.71	19.3
Elliot & Davison (1975)	-	-	-	19.61
Ben-Porat *et al.* (1983)	39.74	2.028	0.705	19.60

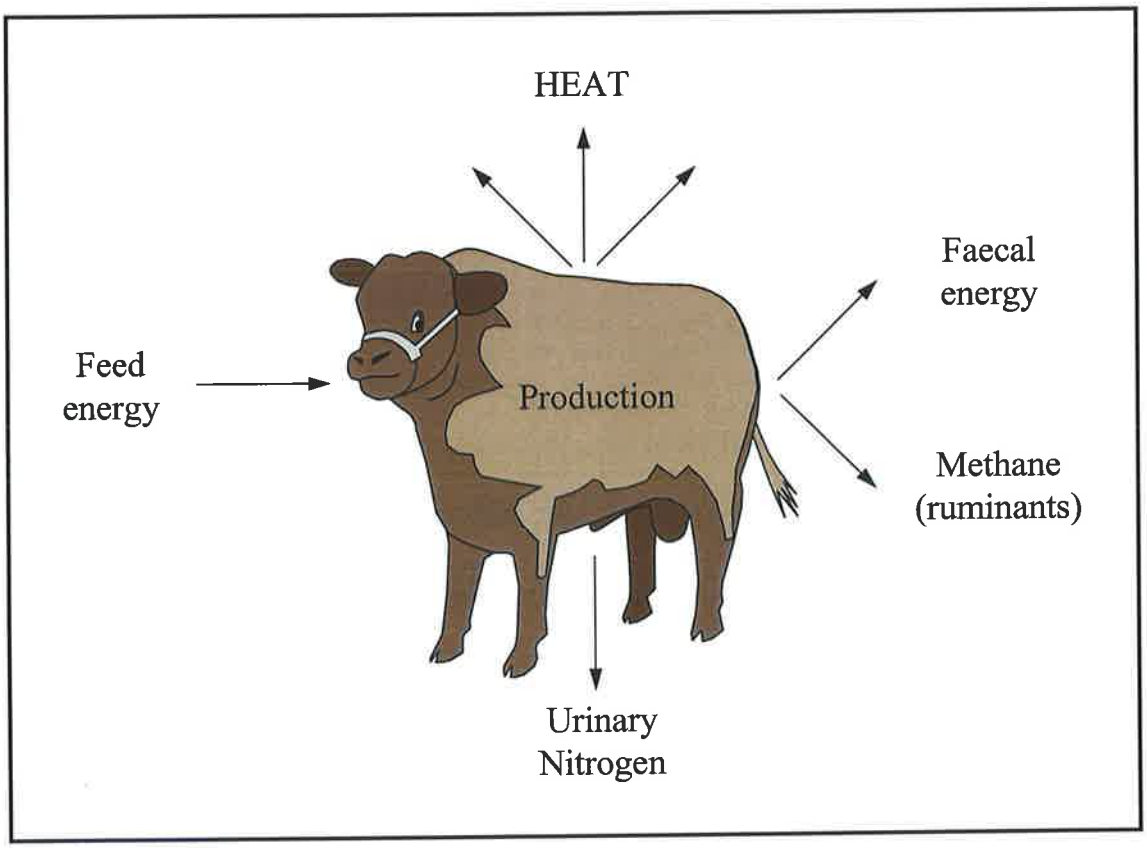
Table 2.4. Calorific factors for partial oxidation of protein to urinary waste

Species Reference	$1/f_{NP}$ Protein-nitrogen ratio	a_N Oxygen consumption (l/g N)	r_N Respiratory quotient	q_N * (kJ/l)
Land Mammals				
Magnus-Levy (1907)	6.24	6.030	0.81	17.8
Peters & van Slyke (1932)	6.25	5.939	0.801	18.77
Lusk (1928)	6.14	5.940	0.802	18.68
Abramson (1943)	-	5.741	0.809	19.3
Kleiber (1961)	6.25	6.7	0.808	18.8
Brouwer (1965)	6.25	5.98	0.809	19.2
Dargol'tz (1973)	6.25	6.45	0.85	19.3
Elliot & Davison (1975)	-	-	-	19.25
Birds				
Dargol'tz (1973)	6.25	5.97	0.74	19.17
Braefield & Llewellyn (1982)	6.27	5.85	0.72	19.43

Energy partition

The partition of the gross energy of food into its major energy sub-divisions is illustrated in Figure 2.2. Some food remains undigested resulting in a loss of energy as faeces. Faeces also contain material originating from the body that has been abraded or secreted in the alimentary tract. The difference between the energy content of the food and the energy content of the faeces is termed the digestible energy of the food. McClean and Tobin (1987) note that this is an apparent value as it includes energy that is not strictly of dietary origin.

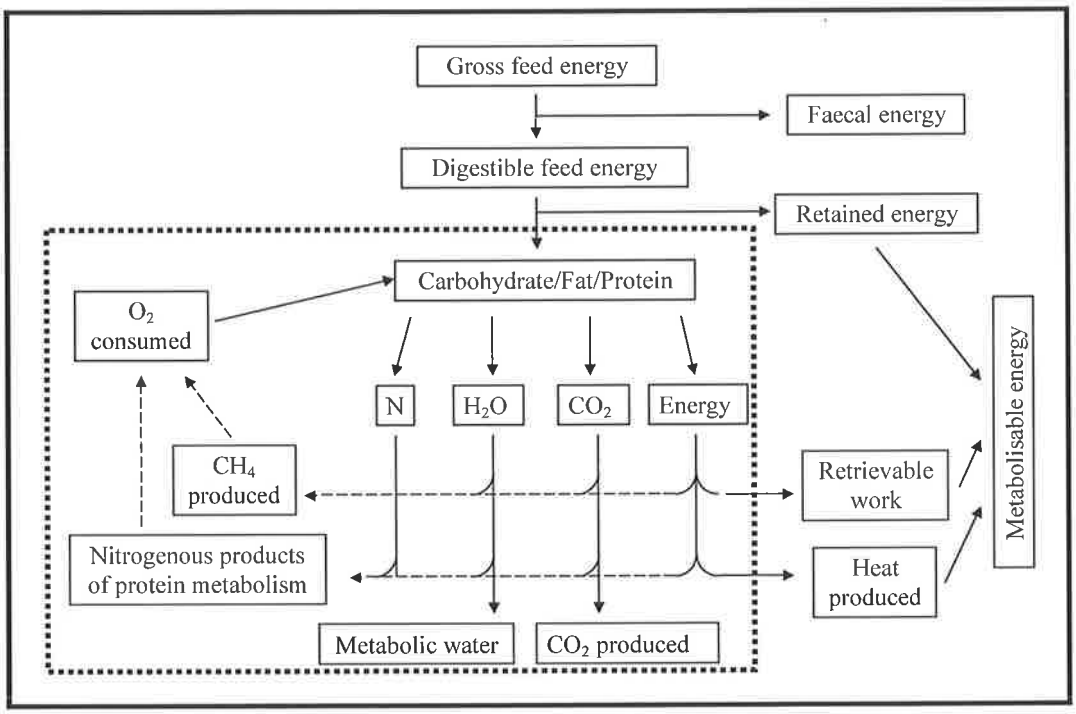
Figure 2.2 The partition of gross food energy



The animal suffers further losses of energy-containing substances in its urine and, if a ruminant, in the combustible gases leaving the digestive tract. The subsequent

metabolisable energy of a food has to provide for the energetic requirements of the body. First, there is the basal energy requirement for the maintenance of respiration, blood circulation and other vital functions. The minimal rate of energy utilisation by a resting subject in a comfortable environment is known as the basal metabolic rate; to this must be added the extra energy cost that occurs after taking a meal (the heat increment of feeding) and any additional energy required for activity, thermo-regulation or other muscular work. Finally, if any of the metabolisable energy is left over from meeting these demands, energy may be retained in the body in chemical form as new tissue growth or as the production of milk or eggs. Figure 2.3 illustrates the net result of food/energy transformations which occur in the body. Rectangular boxes indicate forms of energy. This diagram does not indicate the actual pathways of metabolism, but only pathways which are equivalent according to the Hess' Law.

Figure 2.3. The Energy Partition



Growth, maintenance and energy requirements: energetic efficiency

Biological efficiency in its simplest form is the ratio of the desired form of output energy to the given form of input energy (Brody, 1945) and is thus a measure of 'energetic' efficiency. In animals, the desired form of output energy is milk, meat, eggs, muscular work, wool, and so on; the input is a given category of feed energy, such as gross feed energy, digestible feed energy, metabolisable feed energy and net feed energy. The animal breeder is interested in reducing feed energy inputs, relative to output of saleable product.

Evaluations of animal efficiency have often been based on partitioning feed energy intake between two factors, maintenance and production. Gross efficiency is the percentage of the energy in the given feed category, inclusive of maintenance, recovered in the desired product; net efficiency is the percentage of the energy in the given feed category, exclusive of maintenance, recovered in the desired product. Koong *et al.* (1985) noted that partitioning energy intake has been a convenient and useful means to study whole-animal energy metabolism, and has proven useful in the development of recommendations for feeding standards for practical animal production.

Growth and production

Energy supplied by food in excess of that required for maintenance is used for the various forms of production. Essentially, above-maintenance energy is the basis for the 'work' of growth and morphogenesis. This is associated with both the transformation of the original feed into the final productive precursors circulating in the blood stream or in temporary storage in the body, and with the transformation of

the relatively simple, amorphous precursors into a complexly organised and thermodynamically improbable living organism and related biologic products (Brody, 1945).

A young growing animal will store energy principally in the protein of its new tissues, whereas an adult will store relatively more energy in fat and a lactating animal will transfer food energy into the energy contained in milk constituents. Other forms of production include muscular work and the formation of eggs and wool.

Maintenance energy overhead

An animal deprived of food continues to require energy for those functions of the body immediately necessary for life: the mechanical work of essential muscular activity; the chemical work involved in moving dissolved substances against concentration gradients and the like; and the synthesis of expended body constituents such as enzymes and hormones. In a fasted animal, the energy required for these purposes is obtained by the catabolism of the body's reserves, first of glycogen, then of fat and protein (McDonald *et al.*, 1988). In the fed animal, this demand for body maintenance is met primarily by the energy of the food, avoiding catabolism of the animal's tissues.

When the chemical energy of the food is used solely for muscular and chemical work involved in maintenance, the animal does no work on its surroundings and the energy used is converted into heat. Energy used in this manner is regarded as having been expended, since heat energy is useful to the animal only in maintaining body temperature. In a fasting animal the quantity of heat produced is equal to the energy

of the tissue catabolised and when measured under specific conditions, estimates the animal's basal metabolic rate.

The cost of maintenance

Maintenance requirements constitute a large proportion of total feed costs in animal production systems. Dickerson (1978) estimated that over 50% of total feed intake was used solely for the body maintenance of adult and slaughter animals. Holmes (1977) estimated that only 5.2% of the metabolisable energy fed in beef production systems is recovered as edible energy output.

In a typical beef breeding herd the feed energy for maintenance represents 65% to 75% of the total energy requirements of individual breeding cows (Ferrell and Jenkins, 1985; Davis *et al.*, 1985; Montaño-Bermudez *et al.* 1990). Furthermore, it has been estimated that the cow herd uses between 65% and 85% of the energy required for beef production (Klosterman and Parker, 1976; Montaño-Bermudez *et al.*, 1990). Thus, Dickerson (1970) estimated that at least 50% (~ 70% x 70%) of total feed intake was used solely for body maintenance of adult and slaughter animals. Furthermore, Holmes (1977) estimated that only 5% of the metabolisable energy fed in beef production systems is recovered as edible energy output. In contrast to beef cattle, pig and poultry breeding feed costs represent only around 10% of the total feed cost (Large 1976), a function of the relatively high numbers of progeny per breeding unit in these species.

Thompson and Barlow (1986) modelled the effect of changes in feeding and growth parameters on the efficiency of the cow/calf unit. They estimated that the food costs of the dam represented as much as 89% of total food costs in cattle production. The

discrepancy between this and other estimates in the literature was attributed to the failure of other researchers to allow for the cost of replacing the female and her food consumption from birth to sexual maturity. They 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.

Large (1976) compared the efficiency of meat production systems in five domestic species with that of individual animals from each species, which was taken as the maximum efficiency possible within the species. Reproductive rate was the most important determinant of efficiency, as dam maintenance costs were spread over a greater number of progeny. Poultry, rabbits and pigs under contemporary production systems achieved efficiencies of around 90% of individual animal levels (i.e. slaughter progeny consumed 90% of the food). Corresponding figures for sheep and cattle were around 30% and 50%, respectively. An increase in reproductive rate in cattle from 1 to 2 progeny per year was estimated to give a 30% increase in efficiency. Cattle had a higher calculated efficiency than sheep despite having a lower reproductive rate. This was a result of higher slaughter weights relative to dam size, resulting in a greater proportion of feed going to saleable product.

Measuring maintenance requirement

A range of techniques has been used to estimate maintenance requirement, including:

a) partitioning energy intake between maintenance and growth, either statistically or with assumptions about the cost of various metabolic processes;

b) measuring fasting heat production;

c) measuring feed intake at some equilibrium state.

No function can be said to have absolute priority for food energy. For example, a young animal receiving adequate protein but insufficient energy for maintenance may still store protein while drawing on its reserves of fat (McDonald *et al.*, 1988). Similarly, some wool growth continues to take place in sheep with sub-maintenance intakes of energy. As such, dividing energy metabolism into the components required for maintenance and production is purely artificial; energy balance is in constant flux and reflects all physiological processes and environmental influences. Such a partition is further complicated in growing animals, where the metabolic 'machinery' of growth imposes a maintenance overhead over and above that of normal bodily functions, and this extra load is not readily quantifiable.

Turner and Taylor (1983) suggested that fasting metabolism overestimates basal metabolic rate, because it is a reflection of the animal's metabolic rate before fasting was imposed. This agrees with the conclusions of Webster *et al.* (1974), who described measurements of fasting metabolism as "irrelevant" when used to predict energy retention during growth in cattle. Stephens (1991) noted that physiological responses of an animal to a sudden shortage of food are difficult to predict, and

measurements of fasting metabolism in growing animals are usually preceded by a period during which feed intake is restricted to a level close to "maintenance". Food is then removed and fasting metabolism measured on the third and fourth day of fasting (ARC, 1980).

Webster (1978) described maintenance as the physiologically normal state of adult animals, which implies that at maturity, animals have reached their genetically defined equilibrium and in the absence of physiological stress their food intake defines maintenance requirement. Turner and Taylor (1983) suggested that heat production at energy equilibrium in immature animals is simply the endpoint of a continuous gradient of heat production at higher feed intakes, and that the increase in maintenance requirement due to growth is simply a cost of production. Growing animals are simply expressing their innate impetus for growth, and an artificially imposed environment, under which this cannot be expressed, is not "physiologically normal". The extra increment of heat production which feeding at a maintenance level of nutrition attempts to remove is a real metabolic cost of growth, and heat production in its absence is not biologically meaningful in terms of growth. Stephens (1991) noted that this does not mean that fasting metabolism has no biological meaning, rather it reflects the state of metabolism at the time of fasting, and is useful for comparative purposes only if the factors influencing metabolic rate at the time are understood.

Taylor *et al.* (1981) estimated maintenance requirement by keeping Ayrshire heifers on constant feeding levels for two years, until an equilibrium weight was attained. It was reported that there was no systematic change in an individual's equilibrium maintenance requirement per kg body weight in the range 25% to 100% mature. Efficiency of food utilisation for equilibrium maintenance was found to be

independent of age also, except for a small increase at advanced ages beyond 8 to 9 years. This suggests that maintenance requirement of individual animals is under the control of a strong homeostatic mechanism which may be genetically determined (Stephens, 1991). Maintenance requirements per unit body weight at artificially imposed equilibria were identical to those at maturity; all that was needed was a sufficiently long equilibration period to allow the animal's metabolism to settle to the same base level as it would ultimately reach if development were allowed to proceed normally. Turner and Taylor (1983) termed this base level the 'metabolic intensity characteristic' of a genotype.

The work of Taylor and his group on equilibrium maintenance efficiency suggests that individuals have a genetically defined minimum level of metabolism which is expressed at maturity, but which can be reached by immature animals over a sufficiently long equilibration period, during which metabolism is down-regulated from growth levels to true maintenance levels. This minimum level appears to be associated with production potential, and is also influenced by physiological factors such as rate of protein turnover and ionic pumping (Stephens, 1991). Although Webster (1978) described maintenance as the "physiologically normal" state of adult animals, true maintenance will only be expressed if mature animals are artificially isolated from physiological stresses such as gestation and lactation and from behavioural changes associated with breeding. Fasting heat production at any immature stage will reflect both the length of time on 'maintenance' feeding prior to measurement, and the previous level of metabolism. True maintenance requirement is analogous basal metabolic rate, but is not the lowest possible level of metabolism;

metabolic rate is likely to fluctuate diurnally, seasonally and according to hormonal cycles.

Phenotypic variation in maintenance

As part of a multi-breed cattle project for studying genetic variation between breeds, Taylor *et al.* (1986) conducted an experiment based on five breeds to provide a quantitative description of adult body weight as a function of daily food intake and body composition. During the course of this experiment, it was found necessary to introduce a dairy-beef difference in maintenance requirement in order to achieve experimentally pre-assigned 'target' weights and body compositions. In considering data from a range of feeding and fasting trials, a 20% dairy-beef difference in maintenance requirement and the intermediacy of dairy x beef crosses appeared sufficiently well founded to be used to adjust estimates obtained from beef or dual-purpose cattle to a dairy-type basis or vice-versa.

It was noted that the mean maintenance requirement for beef cows from feeding trials (0.57±0.02) was almost identical to the mean value for dairy cows from fasting trials (0.55±0.01), and it was argued that the previous obscurity of a dairy-beef gradient was because the majority of feeding trials had been conducted on beef cattle while virtually all fasting trials had used dairy cattle. Using this reasoning, the authors concluded that accepting the reality of a 0.2-fold dairy-beef difference also necessitated accepting a 0.2-fold average difference between the results of feeding and fasting trials. In other words, an explanation was required for the large (20%) difference between the mean maintenance requirements obtained from fasted and fed dairy cattle.

One explanation is that the fasted-fed difference found for dairy cows also applies to beef cows, with a proportional increment of 35% from fasted to fed cows due to increased activity and the associated stresses of feeding and living. Alternatively, dairy and beef breeds may differ in the efficiency of utilisation of metabolisable energy for maintenance with no significant breed differences in fasting metabolism. Essentially, this indicates that variation in maintenance may result from variation in either basal metabolism or the heat increment of feeding, or from a combination of the two.

Stephens (1991) noted that Taylor and his colleagues based their regressions on data from a large number of feeding and fasting trials on mature and immature animals and consequently it is difficult to draw any firm conclusions. Taylor and his colleagues acknowledged the need for a "crucial experiment" to examine fed and fasted maintenance estimates in mature animals of different breeds.

Maintenance, fasting heat production and the heat increment of feeding

Clearly, maintenance requirement is only easily quantifiable in mature, non-productive animals, or in growing animals maintained on below *ad libitum* diets for sufficient lengths of time. However, even in such animals, measurement of maintenance can only be based on accurate measurement of all the components of the energy balance. Variation in results stems principally from differences in estimating the metabolic heat component of maintenance. Fasting heat production in mature, non-productive animals measures true basal metabolic rate; fed heat production in the same animals measures basal metabolic rate plus the additional heat component associated with taking a meal, often termed the heat increment of feeding. True

maintenance discounts this additional energy increment, however for livestock production it is an important component of the efficiency complex, as animals are not normally maintained on a fasting diet (i.e. maintaining body function through tissue catabolism).

Basal metabolism and body size

Many physiological parameters are allometrically associated with body size. In point of fact, basal metabolic rate was the physiological parameter used originally by Brody (1945) and Kleiber (1961) to derive their respective allometric equations. However, Hayssen and Lacey (1985) have claimed that both equations are based on small and unrepresentative samples of mammals, and are statistically flawed. In a comprehensive study using 3 species of monotrenes, 42 species of marsupials and 248 eutherian species, they demonstrated that no single equation adequately described the relationship between basal metabolic rate and body size in mammals. Variation in the allometry of metabolism was found even within orders of mammals.

Stephens (1991) noted that this in no way invalidates the assumption of a particular allometric relationship between metabolic parameters and body size in mammals, providing the power function of metabolic size is chosen to best represent the breed or species in question.

The physiological basis of metabolic heat production

Ignoring differences in the efficiency of uptake (i.e. energy lost through faeces, methane and urinary nitrogen), the factors which determine maintenance requirement will be those which influence metabolic heat production. There is a large body of literature dealing with this area. Some of the more significant factors are examined below.

Physiological age

Fasting metabolism declines during the growth of sheep from around 580 kJ/kg$^{0.75}$ at 2 months to around 350 kJ/kg$^{0.75}$ at 2 years (ARC, 1980) indicating an effect of physiological age. Ledger and Sayers (1977) reported that the amount of feed necessary to maintain immature steers at fixed live-weight declined with time, and that the proportional decline was greater in less mature animals. This may be related to visceral organ mass/activity. Growing animals have an extra maintenance load imposed by the up-regulated metabolic activity associated with growth. After the point of inflexion in the growth phase, this activity declines with concomitant reductions in growth 'machinery'. In animals where growth rate is declining asymptotically, or where animals are maintained at a constant immature body weight, one would expect to see a gradual reduction in heat production associated with this decline in visceral levels/activity.

Physiological Status

The physiological status of an animal can have a profound effect on maintenance requirement. Heat production of lactating cows can be twice the non-lactating level (Hutton, 1962). When cows are fasted in mid-lactation to the extent that milk

production virtually ceases, their heat production remains significantly elevated above non-lactating levels (Taylor *et al.* 1986). Similarly, the maintenance requirement of growing animals varies according to their feeding level (Blaxter, 1962; Turner and Taylor, 1983). Variation in maintenance energy requirements have also been recorded due to sex, season, temperature and previous nutritional status.

Non-shivering Thermogenesis

Retaining linear proportions (and hence geometric similarity) whilst reducing size results in a larger surface area to volume ratio. One outcome of this 'scaling' effect is that as animal size decreases, the shortfall between the heat produced by essential cell processes and that required to maintain body temperature gets larger. This is made up in homeotherms by the process of non-shivering thermogenesis. Jansky (1973) defined non-shivering thermogenesis as "a specific heat producing mechanism due to processes which do not involve muscular contractions".

Swan (1981) monitored the oxygen consumed in oxidative phosphorylation. He found that at least 75% of the heat produced under conditions of 'basal' metabolism was associated with non-shivering thermogenesis. Experiments involving dogs subjected to hypothalamic lesions (the hypothalamus is thought to control non-shivering thermogenesis) have yielded similar results, where the dogs lost heat rapidly when exposed to cold, but maintained all 'essential' cellular processes and survived if kept warm (Keller, 1938; cited by Swan, 1981).

The proportion of basal metabolism that is attributable to non-shivering thermogenesis would be expected to vary with body size. As basal metabolism is a function of both

body mass and surface area, the exponent used to relate mass to basal metabolism falls somewhere between 0.66 and 1.00.

Body Composition

The energy costs of fat and muscle (protein) deposition in the growing animal are very similar but the maintenance requirement of protein mass is higher than that of fat (Webster, 1980). Consequently, in animals of the same weight but differing in body composition, the leaner animals tend to have higher relative maintenance energy requirements (Russel and Wright, 1983). Thompson *et al.* (1983); Byers *et al.* (1987) and DiCostanzo *et al.* (1990) all reported negative relationships between fatness and maintenance energy requirements of cows. DiCostanzo *et al.* (1990) calculated that of the total energy requirement of non-pregnant, non-lactating cows, 87% was used to maintain body protein, and only 11% was used to maintain body fat.

Solis *et al.* (1988) observed a significant relationship between maintenance requirements and site of fat deposition, and suggested that the site of fat storage had a substantial impact on maintenance requirements. DiCostanzo *et al.* (1991) compared efficient, average and inefficient cows, categorised by their weight change during consecutive test periods either at maintenance or *ad libitum* feeding levels. The most efficient cows tended to have more fat, deposited less protein, had greater liver weights and required less energy for maintenance than average or inefficient cows.

Visceral Tissue

Webster (1989) reviewed the literature and concluded that tissues such as the gut epithelium and liver were major contributors to thermogenesis, and that differences between animals in maintenance requirement are related to the proportion of metabolically active tissues.

Stephens *et al.* (1988) reported that total body weight and visceral weight (around 16% of total body weight) were good predictors of mature food intake in mice. The work of Koong *et al.* (1985) on growing pigs has shown that a relatively small increase in proportional visceral tissue mass due to nutrition results in a significant increase in heat production. Koong *et al.* (1983) reported that the fasting heat production of pigs decreased asymptotically during prolonged maintenance, with associated decreases in the weight of metabolically active organs.

Ferrell and Jenkins (1985) found that the variation between cattle types in their energy requirements for maintenance is greater than the variation in energy requirements for growth, gestation and lactation. More importantly in light of the current discussion, they found that a relatively large proportion of maintenance energy requirements can be attributed to the energy expenditures of visceral organs, especially the liver and gastrointestinal tract. The high rates of energy expenditures of these tissues appears to be directly associated with the high rates of protein synthesis in these tissues.

Genetic Variation in Maintenance

Accurate estimates of genetic parameters associated with maintenance are scarce. This is the result, at least in agriculturally important species, of the difficulty in establishing the 'true' maintenance of an individual. Results to date are in general limited by their method of estimation (if produced from growing animals) or by their failure to measure all the factors contributing to the energy balance (if produced from mature animals). However, they do provide a broad estimate of the amount of genetic variation that is available.

Webster (1989) stated that in a thermo-neutral environment the maintenance requirements of mammals lay in the range 0.4 to 0.6 MJ/kg $W^{0.75}$ per day, and suggested that there appeared to be little scope for increasing the efficiency of growth by reducing maintenance requirement, since much of the variation even within this narrow range was attributable to differences in body composition. There is evidence however that some groups of animals have basal metabolic rates outside the normal range, possibly in response to environmental constraints during evolution. Hudson and Deavers (1976) have reported that basal metabolism was about 40% below the expected level in 8 species of desert-dwelling ground squirrels. It was suggested that this was an adaptation which minimised the amount of water lost in evaporative cooling.

Stephens (1991) cited a number of authors who have reported that the basal metabolic rate of marsupials is about 30% less than for eutherian mammals of the same body weight. However, McNab (1978) suggested that the basal metabolic rate of both marsupial and eutherian mammals was primarily a function of feeding habits,

observing that several eutherians have basal metabolic rates below those of similar-sized marsupials. This evidence again suggests that the relationship between basal metabolic rate and body size is not fixed, but rather the result of physiological adaptation in the course of evolution.

Thonney *et al.* (1976) reported that the maintenance requirements of Japanese Black cattle were significantly less than that of other *Bos taurus* breeds. Frisch and Vercoe (1977) reported that Hereford x Shorthorn cross cattle had 6 to 10% higher maintenance requirements than Brahman or Africander x (Hereford x Shorthorn) cattle. On a fixed level of feed intake the *Bos indicus* cross cattle could maintain at least 10% more live-weight than the *Bos taurus* cattle. Taylor *et al.* (1986) hypothesised that the greater the maximum gross efficiency of a breed for meat or milk production, the lower will be its maintenance efficiency. In other words, the more a breed can 'dilute' its maintenance requirement by having metabolism geared for higher output, the higher its relative maintenance efficiency.

Stephens (1991) produced genetic parameter estimates for maintenance from a series of mouse lines selected for high or low mature maintenance requirement. Realised heritability estimated on the first generation of selection was 0.35±0.18 (or 0.28±0.18 after correction for fat content). However, maintenance was estimated purely on volumetric food intake per unit body weight, taking no account of loss through uptake inefficiency or metabolic heat production.

Ferrell and Jenkins (1985) suggested that genetic potential for production may have an effect on fasting metabolism. Heat production per unit weight or metabolic body size in cows appeared to vary little with size *per se*, but animals with a higher genetic

potential for milk production had higher apparent maintenance requirements, the difference being expressed even when the animals were not lactating.

Gaps in our knowledge

The following areas require further work to determine the mode of action of selection for net feed intake:

1) The physiological basis of net feed intake in terms of maintenance of body tissues, sustaining of body temperature, basal metabolic rate and stress susceptibility, has been investigated in little or no detail yet;

2) The biochemical basis to variation in intake independent of the major biochemical pathways associated with growth and maintenance;

3) The degree to which these relationships are determined by genotyope, versus environmental influences;

4) Possible impacts of selection on reproductive rate;

5) The response in net feed intake and associated traits under different biological constraints (i.e.different production environments).

Summary

During this discussion of animal efficiency, a number of important points have been raised. Selection for growth rate is an effective means of increasing growth rate and efficiency of gain, but results in animals that are larger at maturity with concomitant increases in the maintenance costs of adult stock. Selection for biological efficiency may prove more economically attractive in the future. Identifying sources of intake

that are not associated with production or maintaining body weight has been suggested as a means to improve biological efficiency. However, to date, few studies have examined net feed intake directly and little is currently known about its phenotypic variation, and even less whether it has a genetic basis, although there is some evidence of a heritable component. Furthermore, the relationships between net feed intake and other important determinants of production, such as body composition and reproductive rate, are not well understood. This knowledge will only come through a better understanding of the relationships between the underlying physiological mechanisms that contribute to variation observed in feed intake.

Chapter 3.

Direct response to selection for post-weaning net feed intake and correlated responses in post-weaning growth, intake, gross digestibility and body composition.

Introduction

A number of previous experiments have demonstrated a heritable component of net feed intake (Archer, 1996; Arthur *et al.*, 1997). However, there are relatively few published estimates of realised heritability established from short or long-term selection experiments. Studies such as these are critical to establishing the underlying biological basis of the variation observed in net feed intake, by observing the direct and correlated responses to selection in a range of biologically meaningful parameters.

In animal production environments, selection is generally carried out on individuals at a young age to facilitate a faster generation interval and to reduce costs associated with maintaining unselected individuals. Based on this premise, the current study aimed to replicate real-world conditions by selecting on net feed intake estimated immediately post-weaning over a number of generations. Due to the time and resources required to perform such an experiment in livestock species where maturation is relatively slow, it was decided to use the laboratory mouse as a model species in this investigation. The mouse has been used extensively to examine aspects of feed intake (e.g. Timon and Eisen, 1970; Eisen, 1977; Gunsett *et al.*, 1981; Sharp *et al.*, 1984; Stephens, 1991) and has proved to be a convenient and valuable tool for investigating the biology of growth and in developing concepts applicable to livestock species.

Previous work in mice (e.g. Archer 1996; Nielsen 1998; Bunger *et al.* 1998) has demonstrated a positive genetic correlation of net feed intake or similar measures of intake with maintenance efficiency. Based on this evidence, the current study was progressed until a significant difference in maintenance efficiency was observed between divergent selection lines, to facilitate studies of the underlying biology of maintenance. This chapter reports the effects of seven generations of selection for post-weaning net feed intake on growth and intake traits post-weaning.

Materials and methods

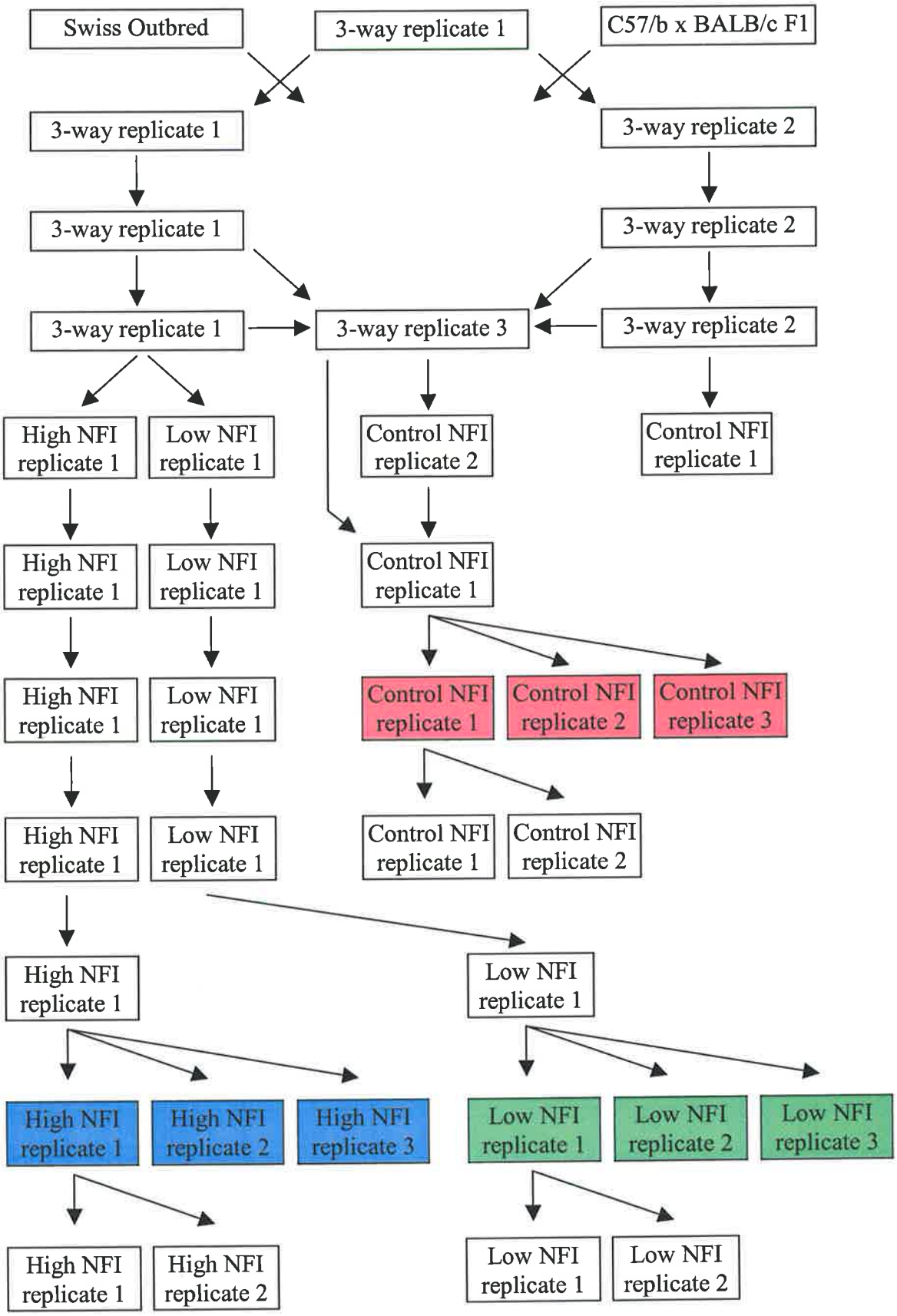
Animals

Animals were sourced from a random-mating population used previously to estimate phenotypic and genetic parameters associated with feeding and growth (Archer *et al.* 1998). The structure of this population is detailed in Figure 3.1. It was originally derived from a three-way cross utilising Swiss out bred males and the F_1 progeny of a cross between BALB/c and C57/bl6 inbred lines. Four generations of random mating were undertaken to examine genetic variation in net feed intake (Archer *et al.*, 1997). Two replicates were maintained for generations 2 and 3, and a third replicate was produced in generation 4. Estimated breeding values for post-weaning net feed intake were calculated for animals in all replicates of generation 4 using DF-REML (Meyer 1993) on the accumulated data set of generations 1 to 4. The 4 highest (most positive) males (excluding full-siblings) and 20 highest females from generation 4, replicate 1 were selected as parents for a high net feed intake selection line. Equivalent selection was carried out on the 4 lowest (most negative) males and 20 lowest females in generation 4, replicate 1 to produce parents for a low net feed intake selection line. Initial for both parental groups was from a total number of approximately 200

animals. Clearly, selection pressure was applied more heavily in males (4/100) than in females (20/100), as is the case in most domestic livestock production systems. There was a trade off between selection intensity and numbers of progeny available for subsequent measurement.

From generations 5 to 10, selection was practiced within line. Post-weaning net feed intake breeding values were re-estimated for all individuals with each succesive generation, based on a continuously accumulating data set leveraging improved relationship information. Selection consisted of the highest (most positive) 5 males/20 females in the high net feed intake line, and the lowest (most negative) 5 males/20 females in the low net feed intake line. In the penultimate generation (10), 3 replicates representing 3 parities for each dam/sire combination were produced within each line to increase animal numbers for subsequent analysis of the reponse to selection (shaded ■ High and ■ Low).

Figure 3.1. Pedigree structure.



Control line

A control line was also derived from replicate 3 of generation 4. Originally it was planned to maintain this line using a slow generation interval so as to reduce the influence of genetic drift and hence use the line as a base-line for comparison of various traits measured at later stages in the selection lines (e.g. metabolic rate). Unfortunately, poor reproductive rates between generation 4 and 5 (only 4 litters produced from 8 individuals) led to a genetic 'bottleneck' and meant that subsequent generations were not truly representative of the original population. In particular, there was a marked increase in weights at all ages. Temporally, generation 7 of the control line was equivalent to generation 10 of the high and low lines. The control line was continued to generation 8, and where possible has been used as a source of comparison (shaded■). However, in most cases it was considered pertinent to place more emphasis on direct comparisons between the selection lines in the absence of a true control, using the methodology of Hill (1972b, d, e) to estimate variances for line differences in the absence of repetition or a valid control line.

Life-cycle

Within the two selection lines, females were generally allocated to males according to rank, e.g. the highest male was mated to females 1, 6, 11, 16 and 21 ranked in order from highest to lowest net feed intake, however half-sib matings were avoided to reduce the rate of inbreeding. Matings were carried out at nine weeks of age. Selected females were removed from communal housing and placed with the appropriate male for a period of seven days to ensure a high rate of conception (females cycle every 4-5 days). Females were then placed in individual boxes with access to paper towelling for nesting material. Gestation length in the mouse is 21

days. To maximise litter numbers and prevent future inbreeding, individual litters were standardized to 5 animals soon after birth, by selecting 2 males, 2 females and a random sex animal. Pups were kept with their mother until 21 days of age, at which point they were weighed and then weaned into individual cages (2 randomly assorted types, one slightly smaller and darker than the other) to undergo a 3 week feed intake test. From this information, another round of selection was initiated. Non-selected individuals were normally euthanased using CO_2 gas asphyxiation. Between the post-weaning test and mating, selected males were housed individually, whilst selected females were housed communally within line in groups of 10. Selection continued for 7 generations, during which a number of other experimental procedures were undertaken to examine correlated responses in a range of traits.

Some deviations from this basic life cycle occurred for specific groups during the course of the experiment. Where this occurred, it is noted in the body of the text. For example, in generation 11 it was decided to examine the interaction between net feed intake and pre-weaning, post-partum nutrient supply. To this end, litters were not standardized to 5, but were reduced or expanded by cross fostering within line at birth to produce a diverse range of litter sizes (3-16) within each line. This experiment was undertaken by another student (Fenton *et al.*, 1999) and hence will only be referred to fleetingly in the body of this text. However, where pre-weaning litter size may effect other measures taken on individuals specific to this study, it is accounted for within the analysis.

Post-weaning growth and intake

During the 3-week post-weaning test, animals were provided with a plastic food hopper designed to minimise spillage and food contamination from bedding (Figure

3.2). This hopper contained a known weight of a standardised ration (Archer, 1996) at the beginning of the test period. At approximately weekly intervals animals were weighed and the hopper was weighed, replenished and re-weighed. This continued for three weeks, at which point the collected data was used to estimate post-weaning net feed intake breeding values for all individuals on a dry matter basis.

Figure 3.2. Food hopper design and caging.

Faecal waste

In generations 10 and 11, faecal waste production was measured on all individuals during the post-weaning test period. This was achieved by housing animals without bedding for the test period and collecting faecal waste at weekly intervals. Samples were dried, weighed, pooled and later analysed for energy content using a ballistic bomb calorimeter. Due to the large sample size and the relatively time-intensive nature of the procedure, it was decided to further pool samples. For generation 10,

samples were pooled across sexes within sire and within management group. Generation 11, designed to estimate the effects of litter size on net feed intake, was pooled across sexes within litter. Energy content of foodstuffs for these generations was also assessed to examine total system energy balances.

Bomb calorimetry

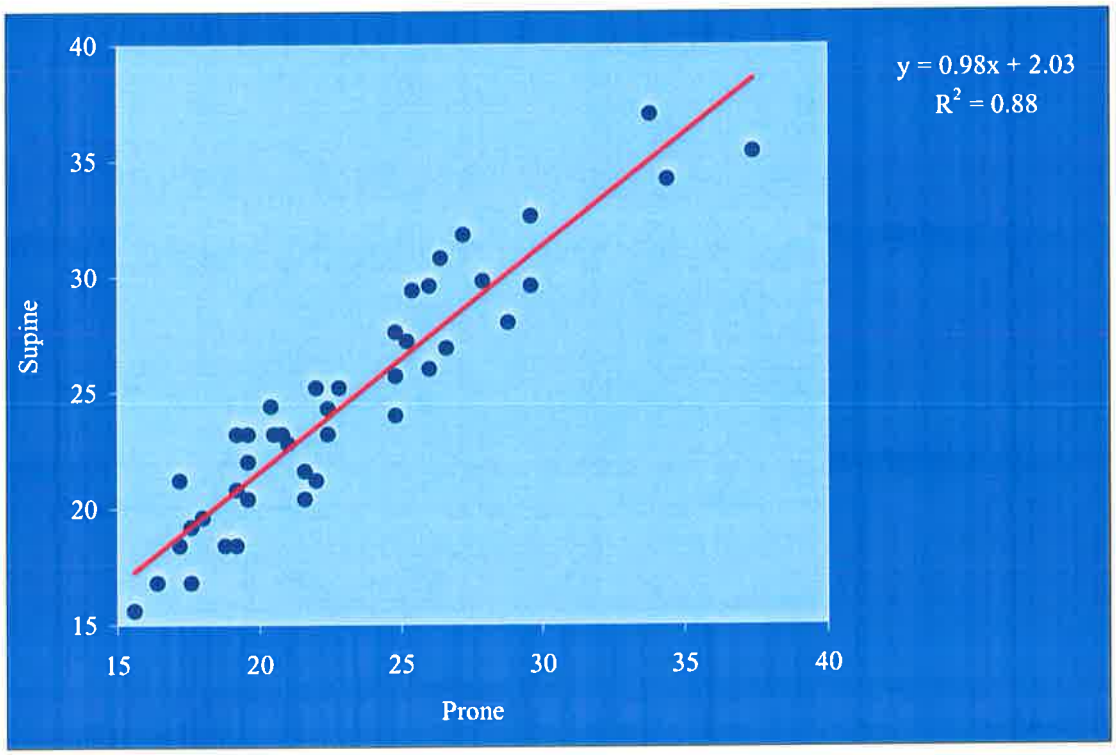
Individual samples were oven dried overnight at 55°C and then grouped on a constant weight basis. They were then ground in a feed grinder followed by mortar and pestle. Each sample was then pelletised using an impact pelletiser. Pellets weighed approximately 0.05 grams. Pellets were weighed and then analysed using a standard protocol for the bomb calorimeter.

Body composition

To assess the impact of selection for net feed intake on body composition, a non-invasive electro-magnetic technique was used to determine body fat percentage in a number of generations both post-weaning and at maturity. The device used was supplied by EM-Scan Corporation (Figure 3.3). This device requires calibration from a series of chemical fat-extractions carried out on the species of interest. A robust calibration curve derived by Merlyn Nielsen (pers. comm.) on mice was used in this instance. The only difference between Dr Nielsen's scanning protocol and that in the current experiment was a change from a supine to prone position. A series of comparative scans using animals of various weights and ages was taken using both methods (Figure 3.4). The intercept of the regression was not significantly different from zero, and the slope was not significantly different from one, indicating that the methodologies were equivalent.

Figure 3.3. EM-Scan device for measuring body composition on live animals.

Figure 3.4. Comparison of % fat estimates produced from prone and supine positions.



Trait definitions

A summary of the traits used in subsequent analyses is given in Table 3.1, and their derivations are given underneath. The data collected post-weaning consisted of weights at days 21 (weaning), 28, 35 and 42, feed intake from day 21 to 28, 28 to 35 and 35 to 42, and body composition at the end of the post-weaning test. The day of the year on which each measurement was made was recorded in Julian days from the 1st of January 1993, and are notated as $DOY_{21....42}$. From this data, other traits of interest were derived. The main period of interest in the post-weaning test was from days 28 to 42, as the first week was considered a pre-test adjustment phase during which the mice were able to adjust to the stress of weaning and adapt to the feeding system. Average daily feed intake during this period, average daily gain, average

daily faecal output (generations 10 and 11), mid-weight, metabolic mid-weight, food conversion ratio and gross efficiency were calculated according to the formulae given below. In addition, the exact age of the mouse at day 28 and at the measurement of body composition were calculated and subsequently used as covariates in the analyses as there was some variation in the actual age of the mice at these times.

Table 3.1. Summary of the post-weaning traits used in the analyses with their abbreviations and units.

Abbreviation	Trait	Units
Wt_{21}	Weaning weight	g(bodyweight)
ADG_{PW}	Average daily gain	g(bodyweight).day^{-1}
MWT_{PW}	Mid-weight	g(bodyweight)
$MMWT_{PW}$	Metabolic mid-weight	g(bodyweight)$^{0.73}$
DFI_{PW}	Average daily feed intake (dry matter basis)	g(feed).day^{-1}
DFO_{PW}	Average daily faecal output (dry matter basis)	g(waste).day^{-1}
NFI_{PW}	Net (residual) feed intake	g(feed).day^{-1}
FCR_{PW}	Food conversion ratio	g(feed).g(gain)$^{-1}$
GE_{PW}	Gross efficiency	g(gain).g(feed)$^{-1}$
$\%F_{PW}$	Percent body fat	%(bodyweight)

Formulae used in the calculation of post-weaning traits

$$DFI_{PW} = \frac{Feed\ Intake_{28-35} + Feed\ Intake_{35-42}}{DOY_{42} - DOY_{28}}$$

$$DFO_{PW} = \frac{Faecal\ Output_{28-35} + Faecal\ Output_{35-42}}{DOY_{42} - DOY_{28}}$$

$$ADG_{PW} = \frac{Weight_{42} - Weight_{28}}{DOY_{42} - DOY_{28}}$$

$$MWT_{PW} = 0.5(Weight_{28} + Weight_{42})$$

$$MMWT_{PW} = (MWT_{PW})^{0.73}$$

$$FCR_{PW} = \frac{Feed\ Intake_{28-35} + Feed\ Intake_{35-42}}{Weight_{42} - Weight_{28}}$$

$$GE_{PW} = FCR^{-1}$$

Calculation of net feed intake

Post-weaning net feed intake was calculated as the residual error term of a linear model (PROC GLM, SAS 1989) fitted to the accumulated data set. The model was fitted to post-weaning daily feed intake and included terms for the class variables sex and management group, co-variables average daily gain and metabolic mid-weight, and the interactions of each class variable with the co-variables. All two-way interactions were retained.

Variance components and breeding value estimation

Variance and covariance components for the randomly mated generations were estimated using derivative-free restricted maximum likelihood (DFREML, Meyer 1993) operated using a front-end program described by Swan (1994). Fixed effects included sex, management group (generation x replicate), parity of the dam, litter size at birth and litter size at weaning. The litter size effects accounted for differences in the pre- and post-natal environments. Age at time of measurement was used as a covariate in the model where appropriate. Random effects fitted included terms for a direct additive genetic effect and the common environment within litters. Variation associated with maternal genotype was examined but was not significant for any trait. Previous work (Archer, 1996) examined sex differences in post-weaning daily feed intake and net feed intake. Correlations between males and females for additive

genetic and common litter environment variance components were close to unity, and hence daily feed intake and net feed intake in both sexes have been treated as single traits in all subsequent analyses. This general model was retained to estimate breeding values for selection in generations 4 to 10.

Alternative calculation of post-weaning net feed intake

For selection purposes, net feed intake was re-estimated from the continuously accumulated data set with each succesive generation. Alternative methods of calculating net feed intake were also carried out. These involved modelling daily feed intake within generation, within line and within replicate, as well as combinations of the three. The effect of these alternative models on overall response was examined.

Inbreeding

Selection within a closed population must take into account any increase in levels of inbreeding. Although matings were designed to minimise the rate of increase in inbreeding, inbreeding coefficients have been calculated for all individuals. These coefficients have been fitted as covariates within line in all subsequent analyses to assess the extent to which inbreeding had an effect.

Analyses

A series of linear models (PROC GLM, SAS 1989) were used to analyse all post-weaning growth and intake traits in generation 10, replicates 1, 2 and 3. A general model was first fitted to all traits. A number of main effects and interactions did not account for a significant proportion of the variance for any trait and were subsequently excluded from the model.

Some consideration was given to the inclusion of inbreeding coefficient in the model. As this was primarily a short-term selection experiment, inbreeding should, by definition, generally correlate with selection differential, and hence the trait under selection (positive or negative net feed intake). As such, when examining divergent selection lines, particularly with a control line, the effect of inbreeding coefficient as a main effect should be negligible, as the most inbred animals tend to have the most extreme values for the trait under selection. Instead, it was fitted as an interaction with line to try and examine the effects of inbreeding on net feed intake generally. One would expect that, particularly for the selected trait, there should be a significant interaction, with the selection lines showing opposite signs for their regression estimates. It is the relative size of the absolute estimates that is of interest with respect to an inbreeding effect.

The final model included:

management group (MGP 1, 2, 3 / 1, 2)
parity (PAR 1, 2, 3 / 1, 2)
age at measurement (AGE 26–33 / 43-70 days)
housing box type (BOX 1, 2 / 1)
litter size (LIT 3-16 pups)
sex (SEX male, female)
line (LIN control, high, low)
management group by sex
management group by line
sex by line
inbreeding coefficient (INC 0-0.37) by line

Where class numbers or covariate ranges differed between post-weaning intake measurement and post-weaning body composition measurement, both are presented in parentheses. A summary of the numbers of mice measured for each trait is presented in Appendix 1, Table A1.1.

Realised heritability

Within line, the cumulative selection differential was calculated as follows:

$$CSD_{G_{i+1}} = \frac{\left[\left(\sum NFI_{Selected\ Males\ G_i}\right)\times n_{G_{i+1}}\right]+\left[\left(\sum NFI_{Selected\ Females\ G_i}\right)\times n_{G_{i+1}}\right]}{2} - \frac{\sum NFI_{G_i}}{n_{G_i}} + CSD_{G_i}$$

Based on the recommendations of Hill (1972a), realised heritability for net feed intake was calculated from regression of cumulative response on cumulative selection differential. Given the lack of replication of the selection process, it was considered appropriate to calculate standard errors using the methods of Hill (1971, 1972a, b, c, d, e) using the associated simplifying assumptions to account for possible genetic drift.

Genetic Drift

Where the selection lines were significantly different for any given variable, it was considered pertinent to assess the extent to which random drift may have contributed to the divergence. Although it was not possible to estimate the response variance empirically, the sampling variance of the response within line was estimated using the approximation:

$$\sigma^2_R = \sigma^2_d + \sigma^2_e$$
$$\cong V_P\left(\frac{th^2}{N_e} + \frac{1}{M}\right)$$

where t was the number of generations (10), N_e was the effective population size of the line, and M was the number of individuals measured within the line at generation 10 (Hill, 1980). Clearly, this was only possible where a previous estimate of the phenotypic and genetic variances was available from the randomly-mated generations.

Results

Phenotypic and genetic parameters

For generations 1-4 (Table 3.3, Archer, 1996) common environmental effects were significant for all traits but only large for weaning weight. Average daily gain was lowly heritable; weaning weight, mid-weight, daily feed intake and net feed intake were all moderately heritable.

Table 3.3. Mean, phenotypic standard deviation, heritability and common environmental effects for post-weaning traits from univariate analyses (Archer, 1996).

Post-weaning trait	μ	σ_P	h^2	c^2
Wt_{21} (g)	14.2	1.7	0.33 ± 0.06	0.48 ± 0.03
ADG_{PW} (g/day)	0.37	0.15	0.14 ± 0.05	0.11 ± 0.03
MWT_{PW} (g)	23.2	2.4	0.35 ± 0.07	0.14 ± 0.03
DFI_{PW} (g/day)	4.65	0.43	0.33 ± 0.06	0.09 ± 0.02
NFI_{PW} (g/day)	0.00	0.31	0.27 ± 0.06	0.16 ± 0.03
$\%FAT_{PW}$	15.3	1.66	0.22 ± 0.10	0.14 ± 0.04

Phenotypic and genetic correlations between post-weaning traits derived from bivariate analyses of generations 1-4 are presented in Table 3.4 (Archer, 1996). Phenotypically, animals that were larger at weaning were also larger throughout the

test period and required more feed to maintain this body weight. Net feed intake was highly correlated with daily feed intake but not growth or body weight.

The two measures of body weight were strongly correlated (0.74). Intake was highly genetically correlated with mid-weight (0.76) but less so with average daily gain (0.36). Percentage body fat was moderately correlated with average daily gain (0.57). Net feed intake was genetically correlated with raw intake (0.64) but not with growth or body weight.

Table 3.4. Phenotypic (above diagonal) and genetic (below diagonal) correlations between post-weaning traits (Archer, 1996).

	Wt_{21}	ADG_{PW}	MWT_{PW}	DFI_{PW}	NFI_{PW}	$\%F_{PW}$
Wt_{21}		-0.16	0.58	0.29	-0.11	0.04
ADG_{PW}	-0.15		-0.01	0.11	-0.04	0.20
MWT_{PW}	0.74	0.49		0.68	-0.02	0.14
DFI_{PW}	0.42	0.36	0.76		0.69	0.11
NFI_{PW}	-0.16	-0.06	0.00	0.64		-0.02
$\%FAT_{PW}$	0.01	0.57	0.24	0.09	-0.10	

Direct response to selection

Cumulative response in net feed intake has been plotted against cumulative selection differential between generations 4 and 11 (Figure 3.5). Between generation 5 and 6 there was only a small selection differential and hence response because of a program sorting error at the time of selection resulting in a generation of essentially random mating. Aside from this, both lines showed a significant and symmetrical response to selection. Realised heritability was 0.27 and 0.26 for the high and low selection lines respectively.

Figure 3.5. Response to selection for post-weaning net feed intake in mice.

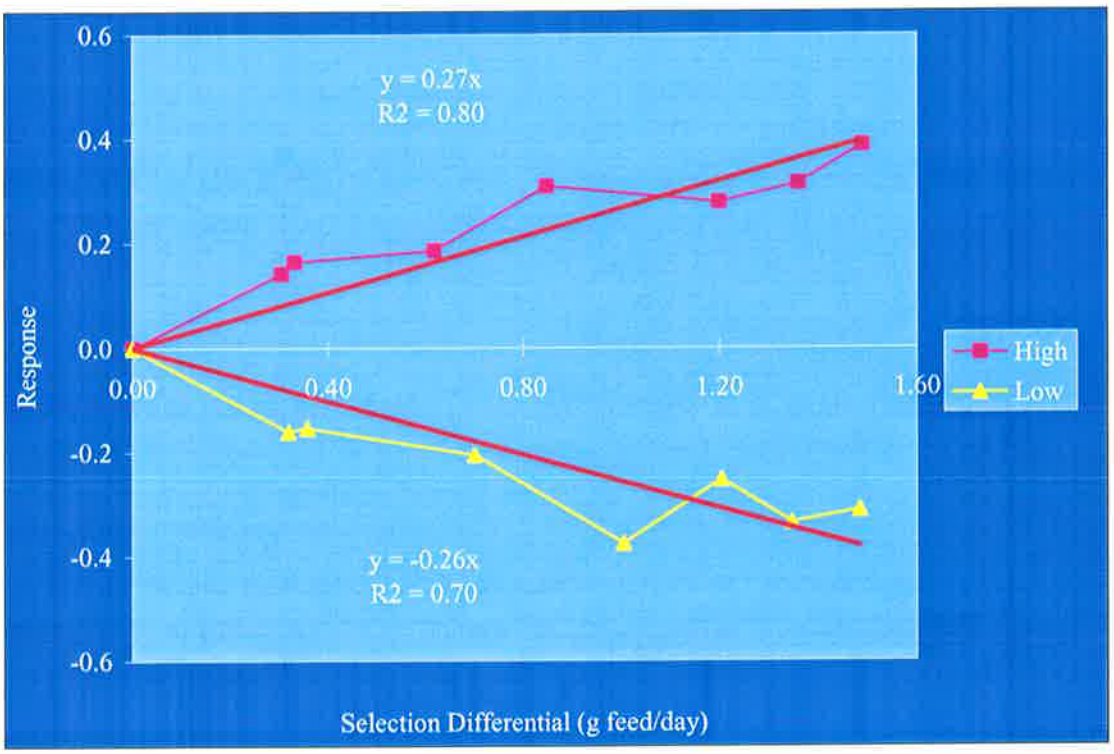
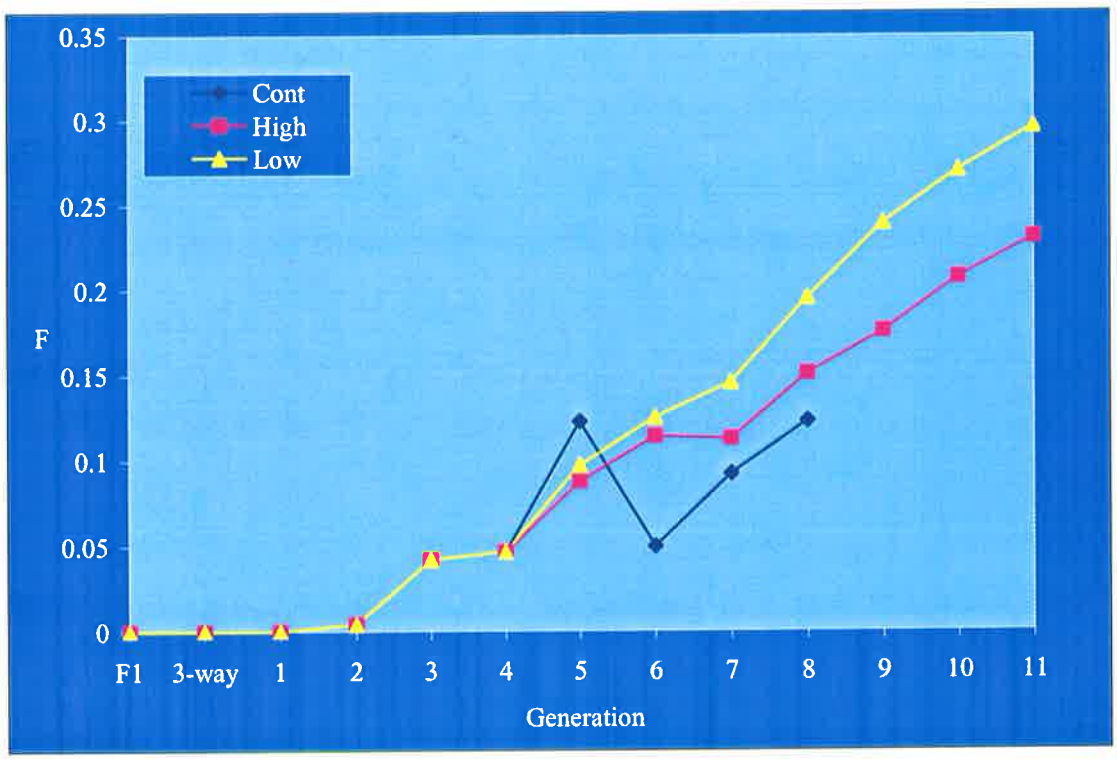


Figure 3.6. Inbreeding Coefficients for Generations 1 – 11.



Cumulative inbreeding coefficients are presented graphically as line averages in Figure 3.6. It was not possible to derive accurate generational averages for the control line as there was significant overlap between generations. Inbreeding within line in the penultimate generation was: Control 0.09±0.05, High 0.21±0.03, Low 0.27±0.03.

Growth and feeding traits

Correlated responses to selection for post-weaning net feed intake in growth and feeding traits were examined for generation 10 (generation 7 for control line) and results from linear models are presented for type III sums of squares. The percentage variance accounted for by the model (R^2), residual coefficient of variation (CV), error degrees of freedom, error mean square and source mean squares are presented in Table 3.5 for each trait when the final general model was fitted.

Table 3.5. Raw means, phenotypic variance and ANOVA table for most traits.

Source	NFI_{PW}	Wt_{21}	DFI_{PW}	DFO_{PW}	ADG_{PW}	MWT_{PW}	FCR_{PW}	GE_{PW}	$\%F_{PW}$
M	0.00	13.6	4.20	1.14	0.28	21.6	20	0.07	15.8
Minimum	-0.95	5.7	2.66	0.57	-0.34	14.0	-250	-0.03	3.9
Maximum	1.56	19.1	5.99	1.89	0.92	34.5	422	0.20	22.3
σ_P	0.30	1.48	0.37	0.14	0.12	2.00	33	0.03	1.58
R^2 (%)	50	27	56	53	28	58	9	30	56
CV (%)	7	11	9	13	43	9	165	41	10
Error DF	722	903	722	710	746	746	722	722	240
Error MS	0.1	2	0.1	0.0	0.01	4	1102	0.001	2
MGP	0.3*	32**	0.3	0.5**	0.01	41**	1315	0.001	10
PAR	0.3*	23**	0.0	0.0	0.02	17*	2732	0.001	3
AGE	2.4**	51**	0.3	0.1*	1.04**	67**	19319**	0.053**	4
BOX	1.4**	21**	3.1**	0.3**	0.19**	26*	106	0.014**	NA
LIT	0.1	61**	0.3	0.0	0.00	40**	1422	0.000	9
SEX	0.0	113**	26.4**	1.4**	1.23**	2733**	2630	0.032**	110**
LIN	0.0	12**	0.3	0.0	0.02	31**	385	0.001	27**
MGPxSEX	0.0	8*	0.2	0.0	0.05*	32**	865	0.001	3
MGPxLIN	0.5**	18**	0.7**	0.1**	0.01	15**	2768*	0.001	1
SEXxLIN	0.2	4	0.3	0.0	0.05*	6	236	0.002*	8*
INCxLIN	0.2*	14**	0.1	0.0	0.02	11*	117	0.002*	18**

* $p < 0.05$
** $p < 0.01$

Litter parity effects

Although partially confounded with management group, parity accounted for a significant amount of variation in net feed intake, weaning weight and mid-weight. Progeny from later parities had more negative net feed intakes (i.e. they were more efficient) and higher weights at all ages.

Inbreeding effects

The main effect of inbreeding was not included in the model. However, there were a number of significant interactions with line, which are outlined in a later section on line effects.

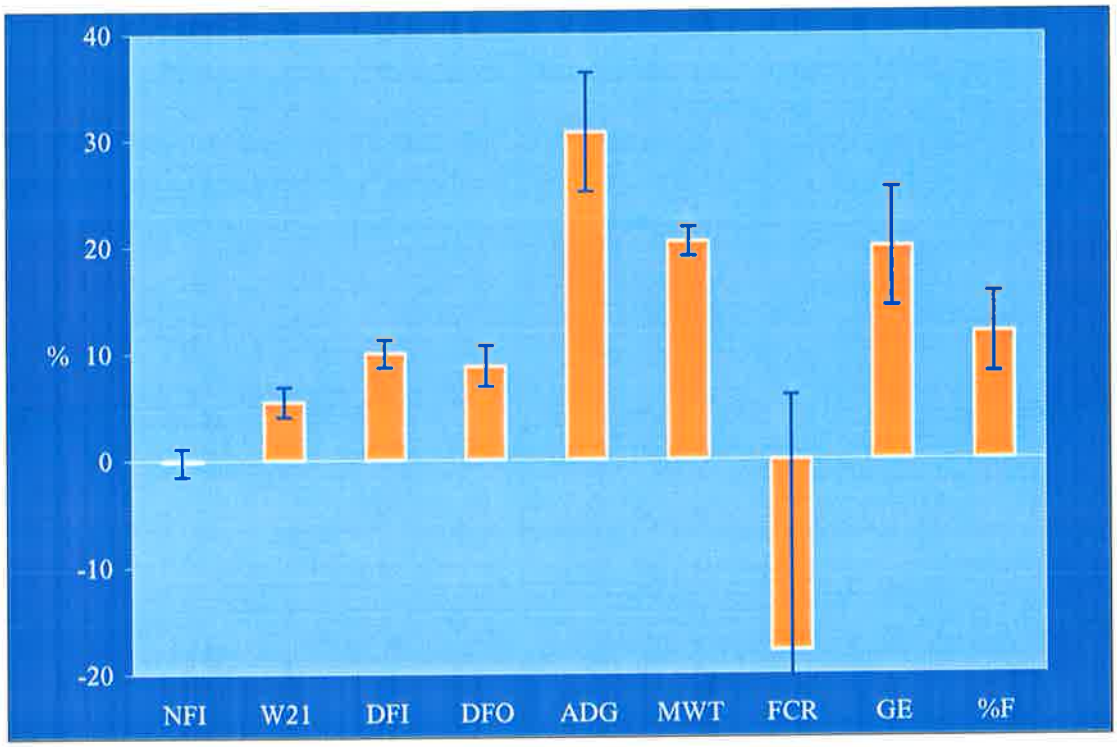
Sex effects

The original model used to estimate net feed intake incorporated a term for sex as well as a sex by management group interaction and subsequent analysis showed no effect of sex on net feed intake within generation 10 (Table 3.6, Figure 3.7). Males ate 10% more than females, and this was reflected in a 9% higher production of faecal waste. Males were 5% heavier than females at weaning and 20% heavier mid-way through the test. However, there was a significant interaction between sex and line for daily gain. Males gained at faster rates than females in all lines, and line rankings were similar in females (C=L>H) and males (C>L>H).

Table 3.6. Least squares means for males and females for post-weaning growth and intake traits.

	NFI_{PW}	Wt_{21}	DFI_{PW}	DFO_{PW}	ADG_{PW}	MWT_{PW}	FCR_{PW}	GE_{PW}	$\%F_{PW}$
♂	-0.02	13.8	4.36	1.16	0.36	23.3	18.2	0.08	17.1
SE	0.03	0.1	0.04	0.02	0.01	0.2	3.5	0.00	0.4
♀	-0.01	13.1	3.96	1.07	0.27	19.3	22.2	0.07	15.3
SE	0.03	0.1	0.04	0.02	0.01	0.2	3.4	0.00	0.4

Figure 3.7. Percentage deviation of males from females for post-weaning growth and intake traits (±SE).



Males had a distinctly higher gross efficiency. There was also a significant interaction between line and sex for gross efficiency. Males were more efficient than females in all lines, and in both sexes line rankings were C=L>H. However, the high line males had a lower gross efficiency relative to the control and low lines when compared to females. The difficulty of using ratios as measures of efficiency on

growing animals was evident in the equivalent results for food conversion ratio, where very large standard errors resulted in non-significant sex differences.

Surprisingly, males were fatter than females. There was a significant interaction between sex and line for percent body fat. Males were fatter than females in all lines, but line rankings were L=C, C=H, L>H in females, and L=C>H in males. Although statistically significant, the similarity between the line rankings implies that this interaction was not biologically meaningful.

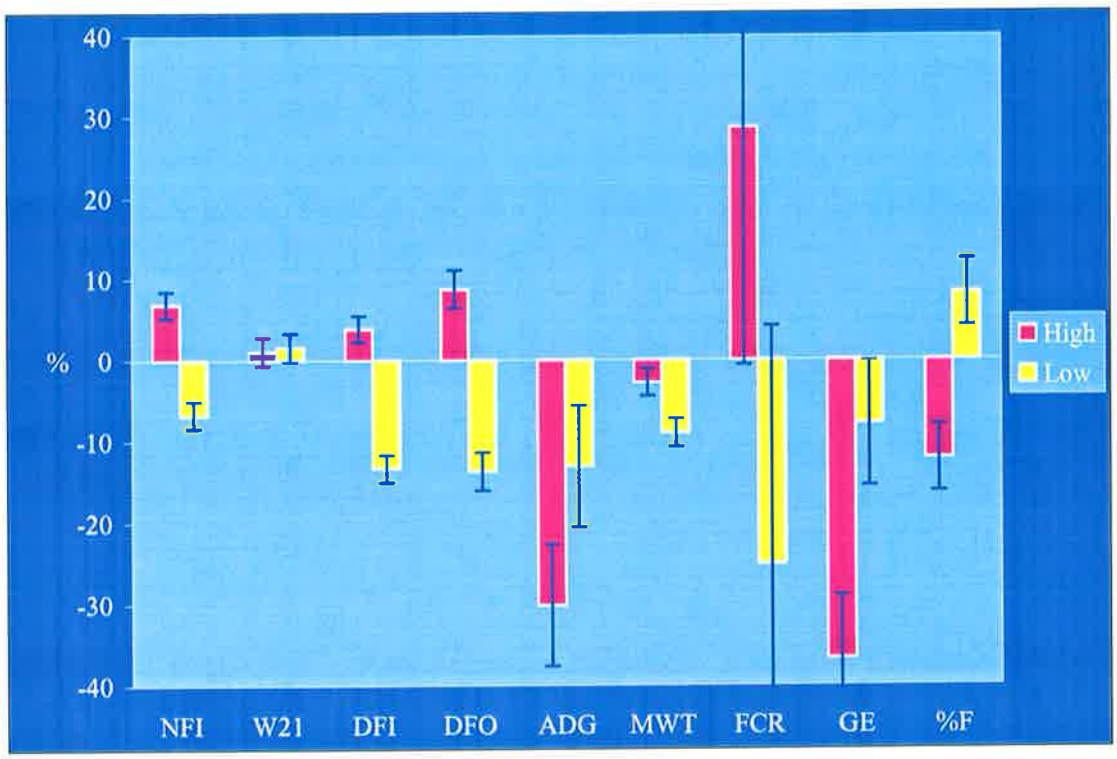
Line effects

Least squares means for lines are presented in Table 3.7. Although the main effect of line was not always significant, the issue is clouded by the presence of the intermediate control line and so differences between the high and low line are presented in Figure 3.8 as the percentage deviations of the low and high lines from the control line. Correlated responses in traits used in the original model to estimate net feed intake were compared to the direct response in net feed intake by examining high and low line differences in genetic standard deviations in Figure 3.9. The sampling variance of the response (an estimate of genetic drift using the methodology of Hill, 1980) was less than 6% of the pooled estimate of the variance for all pair-wise line comparisons of post-weaning traits, hence significant line differences were unlikely to have been due to random genetic drift alone.

Table 3.7. Net feed intake selection lines' least squares means for post-weaning growth and intake traits.

	NFI_{PW}	Wt_{21}	DFI_{PW}	DFO_{PW}	ADG_{PW}	MWT_{PW}	FCR_{PW}	GE_{PW}	$\%F_{PW}$
C	-0.02	13.3	4.30	1.13	0.37	22.1	20.0	0.09	16.4
SE	0.07	0.3	0.08	0.03	0.03	0.4	7.1	0.01	1.0
H	0.28	13.4	4.46	1.23	0.26	21.5	25.7	0.06	14.4
SE	0.02	0.1	0.03	0.01	0.01	0.1	2.3	0.00	0.3
L	-0.29	13.5	3.73	0.98	0.32	20.2	15.0	0.08	17.8
SE	0.05	0.2	0.06	0.02	0.02	0.3	5.5	0.01	0.6

Figure 3.8. Percentage deviation of high and low net feed intake selection lines from control line for post-weaning growth and intake traits (±SE).



The main effect of line was not significant for net feed intake. However, there were differences between all pair-wise comparisons of lines and, as expected, the ranking for net feed intake was H>C>L. The low line had a 7% lower net feed intake than the control line and a 13% lower net feed intake than the high line in generation 10. Clearly, net feed intake demonstrated a substantial direct response in both selection

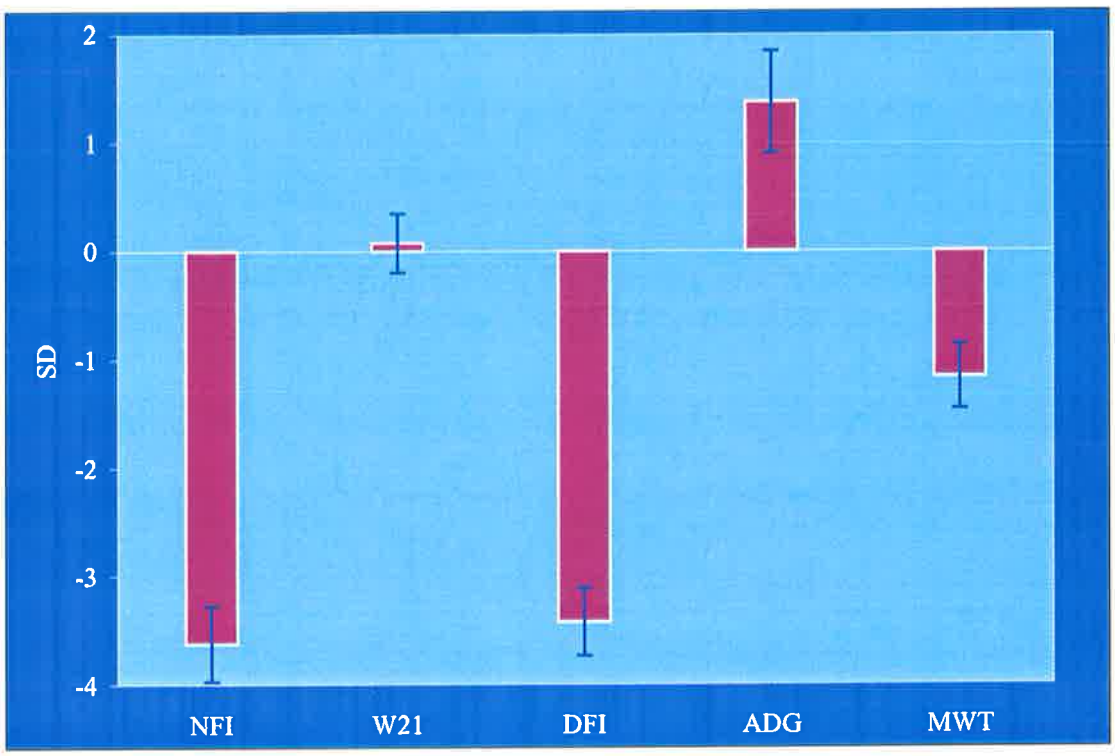
lines. There was also a significant interaction between line and inbreeding coefficient. Neither the control nor low line showed a substantial response to inbreeding. However, the high line became progressively less efficient (i.e. net feed intake became more positive) as inbreeding increased (regression coefficient 1.74 ± 0.83 g.day^{-1}). Similar pair-wise comparisons were observed for daily feed intake, although the control line was generally higher. There was no interaction between line and inbreeding coefficient for intake.

Correlated responses in daily faecal waste production produced similar line rankings with respect to those of net feed intake and daily feed intake. The high line produced 8% more waste than the control line and 26% more waste than the low line (Table 3.7). In terms of gross digestibility, the lines were identical, all retaining 73-74% of their daily intake for the various functions outlined in Chapter 2. Consequently, the high and control lines, which had higher daily intakes, retained approximately 16% more mass than the low line.

There were significant correlated responses in weaning weight, mid-weight and metabolic mid-weight. At weaning, the line rankings were L>H>C, although pair-wise comparisons of least squares means revealed no significant differences between specific pairs of lines. Within the period of the three week test, rankings were re-ordered such that for mid-weight and metabolic mid-weight, C>H>L. In both cases, the low line was approximately 6% lighter than both the control and high lines. There were however specific line differences in daily gain such that the high line gained 25% less per day than both the control and low lines on average. There was also a substantial interaction between line and inbreeding coefficient for both weaning weight and mid-weight. In both cases, the control line tended to be lighter as

inbreeding increased (regression estimates of –10.5±2.4 g.unit^{-1} and –7.0±3.6 g.unit^{-1} respectively). As noted in the section on sex effects, there was an interaction between sex and line for daily gain.

Figure 3.9. Deviation of low NFI line from high NFI in terms of genetic standard deviations for post-weaning growth and intake traits (±SE).



Both the control and the low line had substantially higher gross efficiencies (50%) than the high line. The effect of line on gross efficiency was moderated by sex (see sex effects above). The efficiency of the control line also tended to increase with inbreeding (regression estimate 0.14±0.05 g.g^{-1}). Line differences in food conversion ratio were non-significant due to its large coefficient of variation (164%), again illustrating the difficulty of working with ratios.

There was a significant line effect for body composition. The low line was 23% fatter than the high line, with the control line intermediate, at the conclusion of the test period. However, the low line tended to become leaner as level of inbreeding increased (regression estimate -28.3 ± 6.7 g.unit^{-1}). Although the trend was the same in the high line, greater variation meant that this was not significant. Sex had a significant effect on line rankings for body fat (see sex effects above).

The biological cycle: mass vs. energy

To illustrate the response to selection, a series of crude line-specific daily cycles based on mass and energy transformations were developed (Figures 3.10-3.12). These are also represented graphically in Figures 3.13 (% mass/energy) and 3.14 (absolute mass/energy). Some assumptions were required:

1. The energetic value of feed was 17.7 kJg^{-1} (average figure based on bomb calorimetric measurements).

2. Body fat percentage did not change over the course of measurement.

3. Lean tissue deposited during growth comprised carbohydrate (ash), protein and water in the ratio 4:25:140 by weight (Emmans, 1981).

4. Energetic values for tissue components were:

Fat	39.5 kJg^{-1}
Carbohydrate	17.3 kJg^{-1}
Protein	6.24 kJg^{-1}

(McClean and Tobin, 1987).

Numbers in parentheses are percentages.

Figure 3.10. Control line mass-energy balance.

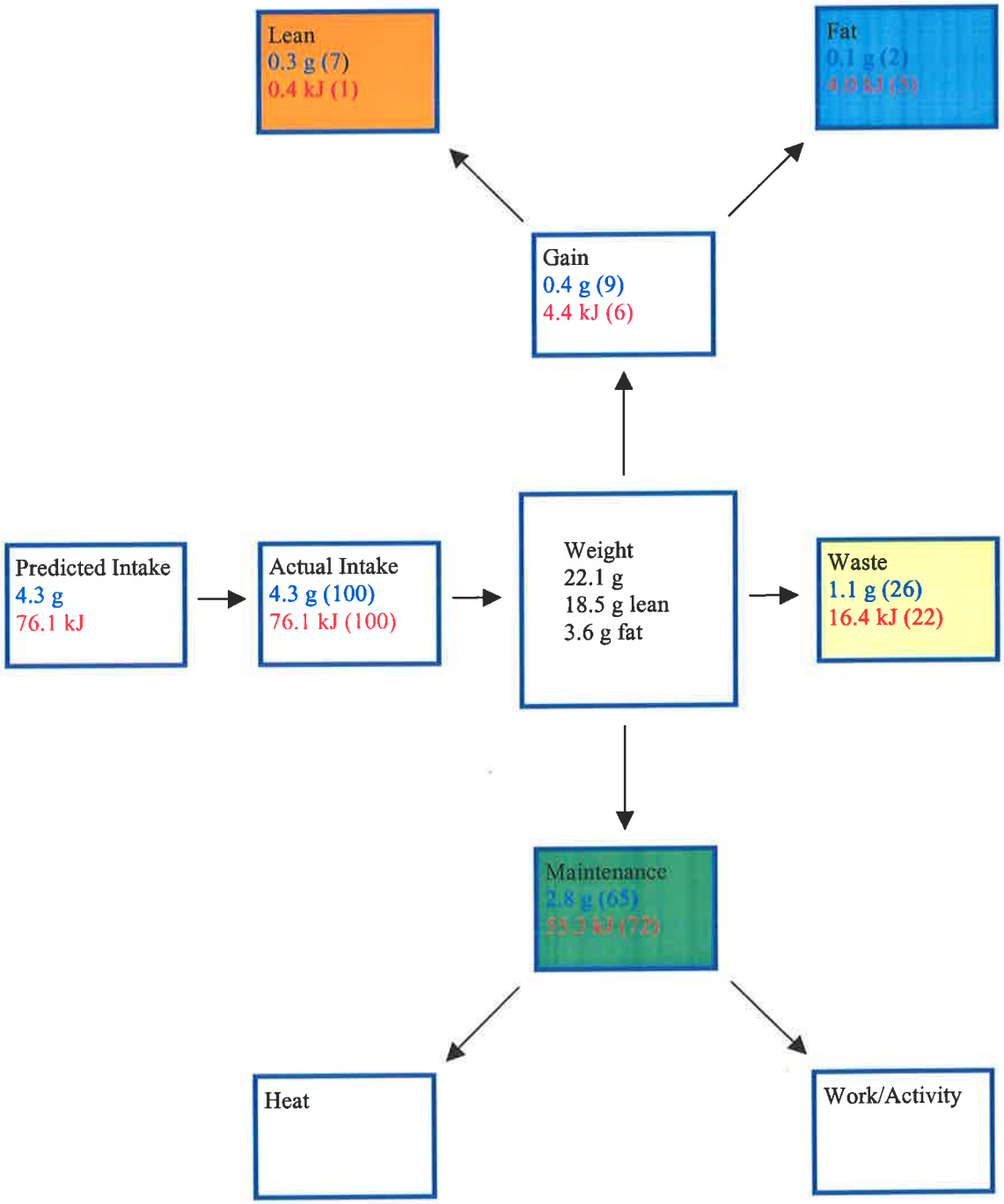


Figure 3.11. High line mass-energy balance.

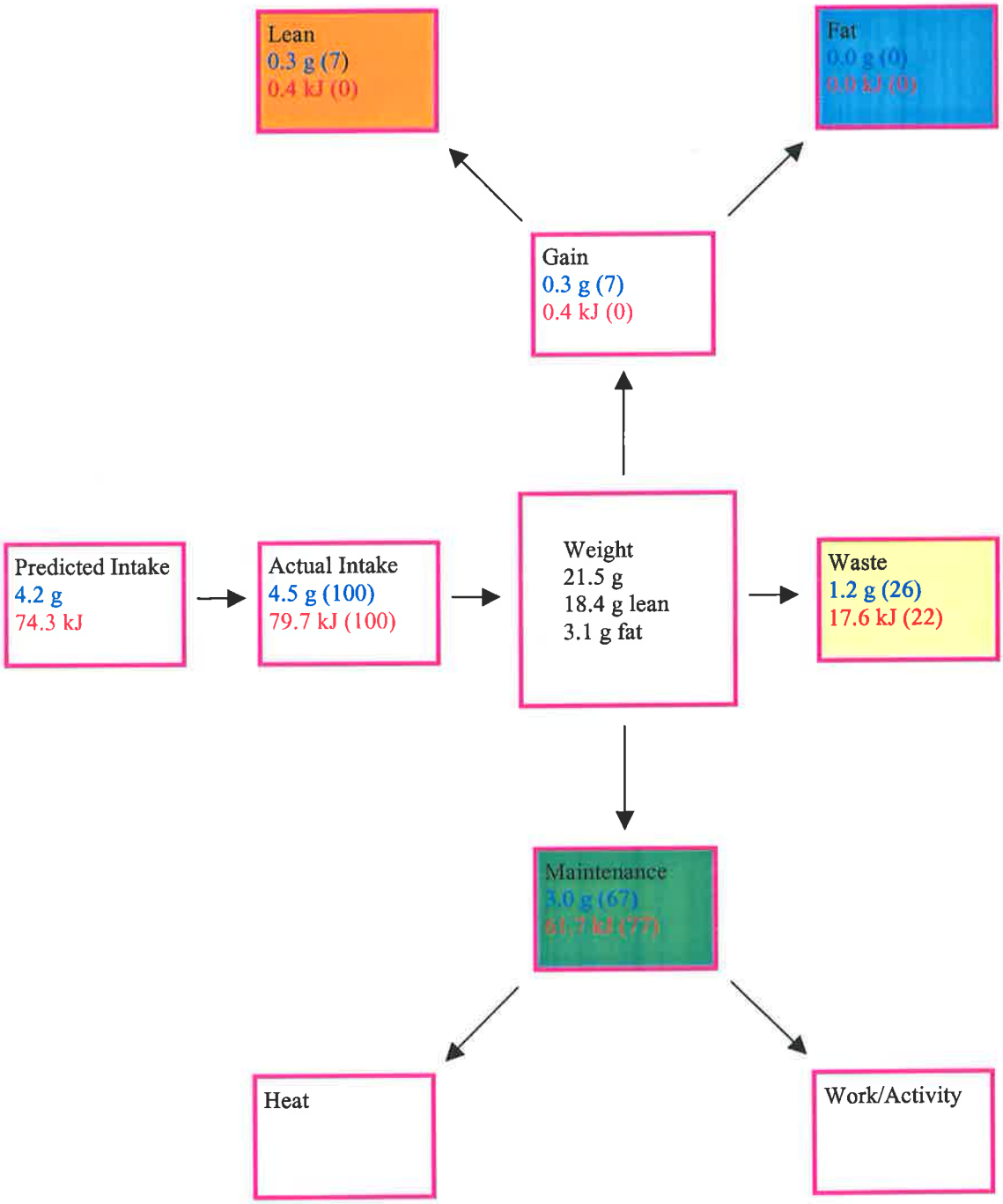


Figure 3.12. Low line mass-energy balance.

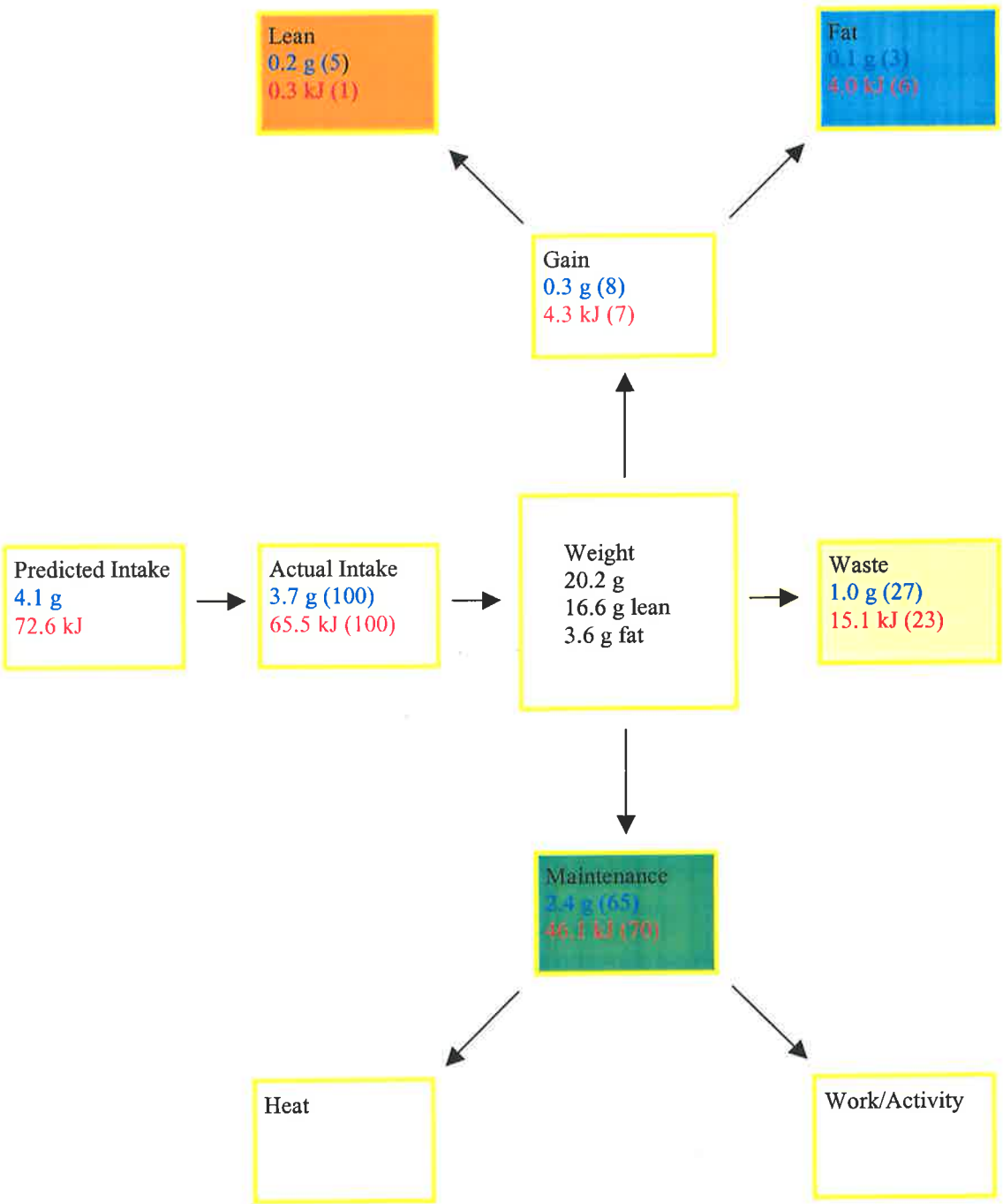
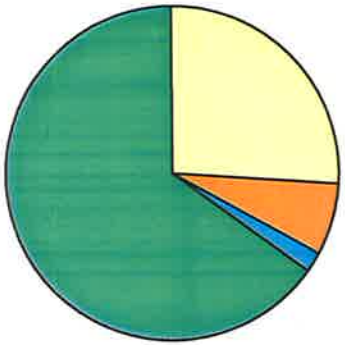


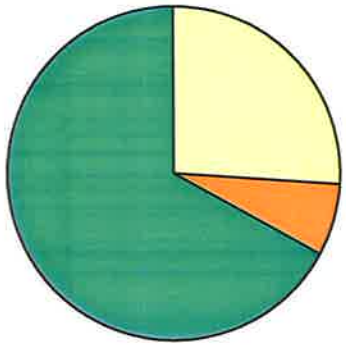
Figure 3.13. Pie charts comparing percentage mass and energy conversions between lines.

Mass

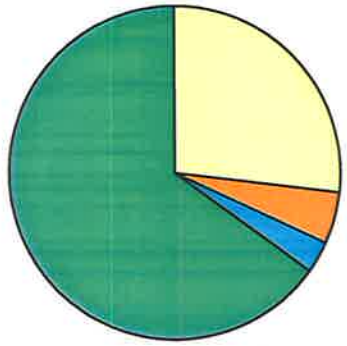
Control



High

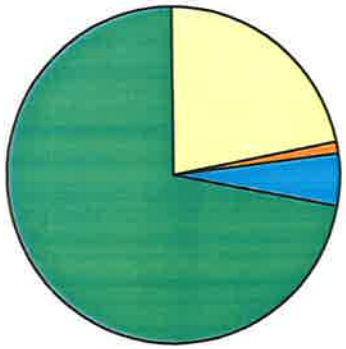


Low

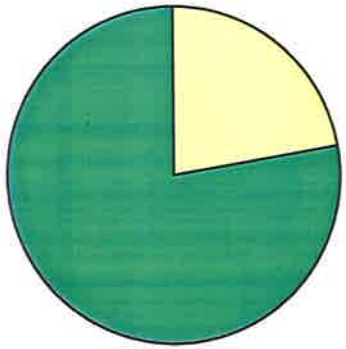


Energy

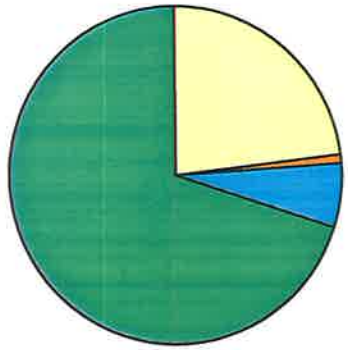
Control



High



Low



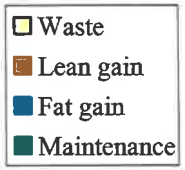
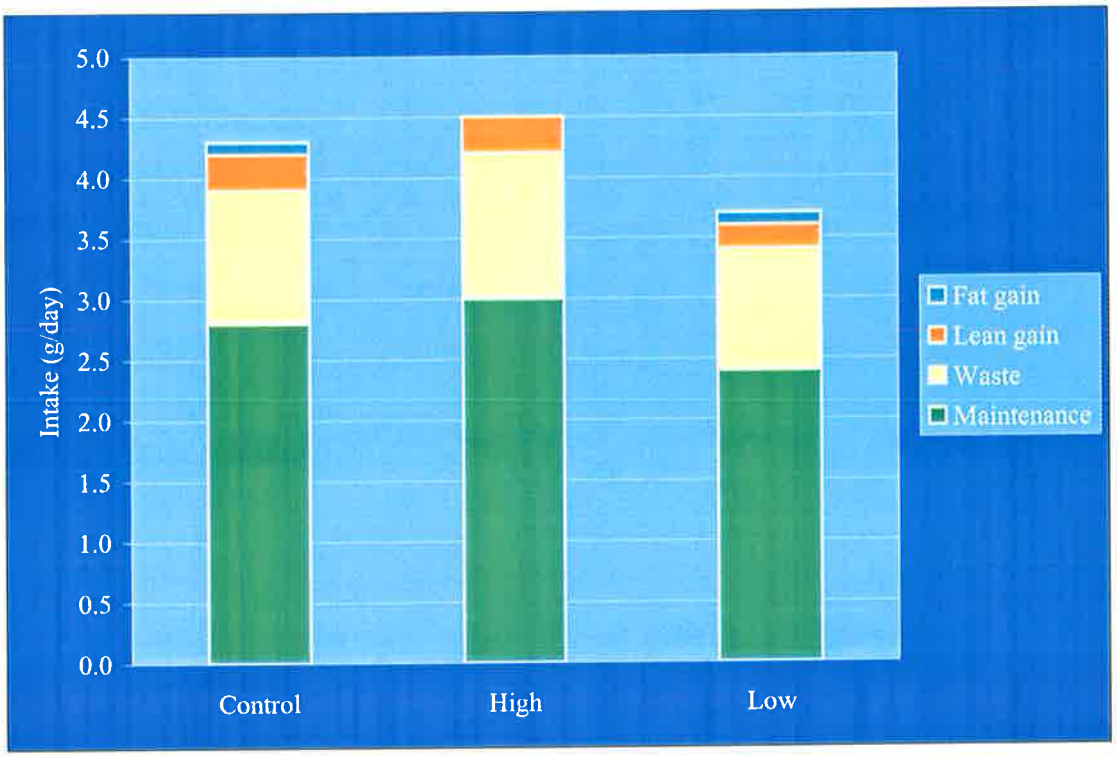
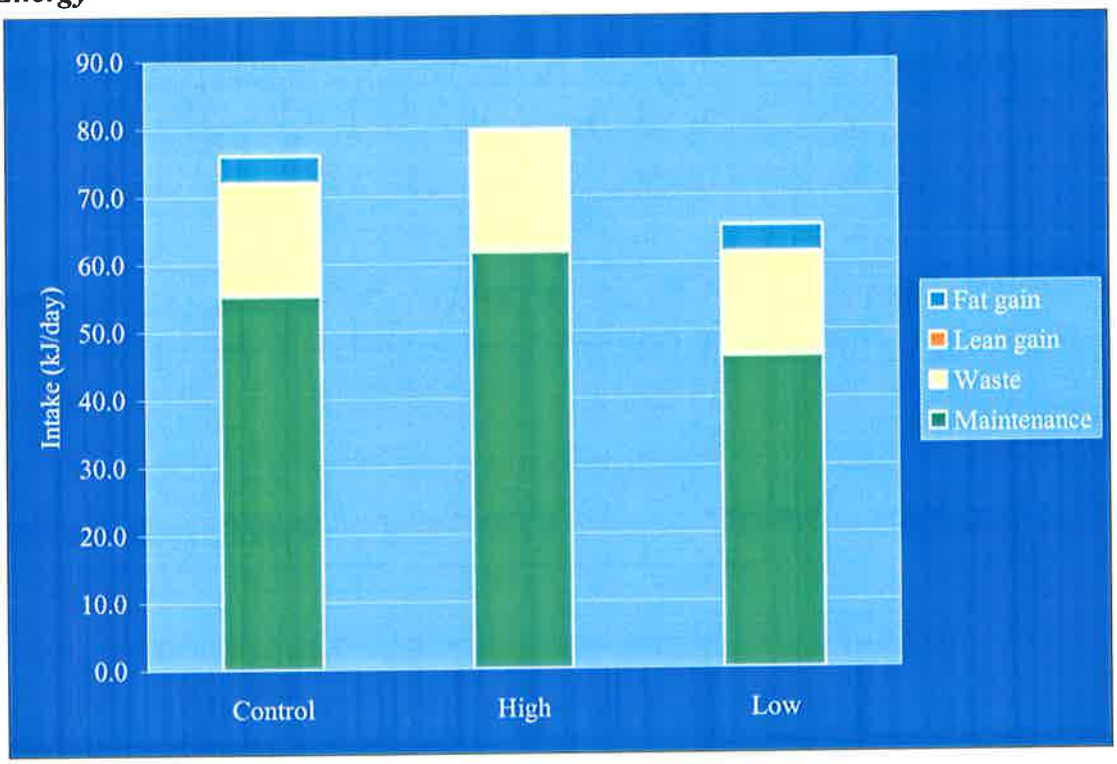


Figure 3.14. Stacked bar charts comparing absolute mass and energy conversions between lines.

Mass



Energy



Discussion

Phenotypic and genetic parameters

Net feed intake is the residual component of a model for intake that already includes terms for growth and body weight, and hence it was uncorrelated, phenotypically, with either daily gain or mid-weight (Table 3.4). However, the model itself was based on phenotypic data and would not have adjusted for any underlying genetic correlations. Kennedy *et al.* (1993) showed that the apparent genetic variation in net feed intake calculated from phenotypic regression may be due to genetic correlations of net feed intake with production traits. However, Archer (1996) noted that for studies where the genetic correlations of phenotypic net feed intake with production traits are close to zero, the results for phenotypic net feed intake would be expected to be very similar to those of genotypic net feed intake. Therefore, the most important finding of the early work (Archer, 1996) in the current study was that net feed intake was genetically correlated with raw intake, but not with growth or body weight, supporting the use of net feed intake to make reductions in intake without the associated costs of increased growth rate and, particularly, body weight. Similar results were observed in beef cattle (Arthur *et al.*, 1996; Herd and Bishop, 1999), pigs (Johnson *et al.*, 1999) and laying hens (Luiting, 1991). Veerkamp *et al.* (1994) reported high genetic correlations of net feed intake with live weight change and condition score in dairy cattle. When they estimated energy requirements using coefficients based on partial genetic regressions of energy intake on milk energy yield, metabolic live weight and live weight change, rather than from a phenotypic regression, then the heritability of net feed intake dropped from approximately 0.35 to 0.05. They attributed the difference to (i) antagonistic genetic and environmental

correlations between live weight change and energy intake and (ii) a strong bias downwards in the estimation of the heritability for 'genetic' net feed intake. This supports Kennedy *et al.*'s (1993) theory and validates the findings of Archer (1996).

With respect to genetic correlations, there are a number of other noteworthy results. As expected from the literature (e.g. Brody, 1945), weaning weight and mid-weight were strongly correlated. Intake was highly correlated with mid-weight but less so with average daily gain (Table 3.4). This supports the notion that selecting for post-weaning growth rate will tend to improve gross efficiency (gain/intake) during the growing phase but will also lead to correlated gains in body weight and the associated costs for maintenance (Holmes, 1973; Andersen, 1978; Dickerson, 1978; Fitzhugh; 1978; Barlow, 1984).

Percentage body fat was moderately correlated with average daily gain (Table 3.4). This may indicate that selecting for a faster growth rate post-weaning results in animals that are closer to their mature weight at a given age and hence more likely to be depositing fat (i.e. finishing). However, results from the literature (Brody, 1945; Dickerson, 1978; Barlow, 1984; Salmon *et al.*, 1990), and the moderate genetic correlation between mid-weight and gain, would tend to refute this, indicating that animals with faster growth rates are actually growing to a larger mature weight and hence are more likely to be at an earlier stage of maturity at a fixed age.

Direct response in net feed intake

The present study was not designed to estimate genetic parameters but rather to produce a genetic divergence between lines based on previous estimates derived from the original random-mating population. As predicted, divergent selection on breeding value for post-weaning net feed intake produced marked differences between the

selection lines in feed intake with little impact on the component traits of the model, growth rate and body weight. The moderate heritability of post-weaning net feed intake, presented as both a statistical estimate of the proportion of additive genetic variation and a realised estimate from selection, is in good agreement with other research, particularly within mice. A number of other projects have used the mouse specifically as a model species to examine direct selection using variations of the net feed intake model.

The work of Hill and colleagues in Edinburgh is well documented (e.g. Sharp *et al.*, 1984; Hastings *et al.*, 1997). In one experiment, mice were selected for 4-6 week intake phenotypically adjusted for 4 week weight, with no adjustment for growth rate. The first 12 generations of selection produced a divergence of 17% between the high and low selection lines, with a realised heritability of 15%. This was approximately 5% lower than results based on selecting on raw feed intake in mice (Sutherland *et al.*, 1970) and chickens (Pym and Solvyns, 1979). Sharp *et al.* (1984) suggested that adjusting intake for weight may remove some of the genetic variability of intake, reducing heritability.

There was also a correlated change in body weight, however, such that the high intake lines were heavier. This may be ascribed to both the use of a phenotypic model of intake, which masks underlying genetic correlations, and to the lack of adjustment for growth rate. Hastings *et al.* (1997) noted that this precluded the lines for use as models of the physiology and genetics of food intake as divergence in food intake was confounded with changes in body weight. This issue was addressed in the present study by including growth rate as a variable in the model of feed intake, although there were still small but significant changes in both growth rate and body weight due

to underlying genetic correlations between these variables and net feed intake. A subsequent long term selection experiment by Hill's group (Hastings *et al.*, 1997; Bunger *et al.* 1998), was based on selection of animals at 10 weeks of age when growth rate is diminished, in a successful effort to reduce correlated responses in body weight. However, the results from this study suffer from the difficulty of extrapolating them to young, growing animals and it is more appropriate to compare them to studies of intake in mature animals. This will be addressed further in a subsequent chapter.

Realised heritabilities in the present study (27%, Figure 3.5) are also within the range of published results for other species, although these are predominantly statistical estimates of the proportion of additive genetic variation. They are summarised in Table 2.1 of the literature review.

Archer *et al.* (1997) demonstrated that in cattle, for a given time interval, the accuracy of estimates of net feed intake has a greater dependency on measures of weight gain than on measures of either intake or metabolic body weight. Feed intake in cattle can be measured with moderate repeatability over a period as short as five weeks (Archer *et al.*, 1997). Mean metabolic weight can be measured with high accuracy over the same period, because the errors in individual weight measurements are averaged out by taking the mean of several weight measurements (Robinson and Oddy, 2001). However, unless measured over a long interval of time, accuracy of weight gain may be low (Archer *et al.*, 1997) due to the variation in individual measures of body weight. Robinson and Oddy (2001) extended this by examining the effect on estimates of net feed intake of adjusting weight gain for the amount of feed eaten in the days prior to measurement of weight. They concluded that when feed intake is

being measured in cattle, weight gain can be estimated more accurately by using the amount of feed eaten in the previous 3-5 days as a method of adjusting for gut fill. This helps to reduce biases and increases the accuracy of calculating net feed intake, thus contributing to more effective genetic improvement of this trait. Extrapolating this to mice, where daily intake is much higher relative to body weight (20% in mice at 5 weeks of age vs. 3% in yearling cattle), selection response may have been substantially greater if it would have been possible to conduct intermediate measures of feed consumption and hence derive estimates of daily intake in the periods prior to weight measurement. However, given the allometric scaling effects of body size in which 1 day in a 20 gram mouse is equivalent to approximately 14 days in a 380 kg cow ($\{380/0.02\}^{0.27}$=14.3 days), this would have required multiple measurements of intake in the day immediately prior to weight measurement. Not only was this physically not possible, but it was considered that such regular external interference may have itself biased the results.

Growth and body weight

On first examination, correlated responses in daily gain and metabolic mid-weight were not expected as, theoretically, their influence was removed in the model used to estimate net feed intake. However, this model is based on phenotypic information, and hence any underlying genetic correlation between average daily gain, metabolic mid-weight and net feed intake will be reflected in correlated responses to selection for net feed intake (Kennedy *et al.*, 1993). Based on the small negative genetic correlations estimated on the random mating population, one would have expected the low line to have a higher metabolic mid-weight and daily gain than the high line. This was only true in the case of the gain component. Examining line differences in units

of genetic standard deviations (Figure 3.9) it becomes clear that the primary genetic response to selection has been in net feed intake directly, with a relatively small response in both gain and mid-weight, as expected.

A selection experiment in beef cattle running contiguously to our original experiment in mice has been on-going at the Trangie Research Centre in New South Wales. Arthur *et al.* (2001) reported the direct and correlated responses in post-weaning net feed intake and growth traits resulting from 5 years of divergent selection. Approximately two generations of selection were achieved in both the high and low lines. Although realised heritabilities were not presented, there was a significant divergence between the lines of approximately 13% (high from low) for both net feed intake and daily feed intake, with no observed response in either yearling weight or average daily gain, once again consistent with the fact that, theoretically, net feed intake should be phenotypically independent of test period live weight and growth.

Intake, feed conversion ratio and gross efficiency

The response in net feed intake was associated with a 17% difference in raw daily intake between the high and low lines, slightly higher than the direct response in net feed intake (13%). This is again due to the use of a phenotypic selection index: there was a small positive genetic correlation between net feed intake and daily gain, such that the high line ate more due to having both a higher net feed intake (15%) and a heavier body weight (7%). The marked divergence in intake between the lines, coupled with a small correlated change in body weight, was sufficient for gross efficiency to be significantly higher in the low net feed intake line. Sharp *et al.* (1984) observed the opposite result for gross efficiency in their selection experiment, although gain was not included in their phenotypic index.

In many previous studies (e.g. Holmes, 1973; Andersen, 1978; Dickerson, 1978; Fitzhugh; 1978; Barlow, 1984), improvement in gross efficiency has come at the expense of increased growth rate leading to larger mature sizes. By selecting on a phenotypic index of intake that incorporates gain and weight components, we have effectively uncoupled this relationship. Although the response in gross efficiency would undoubtedly be greater if selected upon directly, using net feed intake we have avoided the complications associated with using a ratio as a selection criteria.

Despite a significant divergence in gross efficiency, there were no line differences in it's inverse, food conversion ratio. This may be explained by relative accuracies of measurement of gain and intake, and the calculation of the respective ratios. Daily gain was determined from only three raw measures of body weight and hence reflects in part any fluctuation in weight on the respective days of measurement. Daily intake on the other hand was determined from cumulative measures of intake and hence is buffered from daily fluctuations. This is reflected in the phenotypic variance of the respective traits, which is considerably larger for daily gain. Hence, when the trait with greater variance is the denominator of the intake:gain ratio (i.e. FCR_{PW}), one expects a much higher standard error, requiring a larger relative difference between lines (sexes, management groups, etc...) to produce a significant difference.

Gross digestibility

Gross digestibility is determined by a series of energy transformations. Recall the energy cycle outlined in the review of the literature (Figure 2.3). In growing mice, intake is:

1. Used to maintain body weight (and subsequently lost as metabolic heat)
2. Used for growth
3. Used for activity and work
4. Lost as faecal waste

The mass:energy ratio varies across these four groups. Furthermore, the relationships between them are potentially quite complex when considering the effect of selection on gross digestibility. However, the method of selection narrows the possibilities substantially. The lines were selected for intake net of growth and body weight on a mass basis, essentially maintaining the same body weight and growth rate. Furthermore, growth in both lines was only a small component of intake both energetically and with respect to mass. This presents three alternative possibilities:

1. Selection for net feed intake does not affect the absolute mass or energy of intake retained for growth and maintenance, but substantially alters gross digestibility. Selection is primarily acting on rate of uptake in the gut. This is summarised in Figure 3.15.

2. Selection for net feed intake alters the absolute mass of intake retained but not the absolute energy retained (i.e. mass:energy ratio of uptake is affected). Gross digestibility on a mass basis remains unchanged. Selection is primarily acting on the energetic efficiency of uptake. This is summarised in Figure 3.16.

3. Selection for net feed intake alters both the absolute mass of intake retained and the absolute energy retained. Gross digestibility remains unchanged. Selection is primarily acting on efficiencies downstream from the gut. This is summarised in Figure 3.17.

Some might also consider a fourth alternative warranted, in which selection for net feed intake alters the absolute energy of intake retained but not the absolute mass retained. However, this would tend to suggest that high net feed intake animals were less efficient metabolically (i.e. had a higher energy requirement for maintenance) but were more efficient at extracting energy from their feed, which is highly improbable energetically.

Figure 3.15. Idealised mass-energy cycle in which equally-divergent selection lines retain the same food content by mass and energy.

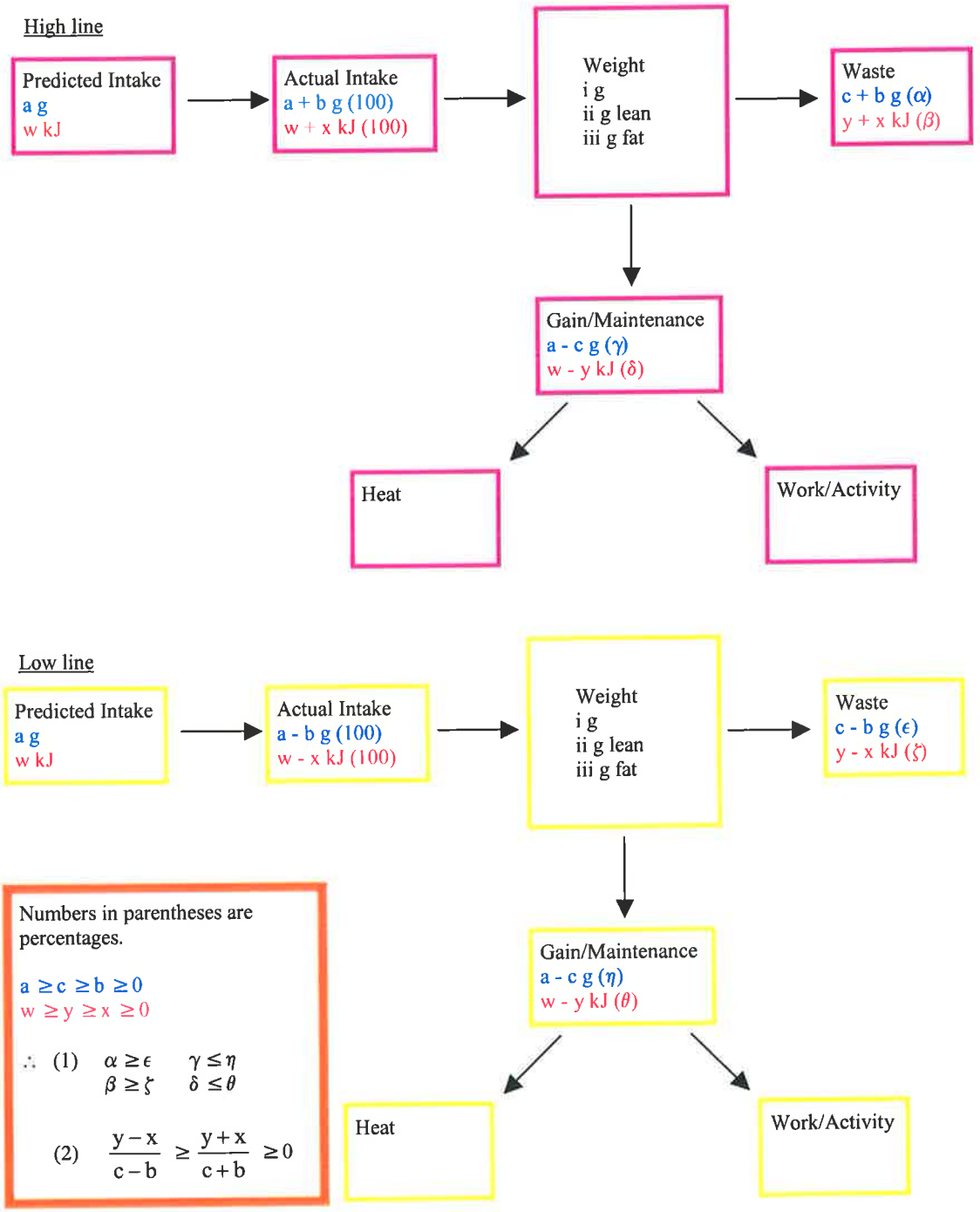


Figure 3.16. Idealised mass-energy cycle in which equally-divergent selection lines retain the same food content by energy but not by mass.

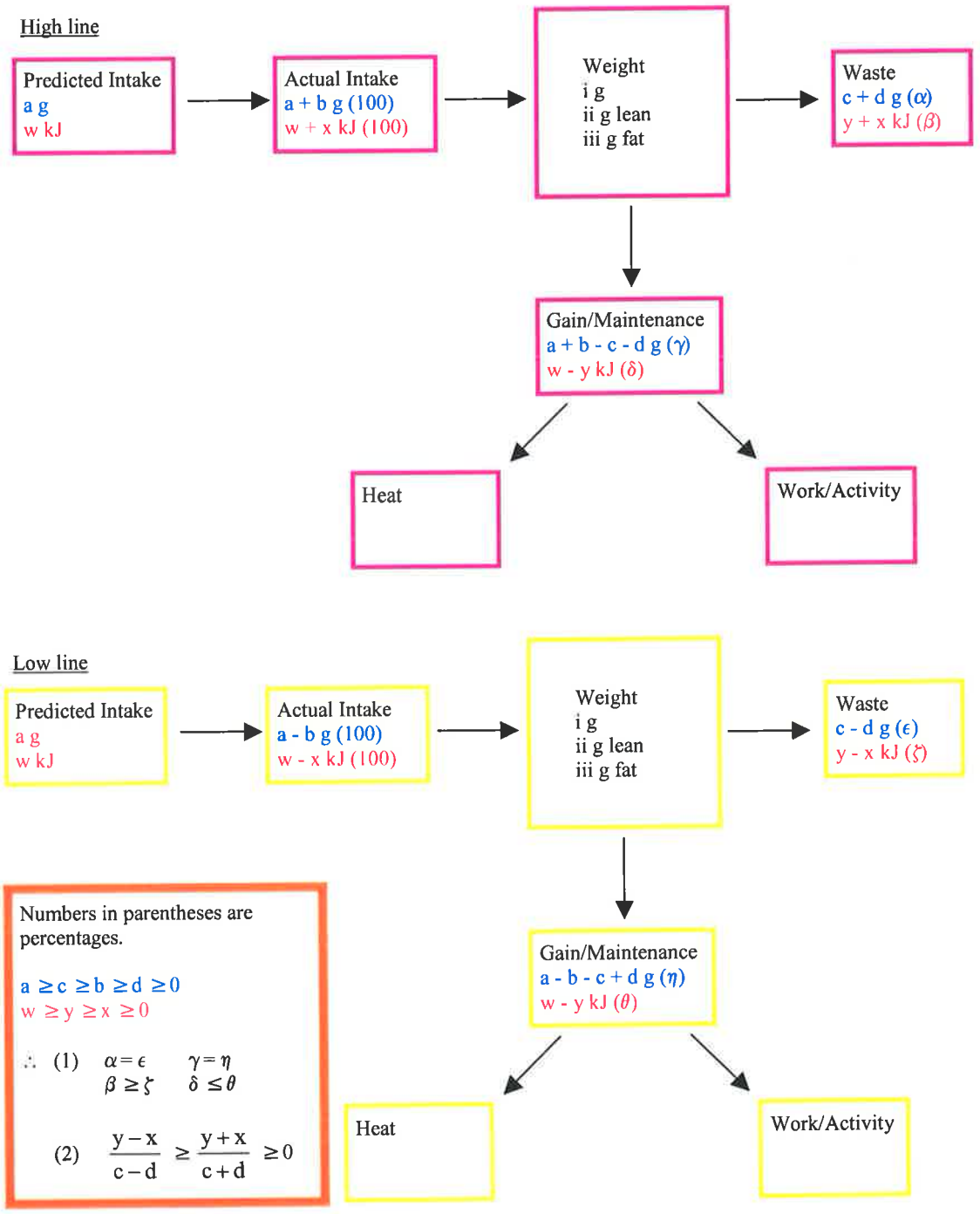
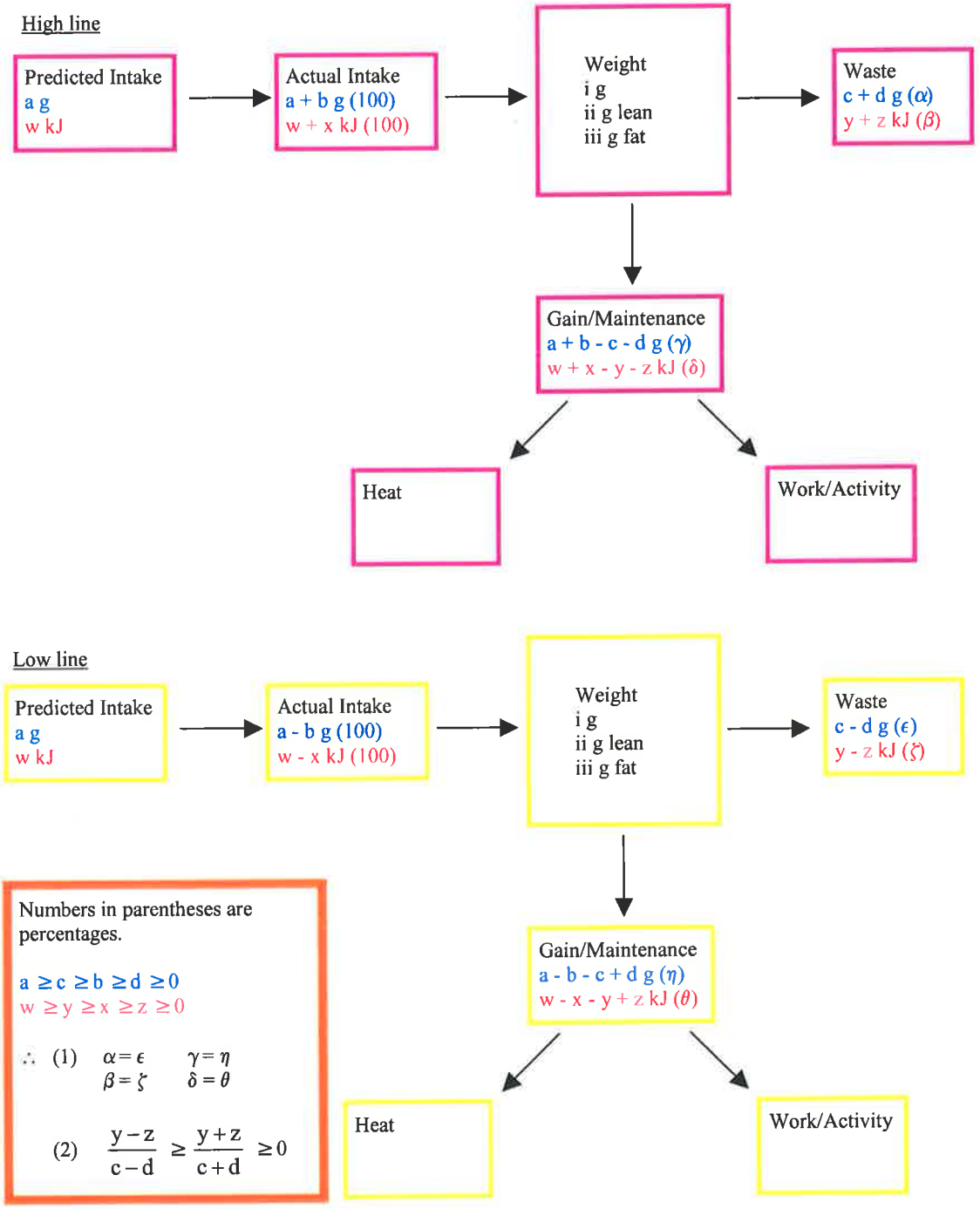


Figure 3.17. Idealised mass-energy cycle in which equally-divergent selection lines retain different food content by mass and energy.



From these three scenarios a series of hypotheses were developed based on the current knowledge of nutrient uptake.

The first hypothesis postulated that selection for low net feed intake resulted in animals with an increased capacity to take up nutrients in the digestive tract. One would expect a decrease in the relative amount of faecal waste, such that the mass of the apparently digested component would be similar between lines in absolute terms. Energy content of faecal waste by mass would be equivalent or higher in the low intake line under this hypothesis.

The second hypothesis was that the rate of passage of feed through the gut was slower in the low net feed intake line, with an associated increase in the mass retained per unit mass throughput. The results would be similar to the first hypothesis.

The partition of intake into a retained component and a waste component was similar between lines by mass, with 30% of intake lost as faecal waste. The high line still retained approximately 17% more feed by mass than the low line (3.23 g/day vs. 2.75 g/day), equivalent to 12% of the average daily intake for all animals. This is not consistent with either hypothesis and appears to indicate that line differences in net feed intake are a function of processes predominantly downstream of nutrient uptake in the gut. However, some inferences may be drawn based upon the energy content of the faecal waste.

A third hypothesis postulated that the lower mass of food digested by the low net feed intake line was nutrient rich, particularly with respect to those nutrients associated with the lean growth that animals were undergoing at the time of measurement.

It was argued that this may have been associated with specific aspects of gut metabolism. Such a scenario would be consistent with no net change in gross digestibility on a mass basis, but would result in a decreased energy content of the faecal waste per unit mass. Such results were observed in preliminary data from the cattle in the Trangie experiment (Richardson *et al.*, 1996), where the low net feed intake animals were able to extract C33 alkane more efficiently than the high line. However, in the current experiment subsequent bomb calorimetric analysis revealed no line differences in energy content of the faecal waste, although there was some concern about the power of the test to detect differences between lines. It appears that the high line animals were retaining more feed both by mass and on an energetic basis. Two remaining factors may explain these differences: there were line differences in the composition of weight gain (energy) and/or there were line differences in metabolic heat loss (energy/mass). The first is discussed below, the second is covered in Chapter 5.

Body composition

Brody (1945) hypothesized that fat tissue is energetically more expensive to deposit than lean tissue, an observation that was confirmed by subsequent experimentation (e.g. Pullar and Webster, 1977; Webster, 1980). The negative correlation between net feed intake and body fat percentage was thus surprising, as it was expected that animals with lower intakes per unit bodyweight would have deposited lean tissue preferentially. However, lean tissue is also physically less dense, and the phenotypic regression of feed intake incorporating a body weight component would act against any decrease in body density. Furthermore, conventional thought supports the idea that lean tissue is energetically more expensive to maintain than fat, and hence the

maintenance component of intake during growth is higher for lean animals. This was supported by the correlated decrease in body fat percentage observed in the high net feed intake line, relative to the low net feed intake line. Similar correlations and/or responses were observed in other experiments with mice (Bishop and Hill, 1985; Nielsen *et al.*, 1997b; Bunger *et al.*, 1998). Sharp *et al.* (1984) also found similar results when selecting on a phenotypic index of intake incorporating only 4 week weight. They suggested that this difference may have been a consequence of the adjustment. Animals that eat the most will normally be those that are heaviest at the start of the test period, but if differences in body weight are taken into account, the animals that eat most may be those that are leanest and therefore have fewest energy reserves.

An alternative interpretation was postulated to explain the observed results. The small negative genetic correlation of net feed intake with growth rate and body weight may indicate that selecting for high net feed intake produced an animal that matured slower and hence was still primarily depositing lean tissue, while a low net feed intake line animal had passed the point of inflection on the growth curve and had begun to deposit fat also. This would be of particular significance in a rapidly maturing species like the mouse and would be associated with a younger age at sexual maturity in the low net feed intake line, and at other significant points along the growth curve.

Arthur *et al.* (1996) found a positive phenotypic correlation of net feed intake with various measures of fat depth (0.24) in beef cattle. Herd and Bishop (2000) found positive phenotypic and genetic correlations between net feed intake and carcass fat in British Hereford cattle. Jensen *et al.* (1992) found a similar phenotypic correlation between net feed intake and percentage carcass fat in young bulls, but the genetic

correlation was negative. Selection in the Trangie cattle produced significant divergence between the lines in both depth of rib fat and depth of rump fat (High NFI > Low NFI). It would appear that, at least in larger ruminants, the relationship between net feed intake and body fat is opposite to that observed in smaller species such as mice, although caution must be used when interpreting the results as they differ substantially in the means used to measure body composition. The effects of selection may also be acting to re-distribute fat between intra-muscular and sub-cutaneous regions, which is an important consideration for economically important species and warrants further investigation.

Ideally, fat mass should have been measured at the both the beginning and end of the test phase in order to determine rates of gain for both fat and lean tissue, so as to produce a more subtle partition of the energy balance. Due to the effect of anaesthesia on intake and gain, and the size of animals at weaning, this was not possible using the EM-Scan procedure. An alternative could be to use serial slaughter and chemical extraction, provided sufficient animals were available to generate useful data.

The energy balance

Stephens (1991) noted that selection for maintenance efficiency, even in laboratory animals, poses a number of difficulties. Problems exist in adequately defining selection objectives and criteria, and this is largely due to the present level of knowledge of nutritional energetics, and the interactions among environment, feed composition, level of production, nutrient partitioning and physiological age that determine maintenance requirement. Particular difficulty is associated with what is referred to as the maintenance requirement of growing animals. Intuitively, a critical

level of substrate utilisation exists for any organism in any physiological state, simply for tissue maintenance. However, immature animals not in positive energy balance are likely to make metabolic adjustments which render estimates of maintenance efficiency suspect. Conversely, in immature animals that are in positive energy balance, the physiological processes which make up maintenance requirement are running at elevated levels (Milligan and Summers, 1986). Brody (1945) puts it more elegantly: "It is not possible to separate or differentiate the heat of morphogenetic work from the heat of maintenance of the formed tissue". However, any considerations of growth efficiency must consider the non-productive component of energy intake. Elucidating the relationship between this quantity and mature maintenance efficiency will be an important step in illuminating the black box of nutritional energetics.

Bünger *et al.* (1998) noted that as the mouse is small, its food intake is large relative to its absolute body weight and, even in the young growing mouse, only a small proportion of the energy input is retained as body tissue. This is supported by the results from the current selection experiment. Although crude, the energy balance diagrams illustrate quite clearly that, in terms of energy, the main effect of selection for net feed intake has been to substantially alter the efficiency with which the lines maintain body weight during the growing period, rather than the efficiency with which they deposit body tissue. Maintenance during growth is quite different to that at maturity: there are substantial energy costs associated with maintaining the 'growth machinery', predominantly associated with a heightened metabolic rate. The comparison of maintenance at weaning and at maturity should produce a more subtle understanding of how selection is acting, and will be dealt with in the next chapter. Specific responses in metabolic rate will be examined in Chapter 5.

Conclusions

Clearly, despite small negative genetic correlations with gain and body weight, it was still possible to make substantial changes in efficiency by selecting on a phenotypic index of net feed intake. Importantly, these changes were generally not at the expense of economically important traits such as gain and body weight. Selection on a genetic index may have produced even stronger responses. However, these responses were not without effect on more specific areas of growth and development. Most notable of these was the composition of gain, with more efficient animals tending to deposit higher levels of fat, at least during the early post-weaning phase. Such results may have implications for the overall energy balance of the biological system. This and other issues will be dealt with in subsequent chapters.

Chapter 4.

Correlated responses in mature growth, intake, gross digestibility and body composition.

Introduction

As illustrated in chapter 2, an improvement in feed efficiency can be achieved in the growing animal by selecting for gross efficiency alone. Such selection is undertaken in the intensive pig and poultry industries, where large, rapidly growing litters consuming a higher proportion of the overall feed intake compensate for the loss in efficiency of the larger, higher maintenance cost dams (Archer *et al.*, 1999). Although such selection is possible in the sheep and cattle industries, their relatively low reproductive rates are antagonistic to any gain in overall efficiency. The argument for selection based on net feed intake rests primarily on the assumption that response in intake will be independent of responses in growth and daily gain. Hence, although response in efficiency in the growing animal may not be as great as selection based purely on gross efficiency, this should be more than offset by the reduced maintenance costs of the breeding herd, improving the overall efficiency of the system.

To test this assumption in mice, animals from generation 10 were maintained through to maturity (approximately 16 weeks) and re-tested. Correlated responses in growth and intake traits are reported.

Materials and Methods

Animals

The main data set consisted of animals derived from generation 10 (high and low lines) and generation 7 (control line) measured concurrently. Mature feed intake was also measured in females in all replicates of generations 2-4, replicate 1 of generation 5, and in males and females of generation 11, replicate 1.

After the post-weaning test, males were housed individually and females were housed in line groups of approximately 10. At 16 weeks of age, all animals were placed back into individual housing for three weeks during which time they underwent a second intake test identical to that post-weaning. At the end of the test, animals were assessed for body composition using the EM-Scan device.

Faecal waste

In generations 10 and 11, total faecal waste production was also measured at maturity using the same techniques as that used post-weaning. Pooling of samples for bomb calorimetric analysis was undertaken using the same logic as for the post-weaning test.

Trait definitions

A summary of the traits used in subsequent analyses is given in Table 4.1. The mature test started when mice were approximately 112 days old and continued for approximately 3 weeks. Measurements of weight were made on approximately days 112, 119, 126 and 133, and feed intake between these days was recorded. Body composition was measured at the end of the test. The first period of this test (day 112

to 119) was used as a pre-test adjustment phase. The data used for the mature traits consisted of that collected between day 119 and 133. Traits calculated for use in analyses were average daily feed intake during this period, average daily gain, average daily faecal output (generations 10 and 11), mid-weight, metabolic mid-weight, maintenance requirement and maintenance efficiency. They were calculated according to the formulae given below. In addition, the exact age of the mouse at day 119 and at the measurement of body composition were calculated for use as covariates in the analyses.

Table 4.1. Summary of the traits used in the analyses with their abbreviations and units.

Abbreviation	Trait	Units
ADG_{Mat}	Average daily gain	g(bodyweight).day^{-1}
MWT_{Mat}	Mid-weight	g(bodyweight)
$MMWT_{Mat}$	Metabolic mid-weight	g(bodyweight)$^{0.73}$
DFI_{Mat}	Average daily feed intake	g(feed).day^{-1}
DFO_{Mat}	Average daily faecal output	g(waste).day^{-1}
NFI_{Mat}	Net (residual) feed intake	g(feed).day^{-1}
MR_{Mat}	Maintenance requirement	g(feed).g(bodyweight)$^{-1}$
ME_{Mat}	Maintenance efficiency	g(bodyweight).g(feed)$^{-1}$
$\%F_{Mat}$	Percent body fat	%(bodyweight)

Formulae used in the calculation of mature traits

$$DFI_{Mat} = \frac{Feed\ Intake_{119-126} + Feed\ Intake_{126-133}}{DOY_{133} - DOY_{119}}$$

$$DFO_{Mat} = \frac{Faecal\ Output_{119-126} + Faecal\ Output_{126-133}}{DOY_{133} - DOY_{119}}$$

$$ADG_{Mat} = \frac{Weight_{133} - Weight_{119}}{DOY_{133} - DOY_{119}}$$

$$MWT_{Mat} = 0.5(Weight_{119} + Weight_{133})$$

$$MMWT_{Mat} = (MWT_{Mat})^{0.73}$$

$$MR_{Mat} = \frac{DFI_{Mat}}{MWT_{Mat}}$$

$$ME_{Mat} = MR^{-1}$$

Calculation of net feed intake

Net feed intake was again calculated as the residual of a linear model (PROC GLM, SAS 1989) fitted both within generation 10/7 and also to the accumulated data set. The first model fitted to mature feed intake was similar to that used for the post-weaning test and included terms for the class variables sex and management group, co-variables average daily gain and metabolic mid-weight, and the interactions of each class variable with the co-variables. Given that the animals were assumed to have reached their asymptotic weight (Hughes and Pitchford, 1995) and supported by the lack of an effect of average daily gain upon the model, it was decided to remove average daily gain and its interactions altogether. Metabolic mid-weight, the two co-variables and their interactions with metabolic mid-weight were retained.

Variance component estimation

Variance and covariance components for the randomly mated generations were again estimated using derivative-free restricted maximum likelihood (DFREML, Meyer 1993) operated using a front-end program described by Swan (1994). Fixed effects included management group (generation x replicate), parity of the dam, parity of the individual, litter size at birth and litter size at weaning. Sex was excluded as

measurements at maturity were only conducted on females in the non-selected generations. Age at time of measurement was used as a covariate in the model. A direct additive genetic effect was included in the animal model for mature traits. Previous analysis (Archer, 1996) indicated that maternal effects and common litter environment effects were negligible at maturity. Traits were again treated as identical between sexes.

Analyses

A series of linear models (PROC GLM, SAS 1989) were used to analyse all mature growth and intake traits in generation 10. The general model was similar to that used for post-weaning traits with a number of important exceptions. It was considered pertinent to remove what were deemed to be the early-developmental effects of parity and number born in litter for the analysis of mature traits, having demonstrated that they were of no significance in preliminary modelling. A number of other main effects and interactions were again removed as they did not contribute to a significant proportion of the variance for any trait. The final model included:

management group (MGP 1, 2 / 1, 2, 3)

age at measurement (AGE 127–154 / 152-378 days)

housing box type (BOX 1, 2 / 1)

sex (SEX male, female)

line (LIN control, high, low)

sex by line

inbreeding coefficient (INC 0-0.37) by line

Where class numbers or covariate ranges differed between mature intake measurements and mature body composition measurement, both are presented in

parentheses. A summary of the numbers of mice measured for each trait is presented in Appendix 1, Table A1.2.

Results

Phenotypic and genetic parameters

For generations 1-4 (Table 4.3, Archer, 1996), common environmental effects were not significant for any trait and were subsequently removed from the model. Average daily gain, daily feed intake and net feed intake were all moderately heritable; mid-weight had a high heritability.

Table 4.3. Mean, phenotypic standard deviation, heritability and common environmental effects for mature traits from univariate analyses (Archer, 1996).

Mature trait	μ	σ_P	h^2	c^2
ADG_{Mat} (g/day)	-0.07	0.15	0.29 ± 0.10	-
MWT_{Mat} (g)	32.0	3.89	0.78 ± 0.09	-
DFI_{Mat} (g/day)	4.25	0.65	0.36 ± 0.09	-
NFI_{Mat} (g/day)	0.00	0.58	0.24 ± 0.08	-
$\%F_{Mat}$	16.6	2.18	0.31 ± 0.11	-

Phenotypic and genetic correlations between mature traits derived from bivariate analyses of generations 1-4 are presented in Table 4.4 (Archer, 1996). Phenotypically, there was a strong correlation between daily feed intake and net feed intake (0.69), and between daily feed intake and mid-weight (0.68), but none between net feed intake and gain or body weight. At maturity, larger animals tended to be fatter.

Genetically, animals that were still gaining weight at maturity tended to have higher intakes. There were strong genetic correlations between daily feed intake and net feed

intake (0.64), and between daily feed intake and mid-weight (0.76), but none between net feed intake and mid-weight. There was also a strong positive genetic correlation between body fat percentage and mid-weight (0.87), and a moderate negative genetic correlation between body fat percentage and gain (-0.42).

Table 4.4. Phenotypic (above diagonal) and genetic (below diagonal) correlations between mature traits (Archer, 1996).

	ADG_{Mat}	MWT_{Mat}	DFI_{Mat}	NFI_{Mat}	$\%F_{Mat}$
ADG_{Mat}		-0.26	0.38	0.00	-0.19
MWT_{Mat}	-0.25		0.68	-0.02	0.58
DFI_{Mat}	0.53	0.76		0.69	-0.16
NFI_{Mat}	0.24	0.00	0.64		-0.23
$\%F_{Mat}$	-0.42	0.87	-0.04	-0.04	

Phenotypic and genetic correlations between post-weaning and mature traits derived from bivariate analyses of generations 1-4 are presented in Table 4.5 (Archer, 1996). Most phenotypic correlations between post-weaning and mature traits were low to moderate, with the exception of mid-weight at both ages, which demonstrated a strong positive association (0.64).

There were a number of significant genetic correlations between post-weaning and mature traits. There were strong positive associations between weights at all ages. There was also a moderate correlation between post-weaning growth rate and mature mid-weight (0.43). Post-weaning intake was moderately correlated with mature intake (0.51), mature net feed intake (0.51), mature weight (0.68) and mature body composition (0.61). There were also moderate genetic correlations between post-weaning net feed intake and both mature net feed intake (0.60) and mature daily

intake (0.50) at maturity, but a negligible correlation between post-weaning net feed intake and mature weight (0.09).

Table 4.5. Phenotypic (upper) and genetic (lower) correlations between post-weaning and mature traits (Archer, 1996).

		Wt_{21}	ADG_{PW}	MWT_{PW}	DFI_{PW}	NFI_{PW}	$\%F_{PW}$
ADG_{Mat}	P	-0.10	0.02	-0.13	-0.02	0.05	-0.02
	G	-0.04	-0.13	0.15	-0.10	0.02	0.31
MWT_{Mat}	P	0.35	0.12	0.64	0.37	0.00	0.22
	G	0.68	0.43	0.85	0.68	0.09	0.37
DFI_{Mat}	P	0.08	0.04	0.20	0.35	0.29	0.03
	G	0.18	0.27	0.30	0.51	0.50	0.13
NFI_{Mat}	P	0.06	0.01	0.07	0.29	0.29	-0.01
	G	0.02	0.21	0.01	0.51	0.60	-0.04
$\%F_{Mat}$	P	0.15	0.05	0.14	0.21	0.06	0.34
	G	0.55	0.44	0.00	0.61	0.17	0.73

Growth and feeding traits

Correlated responses to selection for post-weaning net feed intake in mature growth and feeding traits were examined for generation 10 (generation 7 for control line) and results from linear models are presented for type III sums of squares. The percentage variance accounted for by the model (R^2), residual coefficient of variation (CV), error degrees of freedom, error mean square and source mean squares are presented in Table 4.6 for each trait when the final general model was fitted.

Table 4.6. Raw means, phenotypic variance and ANOVA table for most traits.

Source	NFI_{Mat}	DFI_{Mat}	DFO_{Mat}	ADG_{Mat}	MWT_{Mat}	MR_{Mat}	ME_{Mat}	$\%F_{Mat}$
μ	0.00	4.22	1.15	0.02	33.0	0.13	8.0	14.0
Minimum	-1.27	2.33	0.53	-0.73	22.8	0.06	4.9	3.91
Maximum	1.75	6.14	1.84	0.55	47.1	0.21	16.3	22.3
σ_P	0.46	0.46	0.16	0.11	3.2	0.02	1.2	2.3
R^2 (%)	35	51	54	20	48	39	37	32
CV (%)	11	11	14	460	10	14	15	17
Error DF	242	242	235	250	250	242	242	122
Error MS	0.2	0.2	0.0	0.01	10	0.000	1	5
MGP	0.3	0.4	0.7**	0.00	4	0.000	2	25*
AGE	0.6	0.9*	0.1*	0.03	0	0.001	5	46**
BOX	0.2	1.1*	0.8**	0.06*	15	0.002*	11**	NA
SEX	0.2	5.5**	0.4**	0.12**	614**	0.001	0	115**
LIN	0.2	0.2	0.0	0.01	111**	0.001	3	27**
SEX*LIN	0.7*	1.0**	0.0	0.10**	38*	0.004**	17**	25*
INC*LIN	0.1	0.1	0.0	0.01	59**	0.001*	4*	29**

* $p < 0.05$
** $p < 0.01$

Inbreeding effects

The main effect of inbreeding was not significant for any post-weaning trait, although there were a number of significant interactions with line, which are outlined in the section on line effects.

Sex effects

Least squares means for males and females are presented in Table 4.7. The main effects of sex are presented graphically in Figure 4.1 as the percentage deviation of males from females. The original model used to estimate net feed intake incorporated a term for sex, as well as a sex by management group interaction, and as expected, subsequent analysis of net feed intake showed no main effect of sex on net feed intake within generation 10. There were also no sex differences within line, although there was a significant interaction between sex and line, such that in males the line rankings were H>C=L, whereas in females the line rankings were H=C>L. It is unlikely that this was biologically significant.

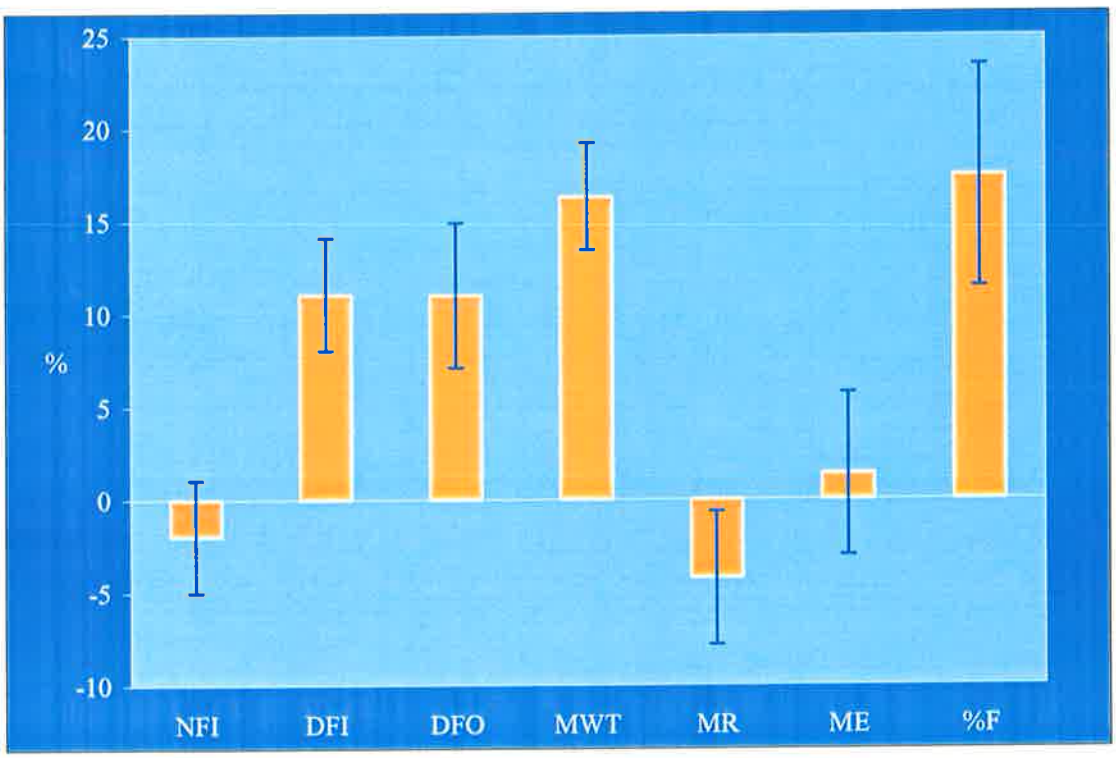
Table 4.7. Least squares means for males and females for mature growth and intake traits.

	Trait							
	NFI_{Mat}	DFI_{Mat}	DFO_{Mat}	ADG_{Mat}	MWT_{Mat}	MR_{Mat}	ME_{Mat}	$\%F_{Mat}$
♂	0.07	4.5	1.27	0.06	33.4	0.14	7.4	16.0
SE	0.08	0.1	0.03	0.02	0.6	0.00	0.2	0.5
♀	0.15	4.1	1.14	-0.00	28.7	0.14	7.3	13.6
SE	0.10	0.1	0.04	0.02	0.7	0.00	0.3	0.6

Males ate significantly more than females at maturity overall, and in both the high and low lines specifically. However, there were no sex differences within the control line, and a significant sex by line interaction produced line rankings of H=C, C=L, H>L in

males, and H=C>L in females. The main effect was duplicated in the results for daily faecal waste production.

Figure 4.1. Percentage deviation of males from females for mature growth and intake traits (±SE).



Although there was a significant effect of both sex and sex by line on daily gain at maturity, these gains/losses were not biologically meaningful, and probably only reflect the accuracy of measurement. As expected, males were significantly heavier than females at maturity, both overall and within line. However, there was an interaction between sex and line such that the low line was significantly lighter than the control and high lines in males, but not in females.

Males and females maintained mature body weight with similar efficiencies. However, there was a significant sex by line interaction for both maintenance

requirement and maintenance efficiency. Males and females were similar in the control line, but females had a higher maintenance requirement and were less efficient in the high line, whereas they had a lower maintenance requirement and were more efficient in the low line. These sex by line interactions also produced the following line rankings: maintenance requirement H=C, C=L, H>L in females, H=L=C in males; maintenance efficiency L>C=H in females, C=L=H in males

Males tended to be fatter than females at maturity, a similar result to that observed post-weaning and quite surprising. This probably reflected the different housing conditions of the sexes prior to measurement at maturity (males were housed individually, females were housed in groups of 10). There was again a significant interaction between sex and line for percent body fat: males were fatter than females in the control and high lines but not the low line, and line rankings were C>L=H in males and C=L>H in females.

Line effects

Least squares means for lines are presented in Table 4.8. Applying the same reasoning developed in the post-weaning chapter, although the main effect of line was not always significant, the presence of the intermediate control line tended to obscure the divergence between the selection lines. As such, differences between the high and low line are presented in Figure 4.2 as the percentage deviations of the low and high lines from the control line. Correlated responses in mature traits are also compared by examining high and low line differences in genetic standard deviations in Figure 4.3. The sampling variance of the response (an estimate of genetic drift using the methodology of Hill, 1980) was less than 7% of the pooled estimate of the variance

for all pair-wise line comparisons of post-weaning traits, hence significant line differences were unlikely to have been due to random genetic drift alone.

Table 4.8. Net feed intake selection lines' least squares means for mature growth and intake traits.

	Trait							
	NFI	DFI	DFO	ADG	MW	MR	ME	% Fat
C	0.14	4.45	1.24	0.06	33.2	0.14	7.5	14.9
SE	0.18	0.18	0.07	0.04	1.3	0.01	0.5	1.2
H	0.45	4.62	1.33	0.03	30.9	0.15	6.7	13.3
SE	0.07	0.07	0.03	0.02	0.5	0.00	0.2	0.4
L	-0.26	3.77	1.04	0.00	29.1	0.13	7.9	14.6
SE	0.11	0.11	0.04	0.03	0.8	0.00	0.3	0.8

Figure 4.2. Percentage deviation of high and low net feed intake selection lines from control line for mature growth and intake traits (±SE).

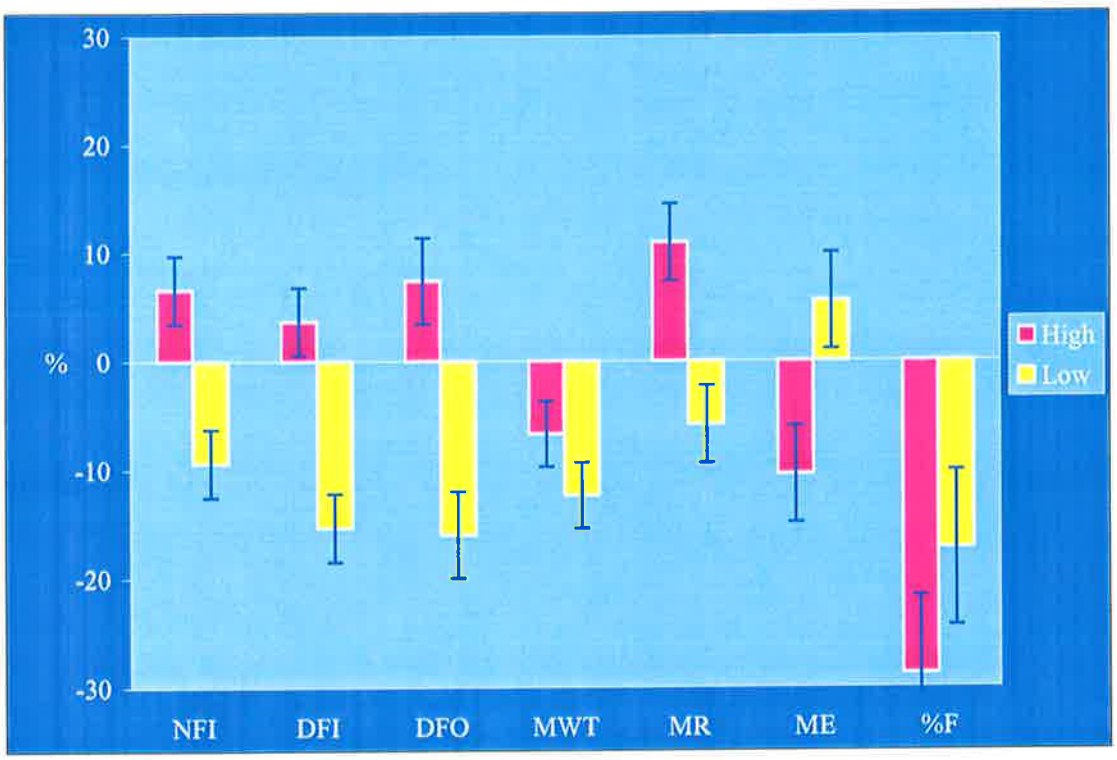


Figure 4.3. Deviation of low NFI line from high NFI in terms of genetic standard deviations for mature growth and intake traits (±SE).

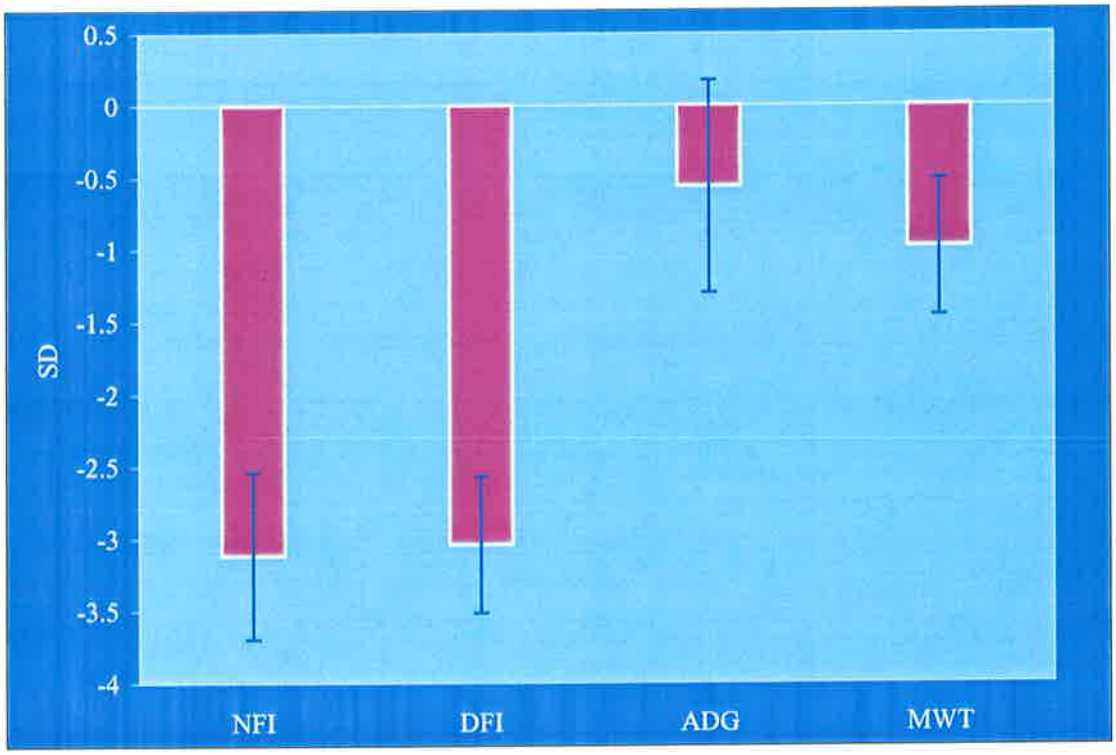
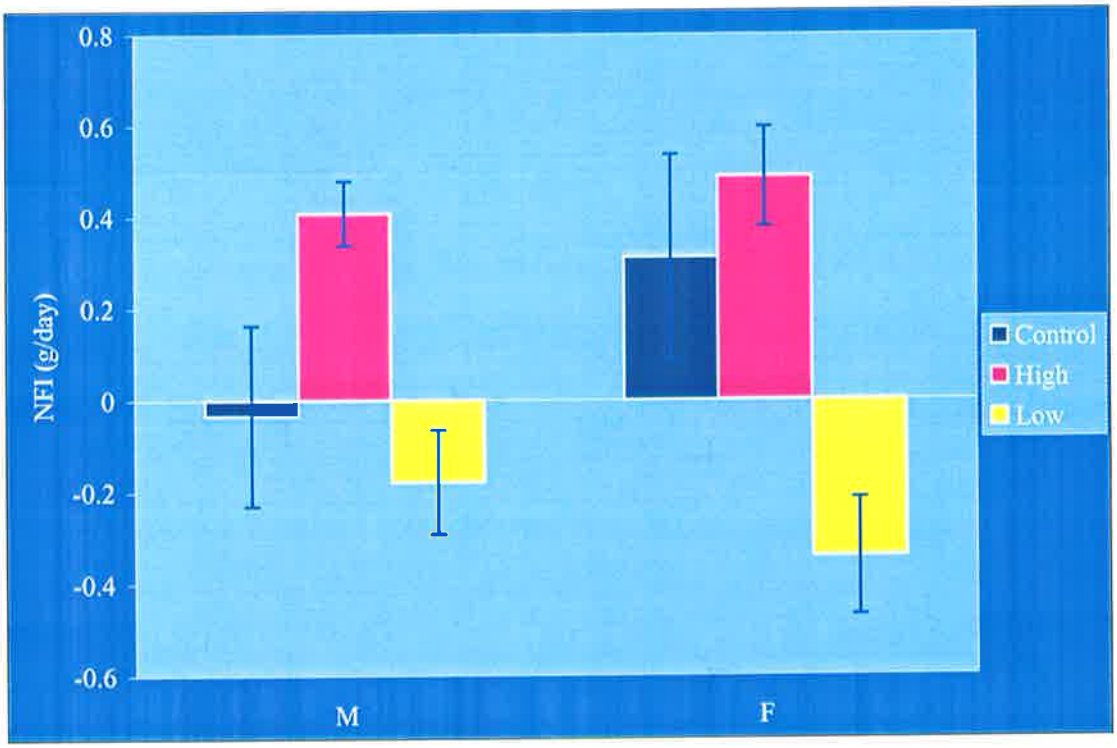


Figure 4.4. Interaction between sex and line in net feed intake at maturity (±SE).



The main effect of line was not significant for net feed intake, although there was a substantial sex by line interaction, as mentioned previously (see Figure 4.4). There were differences between all pair-wise comparisons of lines and the ranking for mature net feed intake was H>C>L. The low line had a 9% lower net feed intake than the control line and a 15% lower net feed intake than the high line in generation 10. Similar results were observed for daily feed intake, although the control line was generally higher.

Correlated responses in daily faecal waste production produced similar line rankings with respect to those of net feed intake and daily feed intake. The high line produced 7% more waste than the control line and 22% more waste than the low line. In terms of gross digestibility, the lines were identical, all retaining 71-72% of their daily intake for the maintenance. In absolute terms, the high and control lines were similar, retaining approximately 19% more than the low line.

There were significant correlated responses in mid-weight and metabolic mid-weight based on examination of the main effect. Rankings for mid-weight and metabolic mid-weight were C=H>L. In both cases, the low line was approximately 5% lighter than the high line. There were also significant interactions between line and sex (see sex effects above) and line and inbreeding coefficient. The high net feed intake line tended to be substantially lighter at maturity as inbreeding increased, whereas the low line tended to be heavier (regression coefficients of –53.5±25.4 g and 39.8±12.3 g respectively). The mature weight of the control line was unaffected by inbreeding, indicative of the lower level of inbreeding in the control line generally – more sires and dams were used per generation and there were less generations in total.

At maturity, the low line was substantially more efficient at maintaining body weight than the high line. The control line was intermediate and not significantly different from either selection line. There were also significant interactions between line and sex (sex effects above) and line and inbreeding coefficient. The low line tended to be progressively more efficient as inbreeding level increased (regression coefficient 12.2±4.5 $g.g^{-1}$). Similar but inverse results were observed for food conversion ratio.

There was a significant line effect on body fat percentage. Line rankings were C=L>H, at the conclusion of the test period, with the low line some 16% fatter than the high line. There were also line by sex (see sex effects above) and line by inbreeding coefficient interactions. The control line tended to become fatter (regression coefficient 21.4±9.2 g) as inbreeding increased, the high line became leaner (regression coefficient –23.7±11.6 g), and the low line remained unchanged.

The biological cycle: mass vs. energy

To graphically illustrate the response to selection, a series of crude line-specific daily cycles based on mass and energy transformations have been developed (Figures 4.5-4.7). Absolute mass and energy transformations are also represented graphically in Figure 4.8. The same assumptions used for the post-weaning results apply here. Numbers in parentheses are percentages.

Figure 4.5. Control line mass-energy balance.

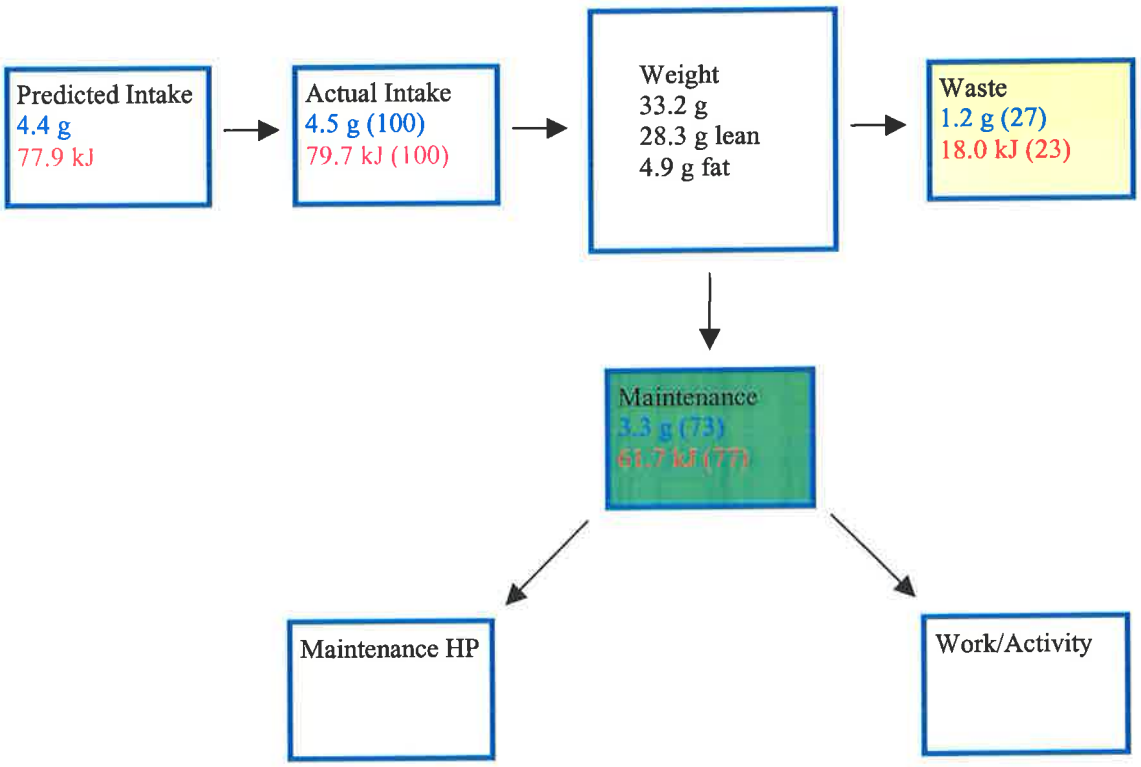


Figure 4.6. High line mass-energy balance.

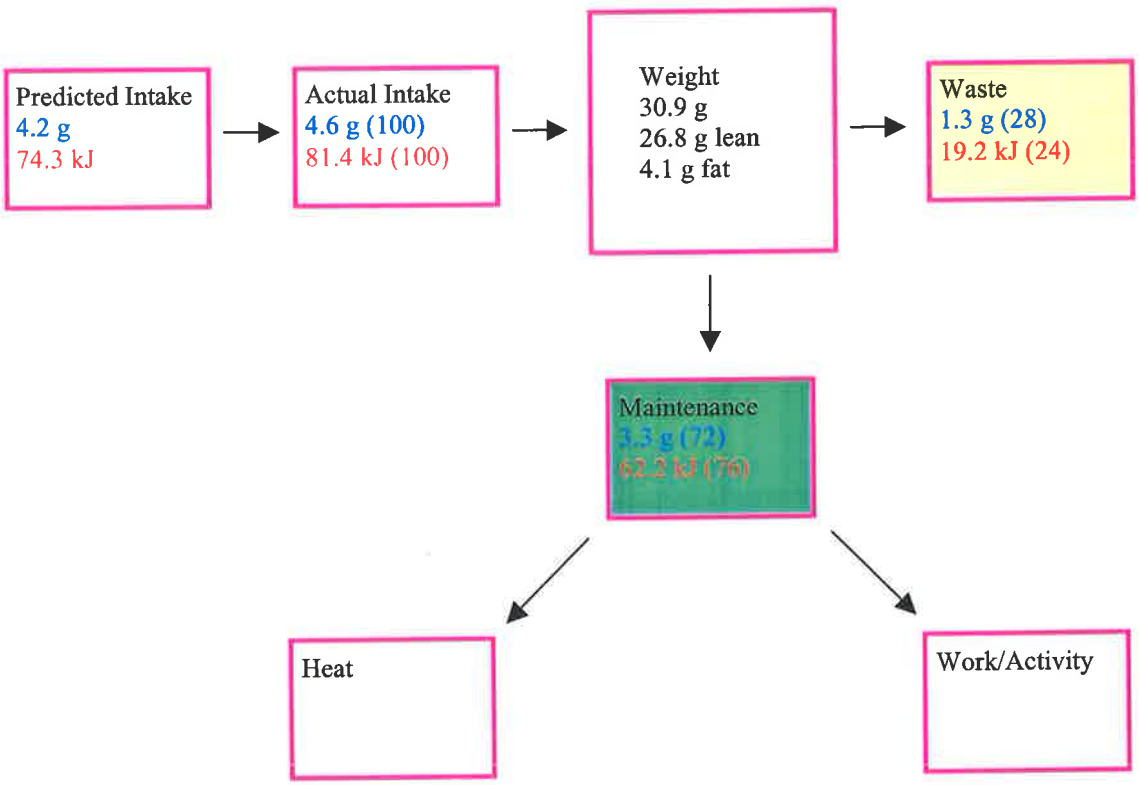


Figure 4.7. Low line mass-energy balance.

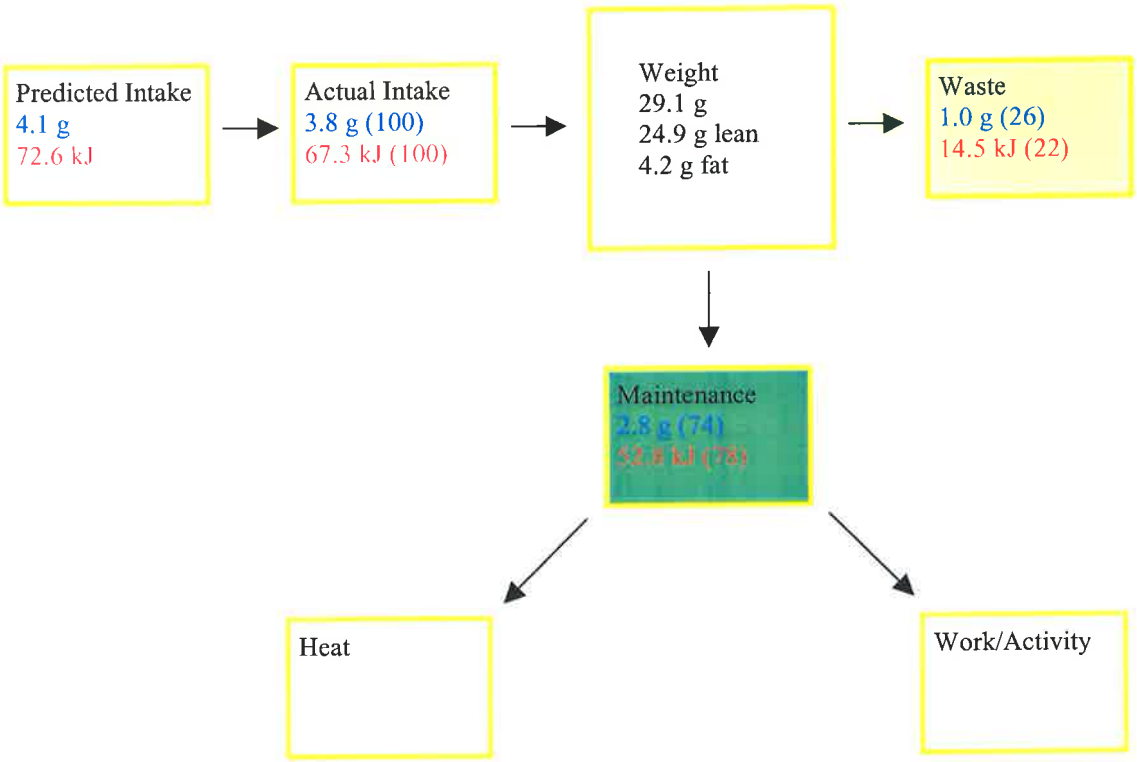
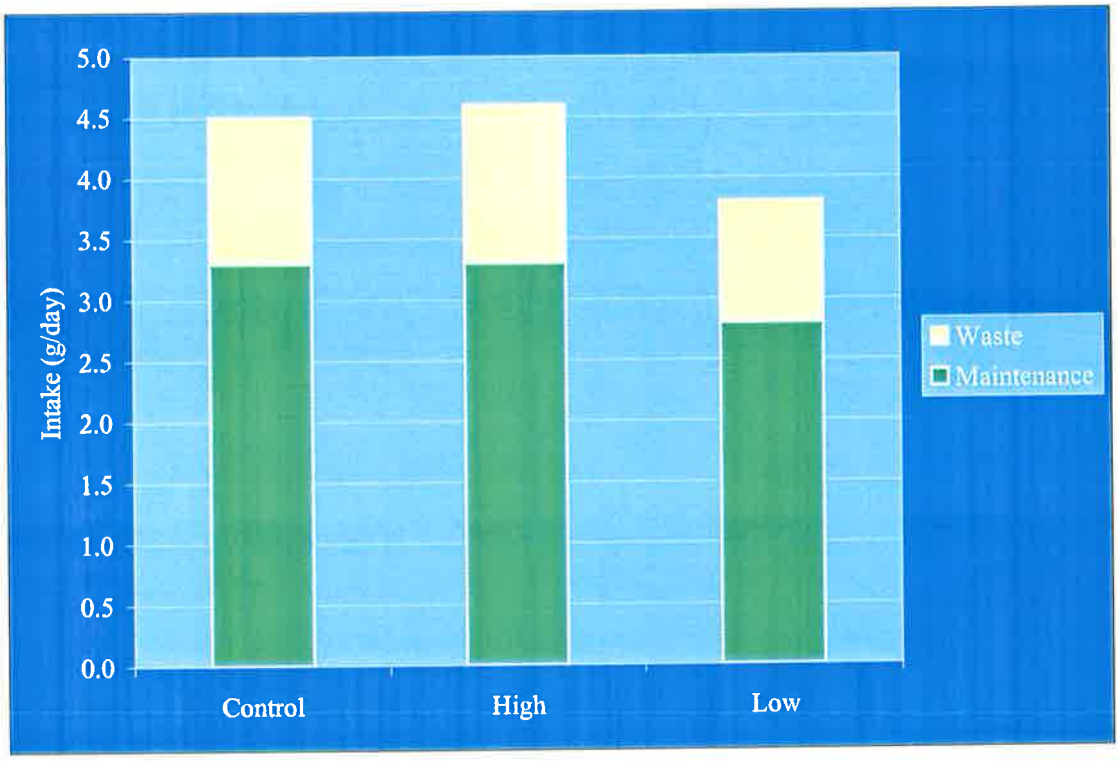
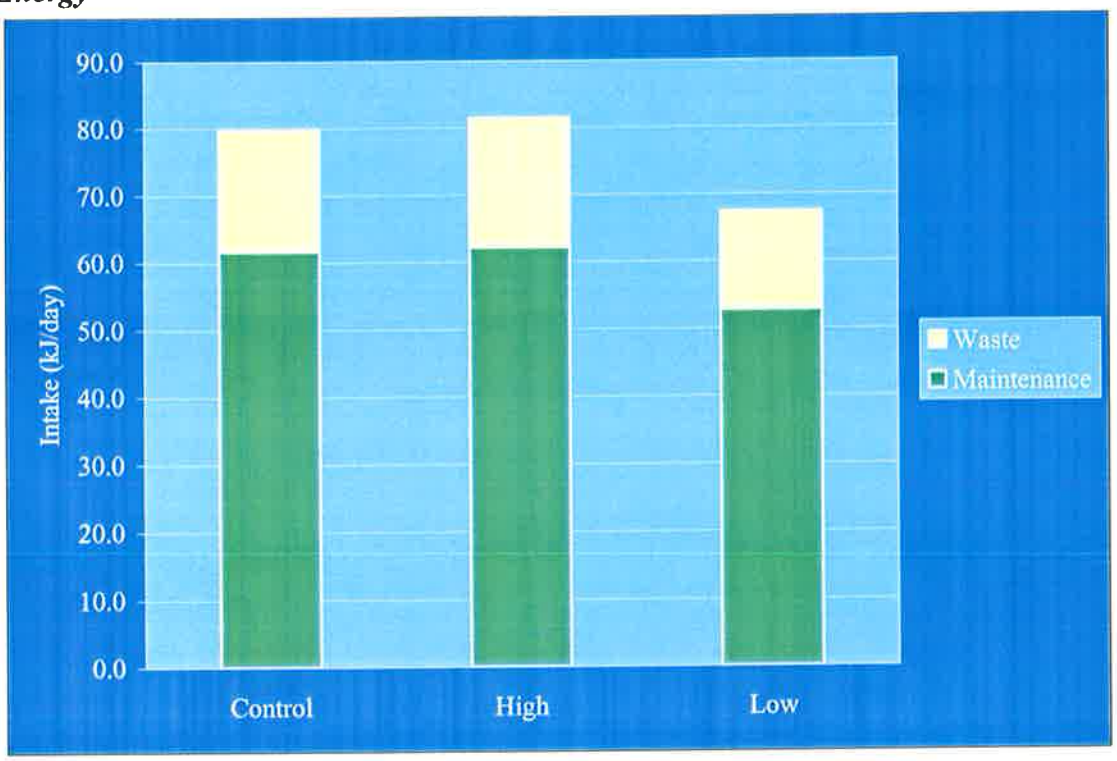


Figure 4.8. Stacked bar charts comparing absolute mass and energy conversions between lines.

Mass



Energy



Discussion

Phenotypic and genetic parameters at maturity

Mature daily feed intake was phenotypically correlated with net feed intake. Again, as for the post-weaning test, this is most likely due to the way in which residual feed intake was calculated, and this is supported by the low correlations of residual feed intake with both daily gain and body weight.

The genetic correlation between net feed intake and daily intake was stronger than the phenotypic correlation, supporting the argument that selection based on net feed intake may be an effective means of reducing maintenance requirements without altering mature body weight.

Theoretically, animals at maturity should have a negligible rate of growth, lying at or near the asymptote of their growth curve, and phenotypically this was the case. This

was reflected in the model for mature net feed intake, which excluded the gain term used previously for post-weaning data. However, there was still a significant genetic correlation between intake and gain. Animals gaining weight close to maturity are primarily depositing fat, and hence have higher energy requirements which this correlation may reflect. Larger animals were also genetically fatter at maturity. This may indicate a genetic 'limit' to the deposition of protein and other non-fat body components.

Phenotypic and genetic parameters across ages

The relationships between post-weaning and mature traits are very important in the context of trying to improve efficiency of mature animals by selection on post-weaning traits. Some of the observed correlations were as expected from the literature: the moderate genetic correlation between post-weaning growth rate and mature mid-weight supported the notion that selection for early gain results in animals that grow quicker to higher mature weights, with a corresponding increase in maintenance overheads. Although post-weaning intake was genetically correlated with intake at maturity, the strong positive correlations with mature weight and body fat suggest that using raw intake as a selection criteria may have undesirable effects on economically important traits.

Of most interest with respect to improving efficiency were the moderate genetic correlations of post-weaning net feed intake with both net feed intake and daily intake at maturity. These, coupled with the negligible correlations with average daily gain, mid-weight and percentage body fat, suggest that early selection for reduced net feed intake may be an effective means to reduce maintenance without impacting on important production characteristics associated with growth and carcass composition.

Correlated response in mature net feed intake

Although the phenotypic correlation of post-weaning net feed intake with its mature equivalent was low, the moderate genetic correlation (0.6) between these two traits led to a substantial change in mature net feed intake after 7 generations of selection for post-weaning net feed intake. This augurs well for using net feed intake of young animals as an indicator of, and possibly as a selection criterion for, the efficiency of the mature breeding herd. Indeed, the line difference at maturity was actually greater than that observed post-weaning. As noted previously, the response post-weaning was predominantly due to a change in maintenance efficiency, which is a greater component of intake at maturity and may go some of the way to explaining this observation. Alternatively, random genetic drift may have caused the response. Without experimental replication, it is difficult to ascertain (Hill, 1971).

Although published results of correlated responses at later ages to early selection for efficiency are scarce, there are a number of studies that have examined the direct response to selection for efficiency at maturity in mice. Stephens (1991) conducted a short-term selection experiment on mice (3 generations). Mature weights and intakes were measured every 3 days for 24 days at 25 weeks of age. Animals were divergently selected on the basis of their individual deviations from the regression of log mean 3-day intake on log mean body weight. Although Stephens termed this 'maintenance efficiency', it differs substantially from the traditional interpretation by excluding intake associated with body weight. Conceptually, it was equivalent to selecting for mature net feed intake in the current experiment. Stephens' approach was successful in generating differences in maintenance efficiency of approximately 20 percent after one generation of selection, although there was a substantial line by

sex interaction. However, after the third generation line differences had regressed to only 3 percent, possible illustrating the effects of random genetic drift and/or management.

Hastings *et al.* (1997) and Bünger *et al.* (1998) report responses to long term divergent selection for food intake over 8-10 weeks of age corrected for weight using the following equations:

♀ adjusted intake = intake – mean body weight

♂ adjusted intake = intake – 1.4(mean body weight)

Although not identical, this experiment provides another basis with which to compare the responses in mature efficiency to both direct and indirect selection. At the equivalent generation in the Scottish experiment, the adjusted feed intake of the low intake line was approximately 30% lower than that of the high line.

It would appear that direct selection for mature 'net' intake produced a more rapid response in mature efficiency than indirect selection based on post-weaning 'net' intake. This is to be expected, given that the genetic correlation between the two traits was observed to be positive, but less than unity. Post-weaning net feed intake is still a useful measurement, as it produces significant response at maturity, whilst allowing selection to progress more rapidly without the requirement to grow measurement animals to maturity. Extended to the commercial world, early selection is particularly useful when combined with many of the advanced reproductive processes available to the modern animal breeder.

Body weight

Line differences were again observed in body weight, although the relative differences between the high and low lines was substantially lower at maturity than immediately post-weaning. Indeed, the absolute difference had only increased by approximately half a gram, from 1.4g to 1.9g. Although selection was based on a phenotypic index, there were no significant correlations, either phenotypic or genetic, observed between post-weaning net feed intake and mature weight in the randomly-mated generations. The observed line differences in mature weight after 10 generations of selection were thus surprising. It should be noted that the high versus low difference was only of borderline significance ($p<0.04$), and may be in part due to genetic drift between the lines, which could not be quantified due to lack of replication. Alternatively, it may have been an indirect effect of the small but positive genetic correlation between post-weaning net feed intake and mature body fat percentage, resulting in an extended 'finishing' phase of fat deposition of the high line relative to the low line, and producing slightly heavier animals during the mature test. The small indirect response in weight relative to the responses in mature net intake and raw intake is again illustrated by comparing the responses in units of genetic standard deviations (Figure 4.3). This once again highlights the utility of selecting on a phenotypic index of intake that accounts for both gain and body weight.

Intake, maintenance requirement and maintenance efficiency

The selection lines were substantially divergent for mature daily feed intake, presumably a result of the positive genetic correlation of 0.50 (Table 4.5) with the trait under selection. Again, the response was greater at maturity than post-weaning, and was primarily a result of a small increase in intake in the high line, the low line

remaining relatively unchanged from weaning to maturity. This serves to illustrate the relationship between maintenance requirement and stage of maturity. By way of explanation, attention is again drawn to the allometric relationship between size and body weight: the mouse is small, and devotes only a small proportion of total intake to growth, the majority going to maintaining body weight (a function, primarily, of a large surface area to volume ratio). Instinctively, one would assume that a larger animal would tend to eat more as it has a greater mass to maintain; however, during growth, many tissues are much more metabolically active and hence are more energetically expensive to maintain. As such, the growing animal tends to have a higher maintenance requirement than the mature animal. In the case of the mouse, the absolute values for intake are approximately equivalent between weaning and maturity.

The lines were similar in weight, and this coupled with the response in intake, produced significant divergence in maintenance efficiency and maintenance requirement. Comparing gross efficiency post-weaning and maintenance efficiency at maturity is a little like comparing apples with oranges. Clearly, the low line had a higher gross efficiency due predominantly to a response in maintenance requirement post-weaning rather than a real change in the efficiency of tissue deposition. This again highlights the difficulty of using a ratio such as gross efficiency as a selection criterion: the trait is subject to influences on both its component traits, which can be complementary or antagonistic. A better comparison between the ages can be gained by examining the energy transformation diagrams, even with their crude assumptions. In both cases, the high line was devoting substantially more, both on an absolute mass basis and on an absolute energy basis, to maintaining body weight, than the low line animals. The response was greater post-weaning, particularly with respect to energy.

This may indicate that selection is acting both on maintenance efficiency generally, and more specifically on the efficiencies of those metabolically active organs associated with growth.

Gross digestibility

The correlated response in gross digestibility at maturity was similar to that observed post-weaning, primarily because the effect of gain on intake post-weaning is so small in the mouse species. Again, the relationship between intake, faecal waste and maintenance was similar between lines on both a relative mass and a relative energy basis, with intake split roughly 3:7 between waste and maintenance. The small percentage contribution of gain to the cycle post-weaning, both on a mass- and energy-basis, appears to have been repartitioned solely to maintenance at maturity, as the waste component remained essentially unchanged in both lines between tests. In absolute terms, the high line again retained more both on a mass-basis and energetically, although the line differences were somewhat smaller at maturity (0.6g/15.6kJ post-weaning vs. 0.5g/9.4kJ at maturity). This slight decrease may have been indicative of the metabolic load of the visceral tissues in the respective lines: at maturity, these tissues were less active metabolically due to negligible growth, and hence line differences associated with gut metabolism were reduced, although not eliminated entirely. Other factors, especially body composition and overall metabolic rate (heat loss/work/activity), may also have been implicated.

Body composition

Surprisingly, both selection lines showed a significant decrease in body fat at maturity relative to the post-weaning test, the effect being greater in the low line. This is difficult to interpret biologically, as animal studies almost universally show an

increase in fatness with age (e.g. Hayes and McCarthy, 1976). There may have been an influence of diet: the compositional characteristics of the basic laboratory ration used in the experiment may have changed with time. However, bomb calorimetric analysis tended to refute this, at least on a total energy basis.

Despite this aberration, the relative results from the mature test were similar to those observed post-weaning: the low line was significantly fatter at maturity. Conventional thought suggests that lean tissue is energetically more expensive to maintain than fat tissue due the high level of protein turnover within the musculature and a much greater vascularization (Pullar and Webster, 1977). The results from the current study appear to support this idea: the more efficient animals tended to be fatter at maturity. The results probably in part reflect the small (0.17) positive genetic correlation between the trait under selection and mature fat percentage. They also likely reflect the continuation of the original line differences observed post-weaning, and emphasize the major influence that immature maintenance, as opposed to gain, has on development at all ages in species with a large surface area to volume ratio such as the mouse. The effect may be entirely different in larger species such as domestic livestock, and warrants careful scrutiny.

The energy balance

The energy balance diagrams from both post-weaning and mature tests were substantively the same. It would appear that selection for post-weaning net feed intake predominantly acted on the energetic efficiency with which animals maintained body weight during growth, and this was effect was maintained at maturity. Although a number of authors (Brody, 1945; Milligan and Summers, 1986; Stephens, 1991) have made the observation that maintenance during growth has a substantially

different basis to that at maturity, the current study appears to indicate that efficiency of those biological processes that are net of differences in growth or body weight remain relatively constant throughout development, a theme which is reflected in the literature (e.g. Brody, 1945; Webster, 1978; Taylor *et al.*, 1981).

Conclusions

Quite clearly, selection for post-weaning net feed intake had a considerable effect on the efficiency with which animals maintained mature body weight. As such, the experiment met one of the prime directives of the overall study, which was to produce lines of mice which differed substantially for maintenance requirement, yet remained similar in both rate of growth and mature body weight. This was to allow further study of what might be termed 'true efficiency'; namely, the innate variation associated with specific metabolic processes, rather than the more abstract concepts of efficiency associated with the scaling effects of elevated rates of growth (i.e. gross efficiency).

Maintenance requirement is traditionally estimated as the gross amount of feed eaten per day per unit body weight. Although the energy balance diagrams presented above were based on a number of important assumptions, they allowed intake to be further partitioned into that used purely to 'maintain' body weight, and that lost as waste in the faecal by-products of digestion. Indeed, in the post-weaning test, it was also possible to further crudely partition intake into a portion for growth and portion for maintenance. In later chapters, variation in intake associated with metabolic heat production, and with activity, will allow a greater understanding of the energy balance.

Overall, the results offer some hope that selection for net feed intake at a young age may be useful in improving the overall efficiency of a livestock production enterprise. Their are a number of important qualifications, however, which relate specifically to extrapolation of the above results to livestock species. The first is that selection had a substantial effect on body composition. This would need careful consideration with respect to applications in livestock production, where increasingly, carcass composition is of as great, if not greater, economic importance than live weight and intake. Secondly, the effects on maintenance at all ages may have been substantially exaggerated relative to larger species, given that intake associated with growth is such a small component of the overall diet in the mouse. Considerations of size-scaling are clearly important when making across-species comparisons. For the purpose of this study, however, it is sufficient to note that if the results obtained in mice have a direct corollary with anticipated results in livestock, then net feed intake is a most promising avenue for improving both biological and economic efficiency at the farm gate.

Chapter 5.

Correlated responses in metabolic rate.

Introduction

Selection for post-weaning net feed intake has resulted in significant changes in feed intake both post-weaning and at maturity, with little or no associated change in either growth rate or mature body weight (Chapters 3-4). What then is the selection process acting upon to produce this change? Previous chapters have highlighted the role of changes in body composition, however this explains only a part of the total change in intake.

Generally, before foodstuffs in their raw state can be assimilated as growth components or utilised for useful work, they must first undergo a form conversion. The underlying basis of food utilisation at its most basic level is the series of chemical pathways associated with the oxidation of foodstuffs to biologically useful products and energy, in the form of carbohydrates, proteins and fats. Due to the intrinsic inefficiencies of oxidative reactions, waste heat is produced and this heat is lost to the environment. There is some evidence in the literature for variation in metabolic heat production (Stephens, 1991; Hastings *et al.*, 1997; Moody *et al.*, 1997; Nielsen *et al.*, 1997a) although little is known about the components of this variation, and whether there is any underlying genetic basis. It would appear that this may be one important pathway upon which selection for net feed intake may be acting, by altering the 'intrinsic' efficiency of an animal's energy generating processes.

This chapter examined selection line differences in a range of metabolic parameters to determine whether significant correlations existed with net feed intake.

Materials and Methods

To examine the correlated responses to selection for net feed intake in metabolic traits, the metabolic status of the lines was assessed using indirect calorimetry. Design and development of a semi-automated indirect calorimeter is described in Appendix 2. This calorimeter was loosely based on designs used by Joy Dauncey (Dauncey *et al.*, 1978; Dauncey and Brown, 1987) at the AFRC Institute of Animal Physiology and Genetics Research in Cambridge, England, and by Russ Baudinette (pers. comm.) while at Flinders University in Adelaide, Australia. An indirect calorimeter utilises the relationship between respiratory gas exchange and metabolic heat production to estimate metabolic rate from measurement of respiratory gases.

Animals

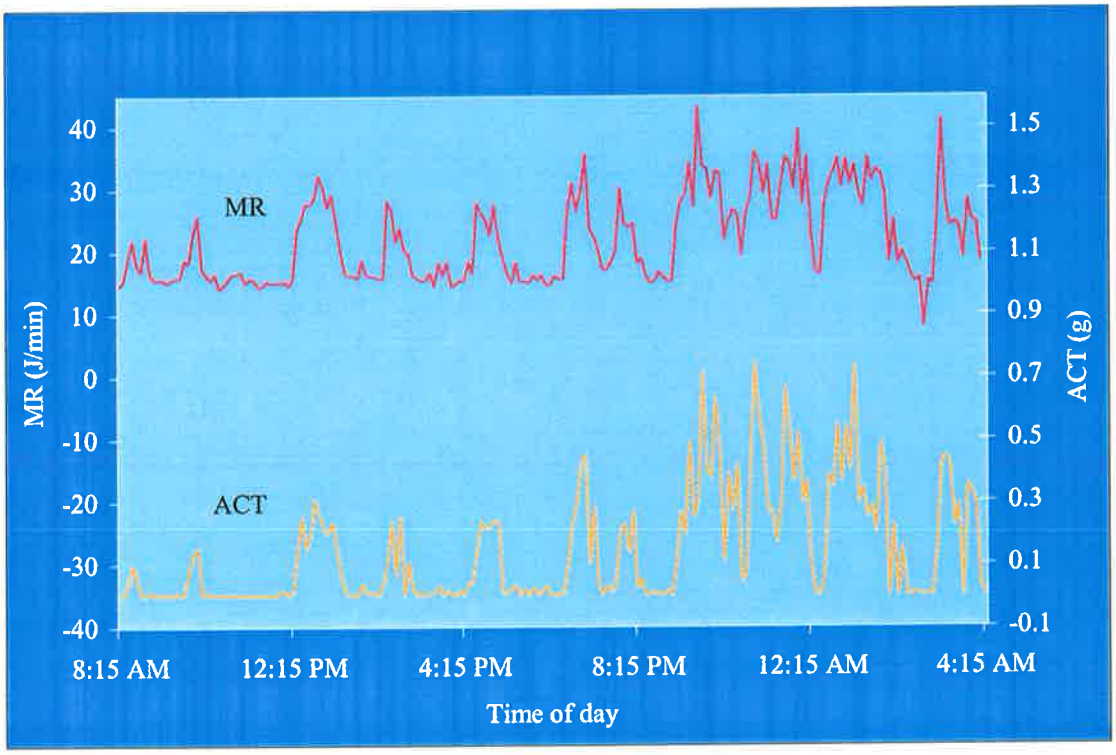
Measurements were conducted on generations 10 and 11 (7 and 8 for the control line), which were deemed to have diverged significantly (i.e. by greater than two genetic standard deviations) for the trait undergoing selection. A large number (410) of individuals were measured. As the animals were first required to undergo a three week intake test for future selection, coupled with a limited throughput of animals per day in the calorimeter, age at measurement was not truly post-weaning, and showed a broad range (77-306 days). Age was instead fitted as a covariate in subsequent analyses to examine age related differences in the metabolic response to selection for post-weaning net feed intake. During non-measurement periods, animals were kept in one of four housing box types.

Calorimetric measurement

The protocol for calorimetric measurement changed substantially over the experimental period and new techniques were adopted as data became available. Such changes meant that comparisons between successive generations were not always straight-forward. Efforts have been made to address this situation within the analysis, but where this has not been possible it is noted in the main body of the text.

A number of important calibration experiments were conducted to assess the calorimeter design. Of note was a series of 24 hour experiments designed to assess the validity of using scales as a means to measure activity, and to observe the accuracy of the calorimeter in detecting diurnal rhythms associated with activity, intake and metabolic rate. Figure 5.1 is a representative graph of the relationship between activity and metabolic rate for a single chamber over 24 hours. It illustrates the elevated rates of activity and metabolic rate typical of an animal that is a predominantly nocturnal feeder. There are a number of other salient features of the graph. The first is the distinct difference between activity levels (and associated metabolic rate) between light (6:30am – 8:00pm) and dark (8:00pm-6:30am) periods. Clearly, even under a stringent fasting regime the diurnal pattern of activity in this nocturnal species was having an effect. This evidence suggests that day-time measures of metabolism are more appropriate for establishing a good estimate of basal metabolism (less 'noise' introduced by activity levels). The second feature of note is the distinct minimum in metabolism in the absence of activity. Clearly, twenty four hours of fasting is sufficient time to reach basal metabolism in the mouse.

Figure 5.1. 24 hour graph of metabolic rate and activity for a single mouse.



There are a number of methods for calculating metabolic rate from respiratory data. The method of choice and the philosophy behind its use is described in detail in Appendix 2. A number of other techniques from various sources (Baudinette pers. comm.; Seymour pers. comm.) were also applied to the data set. These were in good agreement with the final method used.

In the final and most comprehensive protocol, the calorimeter was a three chamber system which allowed measurement of three animals at once, hence all methods refer to groupings of three individuals, where line, sex and age were randomised across chambers and across four successive time-periods. Animals were fasted for 24 hours prior to measurement. They were then placed within the chambers and respiratory gases and activity were monitored for one hour to measure basal metabolic rate. Animals were then given access to food and measured for another hour to observe the re-equilibration of metabolism to a maintenance level. The difference between the

two measures was assumed to be the heat increment of feeding. Total feed consumed during the second hour was also measured. The sampling protocol and calculation of metabolic rate during this two hour period is detailed extensively in Appendix 2.

Activity

Elevated activity is directly correlated with an increase in heat production due to the energy generating reactions occurring within muscle (Dauncey and Brown, 1987; Dauncey, 1991). Whilst it was not possible to quantify the absolute energy expenditure associated with muscular work, the activity monitor enabled metabolic rate measurements to be standardised according to the level of activity. Since activity was measured continuously, whereas metabolic rate was measured discontinuously at four minute intervals (due to chamber switching), some thought was required as to the best use of the activity data. Consultation of Dauncey's publications (Dauncey and Brown, 1987; Brown *et al.*, 1991) indicated that discrete metabolic rate measurements in mice and rats may be affected by activity as much as 10 minutes either side of the measurement. As our measurements were only 4 minutes apart, it was noted that standardisation to 20 minute averages of activity around each metabolic data point would result in elevated correlations between successive data points and would also substantially reduce the data set, so successively smaller averages were applied. It was found that an average of 2 minutes either side of the data point (i.e. 4 minutes total) fit the activity data best. This was in part due to the relatively small time constant of the Waite system.

Analysis

Generations 10 and 11 were analysed as a single group for both activity and metabolic rate. Each individual had 28 consecutive measures of activity, 14 consecutive

measures of basal metabolic rate, and 14 consecutive measures of maintenance heat production. These were analysed using mixed models outlined below (PROC MIXED, SAS 1989). The variable 'metabolic status' accounted for the differences between fasted and fed measurements and allowed the heat increment of feeding to be calculated as the difference. Animal ID was fitted as random effect to account for repeated measures within individuals. A formal repeated measures analysis was attempted initially, but the data structure did not lend itself to this approach.

1. Activity (4 minute averages) model containing:

metabolic status (fasted, fed)
chamber number (1, 2, 3)
time of day (early morning, late morning, early afternoon, late afternoon)
housing box type (1, 2, 3, 4)
parity (1, 2)
sex (male, female)
line (control, high, low)
age (77-306 days)
weight (16.8-60.1 grams)
metabolic status by intake during second half of measurement (0.0-1.7 grams)
metabolic status by chamber number
metabolic status by time of day
metabolic status by housing box type
metabolic status by parity
metabolic status by age
metabolic status by weight
metabolic status by sex
metabolic status by line
metabolic status by intake during second half of measurement by line

2. Metabolic rate model containing

metabolic status (fed, fasted)
chamber number (1, 2, 3)
time of day (early morning, late morning, early afternoon, late afternoon)
housing box type (1, 2, 3, 4)
parity (1, 2)
sex (male, female)
line (high, low, control)
age at measurement (77-306 days)
weight (16.8-60.1 grams)
ln{activity level} (-7.22--0.02 ln{grams})
metabolic status by intake during second half of measurement (0.0-1.7 grams)
metabolic status by chamber number
metabolic status by time of day
metabolic status by housing box type
metabolic status by parity
metabolic status by age at measurement
metabolic status by weight
metabolic status by ln{activity level}
metabolic status by sex
metabolic status by line
ln{activity level} by sex
ln{activity level} by line
metabolic status by intake during second half of measurement by line

Results

General Overview of the Raw Data

There was a substantial decline in metabolic rate over the first hour, a rapid increase in the middle of the experiment when food was provided, followed by a second decline over the final hour (Figure 5.2). There was a progressive decline in activity

level over the course of the experiment, although there was a slight increase at the mid-way point, associated with the addition of food to the chamber (Figure 5.3). There was also a substantial reduction in the variance of activity in the second hour of the experiment (i.e. after feeding).

On closer examination, the relationship between activity and metabolic rate (illustrated in Figures 5.4 and 5.5) appeared to diverge substantially from a simple linear regression. A logarithmic function gave a significantly better fit to the data in both the fasted and the fed periods of the experiment, explaining approximately 45% of the variation in metabolic rate in both periods. The constant was almost identical between the periods (36 J/min). Activity levels (the modulus of the difference in consecutive half-second measures of weight in grams, averaged over four minute intervals, see Appendix 2) were all less than 1, and hence ln(activity) was negative. As such, the lower slope after feeding was associated with a higher metabolic rate (e.g. if activity was, on average, 0.1 g deviation, then the fasted metabolic rate was 27.1 J/min, the fed metabolic rate was 28.3, and the heat increment of feeding was 1.2 J/min).

Figure 5.2. The response (±SE) in metabolic rate over time.

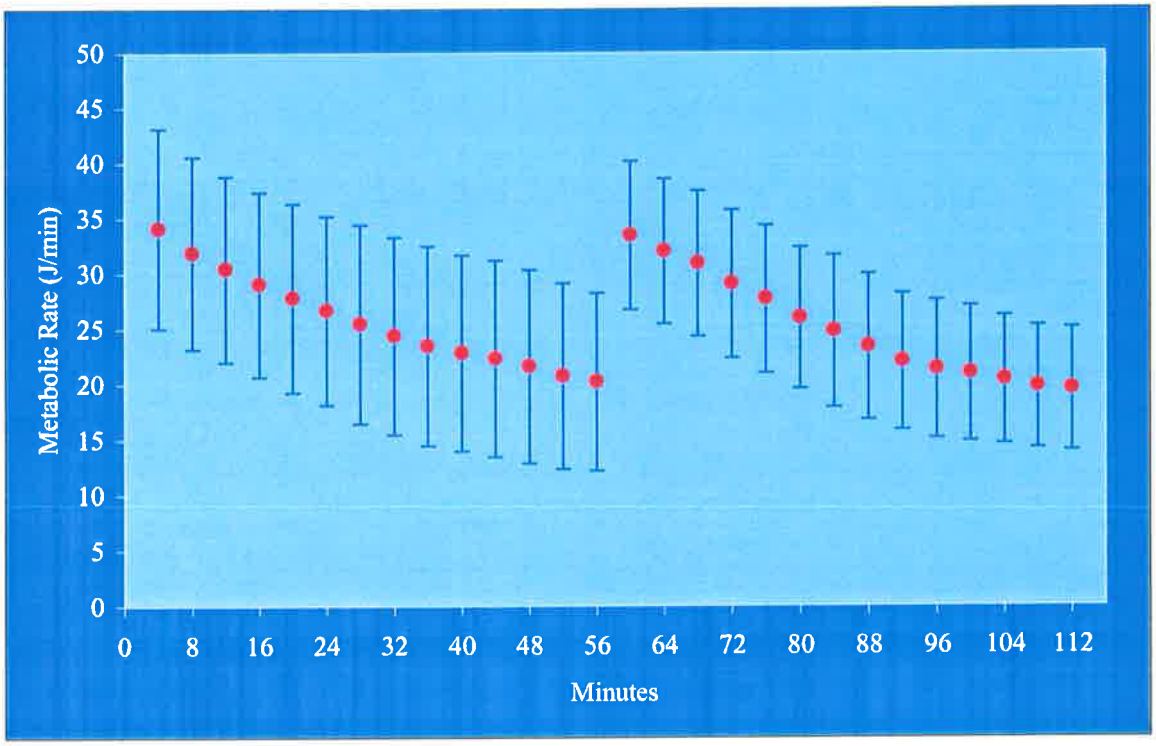


Figure 5.3. The response (±SE) in activity over time.

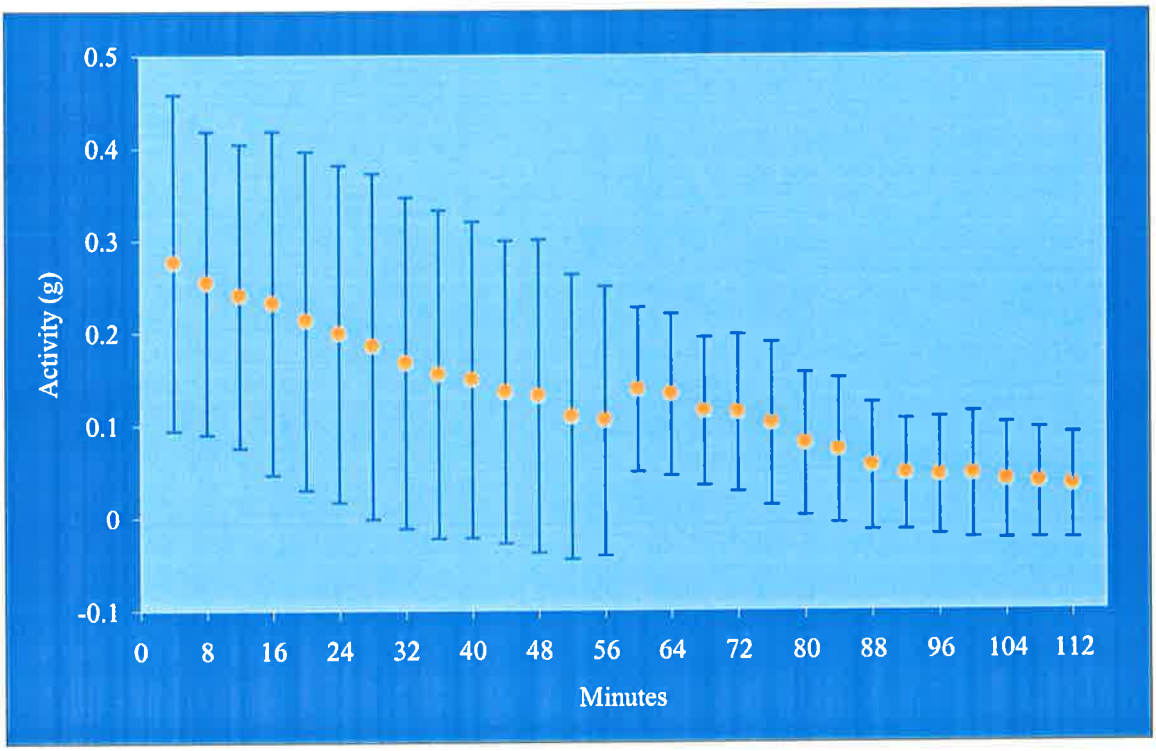


Figure 5.4. The relationship between activity and metabolic rate during the fasted period.

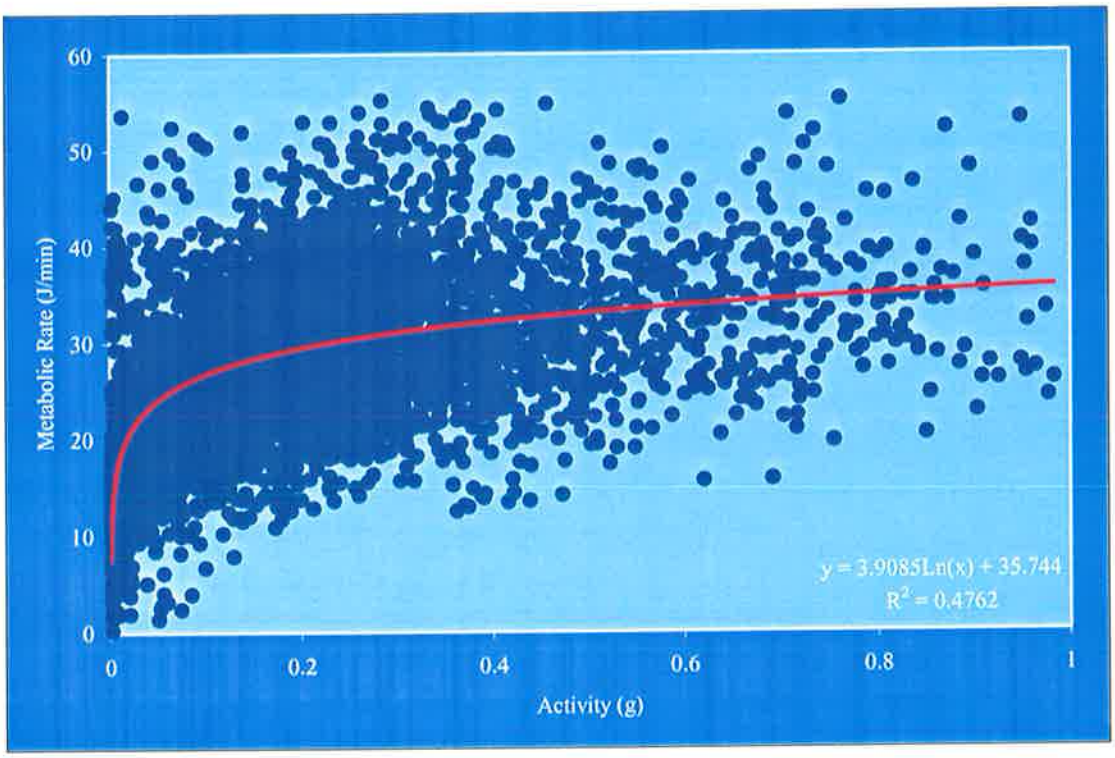
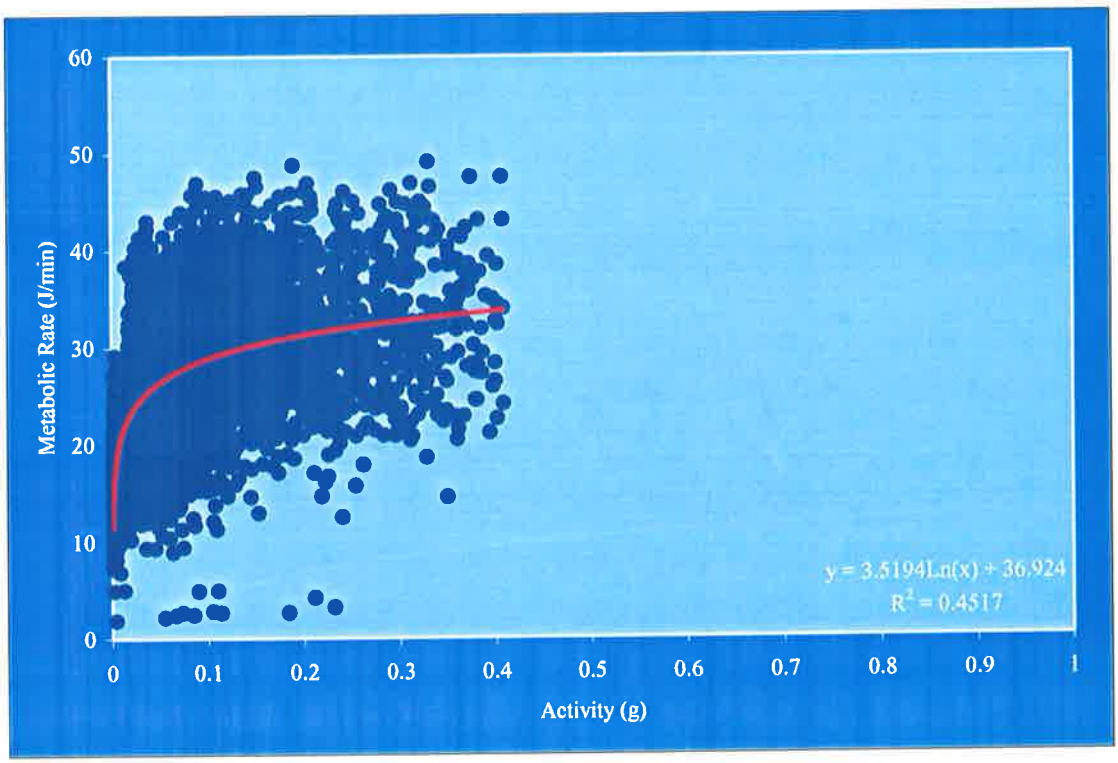


Figure 5.5. The relationship between activity and metabolic rate during the fed period.



Activity

The activity ID variance was 0.004 g^2 and the residual variance was 0.013 g^2. Type III tests of fixed effects used in the final model for activity are presented in Table 5.1, and least squares means and regression coefficients for selected factors and covariates are presented in Table 5.2.

Table 5.1. Type III tests of fixed effects for activity data.

Effect	Numerator DF	F Value	F Prob.
Metabolic status	1	54.36	**
Chamber	2	73.49	**
Time of day	3	3.96	**
Housing box type	3	1.34	NS
Parity	1	0.11	NS
Age	1	0.78	NS
Weight	1	9.92	**
Sex	1	0.66	NS
Line	2	0.16	NS
Metabolic status x Intake	2	0.69	NS
Metabolic status x Chamber	2	132.53	**
Metabolic status x Time of day	3	29.28	**
Metabolic status x Box type	3	8.06	**
Metabolic status x Parity	1	11.15	**
Metabolic status x Age	1	5.80	*
Metabolic status x Weight	1	1.87	NS
Metabolic status x Sex	1	14.66	**
Metabolic status x Line	2	2.19	NS
Metabolic status x Intake x Line	4	3.93	**

** $Pr < 0.01$
* $Pr < 0.05$
NS $Pr > 0.05$

Table 5.2. LS means and regression coefficients for activity data (ln{grams}).

Effect		LSM	SE	Coeff.	SE
Metabolic status	Fasted	0.176	0.005		
	Fed	0.075	0.004		
Time of day	Early morning	0.145	0.007		
	Late morning	0.127	0.006		
	Early afternoon	0.116	0.007		
	Late afternoon	0.115	0.008		

Effect			LSM	SE	Coeff.	SE
Parity	1		0.127	0.008		
	2		0.124	0.005		
Age[a]					0.001	0.009
Weight[a]					0.206	0.081
Sex	Female		0.130	0.007		
	Male		0.114	0.008		
Line	Control		0.122	0.007		
	High		0.127	0.007		
	Low		0.137	0.006		
M_stat. x Intake	Fasted				0.048	0.019
	Fed				0.020	0.019
M_stat. x Time of day	Fasted	Early morning	0.213	0.008		
	Fasted	Late morning	0.171	0.007		
	Fasted	Early afternoon	0.161	0.008		
	Fasted	Late afternoon	0.160	0.009		
	Fed	Early morning	0.076	0.008		
	Fed	Late morning	0.083	0.007		
	Fed	Early afternoon	0.071	0.008		
	Fed	Late afternoon	0.070	0.009		
M_stat. x Parity	Fasted	1	0.184	0.008		
	Fasted	2	0.169	0.005		
	Fed	1	0.071	0.008		
	Fed	2	0.079	0.006		
M_stat. x Age[a]	Fasted				-0.131	0.008
	Fed				-0.001	0.009
M_stat. x Weight[a]	Fasted				0.275	0.080
	Fed				0.206	0.081
M_stat. x Sex	Fasted	Female	0.166	0.007		
	Fasted	Male	0.187	0.007		
	Fed	Female	0.078	0.007		
	Fed	Male	0.073	0.007		
M_stat. x Line	Fasted	Control	0.156	0.008		
	Fasted	High	0.179	0.007		
	Fasted	Low	0.195	0.006		
	Fed	Control	0.072	0.008		
	Fed	High	0.074	0.007		
	Fed	Low	0.078	0.006		
M_stat. x Int. x Line	Fasted	Control			-0.025	0.026
	Fasted	High			-0.004	0.021
	Fasted	Low			0.048	0.019
	Fed	Control			-0.003	0.032
	Fed	High			0.023	0.021
	Fed	Low			0.020	0.019

[a] $x10^{-2}$

Overall, there was a significant effect of metabolic status on activity, such that animals tended to be approximately 2½ times more active in the earlier part of the experiment (fasted) than in the latter half of the experiment (fed) (Table 5.1, 5.2). There were also a number of interactions of status with other variables. The main effect of time of day, associated with the diurnal cycles of normal intake and metabolism, was significant for activity, which tended to decrease from early morning to late afternoon. There was, however, an interaction with metabolic status, such that a consistent decrease in activity with time was observed only in the first hour of experimentation. In the second hour, there was a tendency for activity to peak late in the morning and then decline as the afternoon wore on. Given that activity was measured as the deviation between consecutive measures on a digital scale (grams) , it was not surprising that heavier animals tended to produce greater measures of activity overall (regression coefficient 0.002). Although there was no main effect of age on activity, older animals tended to be less active in the first hour of the experiment (regression coefficient –0.130), but there was no age effect in the second hour. Males tended to be more active than females in the first hour of the experiment but similar in the second hour.

There was no difference between lines in activity, nor was there any effect of line within either the fasted or fed periods. However, closer examination of least squares means indicated that the control line was less active than the low line generally, and during the fasting period line rankings were L>H>C, whereas during the fed period there were no pairwise line differences. Furthermore, when intake during the second half of the test period was taken into account there were significant line differences within the first half. Low line animals that ate more at the subsequent re-feeding were more active initially but were unaffected by intake in the second hour, whereas the

high and control line showed no specific response in activity to intake, either before or after feeding.

Metabolic Rate

The ID variance was 12.0 $(J/min)^2$ and the residual variance was 18.7 $(J/min)^2$. Type III tests of fixed effects used in the final model for activity are presented in Table 5.3, and least squares means and regression coefficients for selected factors and covariates are presented in Table 5.4.

Table 5.3. Type III tests of fixed effects for metabolic rate data.

Effect	Numerator DF	F Value	F Prob
Metabolic status	1	32.31	**
Chamber	2	94.93	**
Time of day	3	1.70	NS
Housing box type	3	0.67	NS
Parity	1	0.08	NS
Age	1	4.64	*
Weight	1	82.14	**
ln{Activity}	1	13630.00	**
Sex	1	0.05	NS
Line	2	0.68	NS
Metabolic status x Intake	2	50.91	**
Metabolic status x Chamber	2	8.11	**
Metabolic status x Time of day	3	2.68	*
Metabolic status x Box type	3	1.87	NS
Metabolic status x Parity	1	0.47	NS
Metabolic status x Age	1	0.48	NS
Metabolic status x Weight	1	65.57	**
Metabolic status x ln{Activity}	1	26.18	**
Metabolic status x Sex	1	0.08	NS
Metabolic status x Line	2	2.00	NS
ln{Activity} x Sex	1	0.08	NS
ln{Activity} x Line	2	35.87	**
Metabolic status x Intake x Line	4	0.24	NS
Metabolic status x ln{Activity}x Line	2	7.89	**

** $Pr < 0.01$
* $Pr < 0.05$
NS $Pr > 0.05$

Table 5.4. LS means and regression coefficients for metabolic rate data (J/min).

Effect			LSM	SE	Coeff.	SE
Status	Fasted		24.31	0.23		
	Fed		26.56	0.23		
Time of day	Early morn.		26.14	0.38		
	Late morn.		25.29	0.34		
	Early aftern.		25.14	0.38		
	Late aftern.		25.17	0.43		
Parity	1		25.36	0.41		
	2		25.51	0.28		
Age[a]					-0.87	0.45
Weight					0.29	0.04
ln(Activity)					3.51	0.07
Sex	Female		25.35	0.37		
	Male		25.53	0.35		
Line	Control		25.65	0.42		
	High		25.53	0.35		
	Low		25.14	0.31		
M_stat. x Intake	Fasted				-0.33	0.96
	Fed				3.05	0.97
M_stat. x Time of day	Fasted	Early morn.	25.17	0.40		
	Fasted	Late morn.	24.15	0.35		
	Fasted	Early aftern.	24.07	0.39		
	Fasted	Late aftern.	23.85	0.45		
	Fed	Early morn.	27.11	0.40		
	Fed	Late morn.	26.44	0.35		
	Fed	Early aftern.	26.22	0.39		
	Fed	Late aftern.	26.49	0.45		
M_stat. x Parity	Fasted	1	24.19	0.42		
	Fasted	2	24.43	0.29		
	Fed	1	26.53	0.42		
	Fed	2	26.60	0.29		
M_stat. x Age[a]	Fasted				-1.02	0.45
	Fed				-0.87	0.45
M_stat. x Weight	Fasted				0.45	0.04
	Fed				0.29	0.04
M_stat. x ln(Activity)	Fasted				3.89	0.07
	Fed				3.51	0.07
M_stat. x Sex	Fasted	Female	24.24	0.37		
	Fasted	Male	24.38	0.36		
	Fed	Female	26.46	0.38		
	Fed	Male	26.67	0.36		
M_stat. x Line	Fasted	Control	24.27	0.43		
	Fasted	High	24.59	0.36		
	Fasted	Low	24.07	0.32		
	Fed	Control	27.02	0.43		
	Fed	High	26.47	0.36		
	Fed	Low	26.20	0.32		

Effect			LSM	SE	Coeff.	SE
ln(Activity) x Sex	Female				3.52	0.08
	Male				3.51	0.07
ln(Activity) x Line	Control				3.58	0.09
	High				3.26	0.07
	Low				3.51	0.07
M_status x Int. x Line	Fasted	Control			0.54	1.35
	Fasted	High			-0.05	1.08
	Fasted	Low			-0.33	0.96
	Fed	Control			3.57	1.66
	Fed	High			2.78	1.09
	Fed	Low			3.05	0.97
M_stat. x ln(Act.) x Line	Fasted	Control			4.10	0.08
	Fasted	High			3.24	0.07
	Fasted	Low			3.89	0.07
	Fed	Control			3.58	0.09
	Fed	High			3.26	0.07
	Fed	Low			3.51	0.07

[a] $x10^{-2}$

There was a significant effect of metabolic status on heat production (Table 5.3). Fasting (basal) heat production was 24.3 J/min, fed (maintenance) heat production was 26.6 J/min, and the heat increment associated with feeding was around 2.3 J/min. There were also a number of interactions of status with other variables. Of note was the interaction with intake. There was no effect of subsequent intake on metabolic rate during the fasting phase of the experiment. However, once re-fed, metabolic rate was elevated at the rate of 3.15 J/min per gram of food eaten.

The main effect of time of day was not significant for metabolic rate. There was, however, an interaction with metabolic status. Animals measured in the early morning tended to have higher metabolic rates under fasted conditions than animals measured in the late morning and afternoon. There was no effect of time of day under fed conditions. Older animals had lower rates of heat production (regression coefficient –0.01). Heavier animals had higher rates of heat production (regression coefficient 0.29), but there was a significant interaction with metabolic status, causing

the effect of weight to be more pronounced under fasting conditions. Activity (ln{activity}) had a large effect on metabolic rate (regression coefficient 3.51), but again there were a number of significant interactions with other variables. The effect of activity was moderated by metabolic status: the effect in the fasting period was greater than that in the fed period.

The main effects of sex and line were not significant, nor were their interactions with status. The overall effect of intake by line by status was also not significant. There was, however, an interaction of line with ln(activity). The response in metabolic rate to activity was highest in the control and low lines, with the high line significantly less responsive. Furthermore, the response to activity decreased substantially in the control and low lines after feeding, whereas the high line remained unchanged.

Discussion

General Overview of the Raw Data: The Basis for the Final Models

Figures 5.2-5.5 are based on the total data set obtained, and illustrate some of the ideas that went into the development of the final analytical models used to describe metabolic rate in the three lines. When comparing the response in metabolic rate (Figure 5.2) with the response in activity (Figure 5.3), it became clear that although there was a strong linear relationship between metabolic rate and activity, there was also a substantial response in metabolic rate in response to re-feeding which was independent of activity. This was most encouraging, indicating that the Waite indirect calorimeter was sufficiently accurate to detect the (relatively) small response associated with the heat increment of feeding in mice over a short time period.

The response in activity (Figure 5.3) was also of interest in its own right. Clearly, there was a marked decline in activity over the first hour, assumed to be associated with the animal becoming accustomed to its surroundings within the calorimetric chamber after an initial period of adjustment. Although there was some concern that the decline in activity generally did not stabilise fully within the first hour, the subsequent use of activity as a covariate within the model for metabolic rate addressed this issue. As expected, activity again increased directly after introduction of food into the chamber as animals sought to sate their hunger from the enforced fast. Two aspects of this second phase of activity were of interest. Firstly, the maximum level maintained post-feeding was substantially lower on average than that prior to feeding, and soon declined to what appeared to be a relatively stable asymptote. This was presumably due to the onset of 'post-prandial stupor' associated with the metabolic effects of digesting a meal, particularly after a substantial fast. Second, and perhaps of greater interest, was the marked decline in the variability of activity post-feeding. It is hypothesized that this was due to specific differences in the nature of the activity pre- and post-feeding. In the first hour, movement would have been predominantly associated with both nervous movement (initially) and exploratory behaviour (subsequently), both caused by introduction to a substantially new environment, and both associated with movements that were larger in scale. During the second hour, movement would have been associated with the acquisition and mastication of foodstuffs, both presumably substantially smaller ranges of movement than those in the first hour. Ideally, closed-circuit video could have been used to establish the real basis behind this apparent difference, but this was beyond the scope of the current experiment.

The relationship between metabolic rate and activity is of paramount importance if one is to establish credible measures of metabolic rate for comparison between lines. The muscular movement associated with physical activity can have a significant impact on 24 hour energy expenditure (Dauncey, 1990). Activities to be considered range from incidental activity due to muscle tone and 'fidgeting', to sitting and standing, walking and running, and major activities such as exercise in man and flying in birds. The energetics of muscular work has been discussed in detail by Blaxter (1989) and aspects of research on the influence of muscular activity and exercise on energy expenditure have also been reviewed (Dauncey and Blaxter, 1991). Dauncey (1991) detailed many of the associated variables that may influence activity directly, and also affect its relationship with metabolic rate. Within the current experiment, many of these issues were addressed directly by standardizing the measurement environment (nutrition, environmental temperature, etc...). Where this was not possible, the effects were modelled concurrently with activity and metabolic rate.

Some studies in mice (e.g. Dauncey, 1986) have indicated a simple linear relationship between activity and metabolic rate. The current data set, however, deviated substantially from a simple linear regression (Figures 5.4 and 5.5). Importantly, although the majority of measurements were clustered near to zero activity, there was a substantial amount of data at higher levels of activity. Much of the current literature has been based on substantially smaller data sets and has tended to concentrate on resting levels of activity, and hence have given little weight to those measurements occurring at the extreme upper end of the full range of movements. Given the size of the current data set, the extreme measurements had considerable weighting on the regression, and overall the relationship between activity and metabolic rate appeared to be best described by a logarithmic function. This was similar to results obtained in

rats (Brown *et al.*, 1991). In that study, the researchers examined the influence of acclimation to mild cold on 24 hour heat production and motor activity. They found that heat production over 24 hours in individual rats at thermoneutrality (28°C) was best described by a two component model. The first component incorporated a fourth order polynomial to describe the response in time of heat production during low levels of activity, termed underlying thermogenesis (UT):

$$UT = \mu_0 + \mu_1 t + \mu_2 t^2 + \mu_3 t^3 + \mu_4 t^4$$

The second component incorporated a curve of ever decreasing slope, which eventually reached an asymptote (a) for large values of measured movement (m). This was termed measurement induced thermogenesis (MIT):

$$MIT = a(1 - e^{-bm})$$

where b was the rate constant. Combined, the two terms described metabolic rate over 24 hours quite adequately:

$$HP = UT + MIT + \varepsilon$$

The researchers also examined a power function for the relationship between activity and metabolic rate:

$$MIT = am^b$$

Although the fit was poorer than that for the exponential function, the authors noted that their experiment was conducted under relatively low levels of activity due to the restriction of the dimensions of their metabolic cages. At much higher levels of movement, it is possible that a different model, such as the power curve, could be more appropriate.

Brown *et al.* (1991) also highlighted the significant effect of external temperature on core body temperature, and its consequences for metabolic rate. For those animals acclimated to mild cold (21°C) over two weeks prior to measurement, a third term was required in the model to account for the effects of non-shivering thermogenesis during prolonged periods of inactivity.

In the current experiment, it was desirable to include activity directly as a covariate in the analysis of the full data set, rather than fitting non-linear functions such as those used above to individual mouse data prior to analysis and subsequently including their parameter estimates in the final model. As such, it was decided to proceed using a logarithmic function of activity within the final model. It should be noted that a power function of activity did not provide a significantly better overall fit to the data.

Some thought was given as to the basis of the logarithmic relationship between metabolic rate and activity. It was concluded that the early, substantially linear phase of the curve, where most of the measurements lay, was associated with basic aerobic metabolism. This was the domain of the indirect calorimeter for measurement of heat production. When animals carried out substantially higher levels of activity for considerable periods of time (bearing in mind that each datum is a four-minute average of half-second point-measurements), anaerobic glycolysis associated with the catabolism of body fat and residual glycogen would have had a significant impact on the energy requirements for movement. Indirect calorimetry, based as it is on the measurement of O_2 uptake and CO_2 production, cannot estimate the heat production from anaerobic metabolism, and as such the relationship between activity and metabolic rate as measured by the Waite calorimeter would have broken down. This was demonstrated by the logarithmic decline in response at higher levels of activity.

An alternative hypothesis developed to address similar observations in kangaroos (Baudinette, pers. comm.) is that the larger musculature and tendons, when hyper-extended during extreme movements (e.g. rapid hopping in kangaroos), have an inherent elasticity, such that during both elongation and contraction, there is a degree of conservation of kinetic energy which would be unaccounted for in a simple indirect calorimetric assessment of metabolic rate. However, there is evidence that this is an evolutionary consequence of the kangaroo's specific bi-pedal locomotion, and hence it is inapropriate to extrapolate such a hypothesis to mice.

Correlated responses to selection: activity

The findings from the raw data were generally well supported by the subsequent analysis. Not surprisingly, there was a substantial reduction in activity between the first and second hours of measurement, associated with acclimation to the chamber surrounds and a tendency to reduce activity after re-feeding at the end of a significant fasting period. There may also have been an influence of ambient temperature. In their normal environment, the animals were exposed to a substantially variable thermal environment. Although air temperature was maintained as accurately as possible at the mouse's thermoneutral zone ($27^{o}C$), other factors that affected the thermal environment remained variable, and as such, normal activity levels may have been elevated to maintain core body temperature. In the confines of the calorimetric chamber, many of these variables were better defined. Specifically, the requirement to remain active for maintenance of core body temperature was substantially reduced in the confines of the calorimetric chamber, leading to a progressive decline in activity over the two hour period.

Mice are primarily a nocturnal species, and activity levels are normally reduced during the daylight hours in which the measurements were conducted (Hastings *et al.*, 1997). It was thus not surprising to observe a decrease in activity from early morning to late afternoon. The interaction of time of day with metabolic status was almost certainly due to the 24 hour fasting period prior to measurement. Under fasting conditions, animals would still have been subject to the behavioural processes associated with circadian rhythms, and this was reflected in the pattern of activity observed throughout the day in the first hour of measurement. However, re-feeding following a fast would generally have overridden these behavioural controls as animals responded to the presence of food. The exact pattern of activity throughout the day in the second hour of measurement is of unknown origin.

The regression of activity on body weight was positive. There was some concern that the observed influence of weight on activity may have been due to the relationship of mass and gravity in the point measurements of weight used to assess activity: larger animals with greater mass tend to produce larger deviations in response to similar levels of activity. Conversely, this measure of activity may be better suited to adjustments of metabolic rate, as larger animals tend to exert greater levels of muscular energy to displace their mass against gravity.

Age may have been partially confounded with weight, but there was still a significant effect of age under fasted conditions, in which older animals were generally less active. Older animals may have suffered (energetically) more as a consequence of the previous 24 hour fast and hence reduced energy-burning activities. Alternatively, older animals may have been less active generally, but after re-feeding following a fast, were equally active in seeking food within the metabolic chamber as their

younger counterparts. A second alternative was that younger animals may have been more susceptible to the stress caused by an unfamiliar environment.

The effect of sex may also have been partially confounded with weight, however an interaction with metabolic status was only observed for sex. Both physiological and behavioural causes may explain the differing sex responses within fasted and fed periods. In the early period, the tendency of males to exhibit greater exploratory behaviour and hence higher levels of activity was consistent with traditional male/female behavioural characteristics. Alternatively, the larger fat depots observed in males previously (Chapters 3 and 4) may have given them a greater ability to liberate body energy stores to maintain a normal level of activity. Presumably this would also account for the results observed post-feeding: males required less intake per unit body weight to recover from fasting conditions and hence were less active during the hour following application of food.

Activity was considered one of the primary means through which selection for net feed intake may be acting, independent of those issues already outlined previously (e.g. body composition). Surprisingly, there were no specific line differences in activity overall, and particularly between the two selection lines. There were, however, specific differences between the lines following fasting, with the low line considerably more active than the high line. As the low line animals were more efficient at maintaining body weight, it could be argued that they were better able to cope with the prolonged fast and hence were able to maintain near-normal levels of activity, even after 24 hours without food. However, after feeding the line differences disappeared. It may have been expected that selection would cause the high line

animals to be more active under ideal (i.e. fed) conditions, effectively 'wasting' intake through greater spontaneous motor activity.

It must be noted that the present experiment was not designed to measure line differences in activity directly. Rather, it was set up to examine line differences in metabolic rate, and measurements of activity were of secondary importance as part of the analysis. As such, the measurement of activity was not conducted under ideal conditions, but rather under the quite extreme conditions of a lengthy fast followed by a rapid re-equilibration following feeding. Ideally, activity would have been measured on the animals under steady-state 'normal' conditions. Indeed, subsequent measurement of the lines under just such conditions by other researchers (Oddy, pers. comm.) have revealed substantial line differences in levels of spontaneous motor activity. These results were in good agreement with those observed by both Hastings *et al.* (1997) and Bünger *et al.* (1998), in which lines of mice were divergently selected for intake adjusted for body weight only at 8-10 weeks of age. The high line exhibited substantially higher levels of activity than the low line. The authors noted, however, that the contribution of activity to differences in observed fasting heat production was less than 5%, and hence it did not contribute greatly to the divergence in food intake. With respect to the relationship between net feed intake and activity, it was of equal importance to examine line responses to activity in the liberation of waste heat energy, and these are detailed in the following section.

Correlated responses to selection: metabolic rate

The raw data observations were again broadly confirmed by subsequent analysis. Comparable estimates of average heat production from the literature, standardised per

unit metabolic body weight (weight$^{0.67}$), are presented along with the current data in Table 5.5. The current data fell well within the range of published estimates.

Table 5.5. A selection of current and published estimates of average heat production in rodents.

Author(s)	Species	°C	Age (Weeks)	Type	HP ($Jg^{-0.67}min^{-1}$)
Brown *et al.* (1991)	♂ Wistar rats	28	100g	Indirect	3.37
Moody *et al.* (1997)	♂ & ♀ mice, controls	22	9-11	Direct	3.90
Moody *et al.* (1997)	♂ & ♀ mice, C57BL/6	22	9-11	Direct	3.25
Dauncey (1986)	♂ & ♀ mice, C57BL/6	28	Young	Indirect	2.27
Dauncey (1986)	♂ & ♀ mice, C57BL/6	28	Mature	Indirect	2.23
Hastings *et al.* (1997)	♂ & ♀ mice, controls	22	9-10	Direct	1.70
Bunger *et al.* (1998)	♂ & ♀ mice, controls	22	9-10	Direct	4.87
Current work	♂ & ♀ mice	28	Mature	Indirect	2.69

The effect of metabolic status on heat production was such that a positive estimate of the heat increment of feeding was established from the measures of fasted and fed metabolic rate, lending further weight to the accuracy of the calorimetric device in use. The average estimate for all animals of 2.25 J/min (Fed 26.56 – Fasted 24.31) was similar to previous estimates of the heat increment of feeding in mice (Hastings *et al.*, 1997; Moruppa, 1990) on a per unit body weight basis. Not surprisingly, there was a substantial effect of the mass of food eaten in the second hour on the rate of heat production in excess of basal metabolism (3.15 J/min/g eaten). In adult man, the resting metabolic rate may be elevated even when measured at least 14 hours after the last meal (Dauncey, 1980). Similarly, in the young pig, this rate of metabolism can

remain elevated for at least 20 hours after feeding (Dauncey and Ingram, 1979). The extent to which this elevation is due to post-absorptive, as distinct from absorptive, processes is not known. Nevertheless, the post-absorptive state is not thought to be reached until about 3-5 days in ruminants, 2 days in chickens, and 10-20 hours in small omnivorous animals (Mitchell, 1962). Assuming that a single meal elevated metabolic rate maximally over the first 10 hours post-feeding, and considering that the energetic value of the feed was approximately 17.7 kJ/g, then based on an average daily intake of 4.2 g, the heat increment of feeding accounted for approximately 11% of total digestible energy intake.

Variations in deep body temperature, metabolic rate, thermal conductance and heat loss occur cyclically over 24 hours in most species (Ingram and Mount, 1975; Ingram and Dauncey, 1985). These appear to be innate and occur independently of nutritional status and physical activity. In resting man, circadian variations of heat loss are responsible for about 75% of the range of oscillation in core temperature, while the variation in heat production contributes only about 25% (Aschoff and Heise, 1972). In the current experiment there was a significant effect of time of day on metabolic rate. Under fasting conditions metabolic rate tended to decline from morning to afternoon, as one would expect for principally nocturnal species, and supported by previous results in mice (e.g. Brown *et al.*, 1991). However, after feeding, the circadian effect all but disappeared, indicating that there was at least some influence of nutritional status on these cyclical variations.

Although partially confounded with activity, both age and weight played significant roles in defining metabolic rate. There is much evidence in man and other species that metabolic rate generally tends to decline with age (e.g. Piers *et al.*, 1998;

Roubenoff *et al.*, 2000), although there is scant evidence in the literature of the specific effects of age in mice. The effect of weight, on the other hand, has been well documented. Clearly, one would expect larger animals to have greater heat loss in absolute terms, and this is indeed the case in the current study (Table 5.4). However, it is due to the specific relationships of body weight and size with rate of heat production that many of the empirical size-scaling rules associated with 'metabolic' body weight have been developed, most famously in the definitive work of Brody (1945), "Bioenergetics and Growth". Generally, most traits associated with growth and development are not linearly related to body size, but rather follow an allometric relationship based on a power function (Reiss, 1989). This is thought to be due, at least in part, to the relationship between surface area to volume ratio and heat loss. Many studies have attempted to produce definitive evidence of the underlying power relationship, but the consensus is that it lies somewhere between 0.60 and 0.80, and is invariably species and/or trait specific. Initial examination of the relationship between basal metabolic rate and weight in the current study produced the following models:

$$\text{Basal Metabolic Rate} = 2.4(\text{Weight}^{0.69})$$

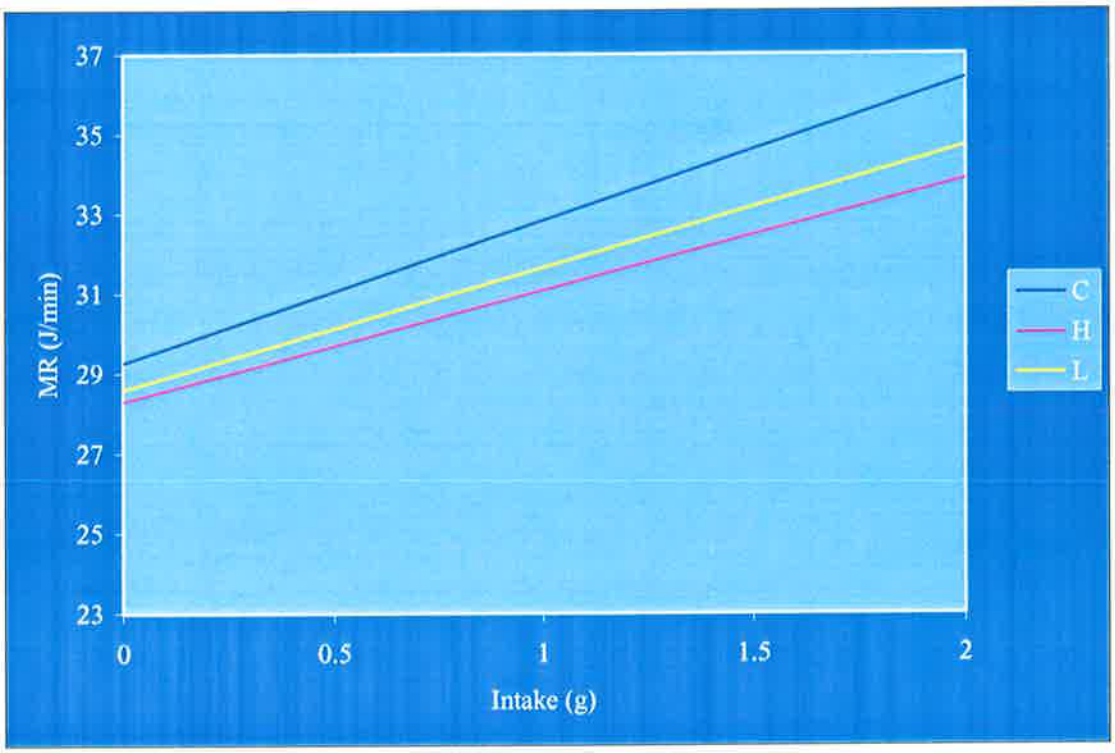
Clearly, the power function fell well within the established limits, although the level of fit was relatively poor (R^2 18%) on account of variation due to other factors. However, given that the current study examined the differences between lines that were of essentially the same size, it was decided to fit raw weight instead of metabolic body weight in the final analysis of heat production.

The rationale for fitting the natural logarithm of activity has been described earlier in this chapter. The relationship between activity and metabolic rate was moderated by

status, providing evidence that nutritional status is of importance to heat production both directly and indirectly. An important consideration in relation to activity is that the heat it produces can substitute for thermoregulatory thermogenesis (Dauncey, 1991). This may partially explain the marked difference between the fasted and fed periods: under the fasting regime, the absence of a heat increment associated with absorptive processes tended to increase an animal's reliance on activity for thermoregulation, whereas after feeding, the reliance on activity was reduced and as such, heat generation associated with activity (as well as activity in its own right) was moderated downwards. How this was achieved is difficult to establish, but was most likely related to specific types of movement undertaken before and after feeding, and specifically to the use of different types of musculature associated with different levels of efficiency (e.g. slow-twitch vs. fast-twitch fibre types).

There were no clear overall distinctions between sexes or lines for basal metabolic rate or maintenance heat production. There was also no difference between the high and low lines for the heat increment of feeding, although both were significantly lower than the control line ($p<0.05$). Line responses in metabolic rate per unit intake in the second hour were similar in both selection lines (Figure 5.6).

Figure 5.6. Line responses in metabolic rate to total intake after re-feeding.



It appears that selection for net feed intake did not alter the specific efficiencies of absorptive processes. This was at first counter-intuitive, as it was expected that more efficient animals would have a lower heat increment of feeding per unit intake, associated with more efficient absorption. However, it was important to consider the data in the context of the previous 24 hour fast. Given that all animals, even those from the low line, were assumed to have reached a basal state after 24 hours, it may have been that individuals from both the high and low lines initially overate, relative to their normal intake, as a means to counter the effects of the fast. Closer examination of the total intake in the second hour revealed no difference in the average amount eaten between the two lines. The high line animals may have used most of their energy intake for maintenance and as such, were operating at their optimal 'phenotypic' efficiency. The low line animals may have instead used a

proportion of their intake for maintenance with the rest lost as 'waste' heat to compensate for overeating, and as such were operating at a sub-optimal level of their 'phenotypic' efficiency. This illustrates the difficulties inherent in trying to establish normal differences under abnormal conditions, and it is a challenge to determine how this might best be addressed in future studies. One alternative may have been to provide food to individuals on a line specific basis (i.e. low line animals would receive an amount based on low line intake per hour). For this to be effective, greater understanding of circadian feeding patterns, and particularly line differences in these patterns, would have been required. This was beyond the scope of the current experiment. The underlying physiological basis for activity-derived thermogenesis may to have been influenced by selection for net feed intake. Line-specific results from the current study are illustrated graphically in Figures 5.7 and 5.8.

Surprisingly, the high line animals were more efficient (lower metabolic rate) at higher levels of activity during fasting than either the control or low lines. Given that the high line animals also demonstrated significantly less activity during this period, it could be argued that they were undertaking a substantially different type of activity in the first hour. This was alluded to earlier, and may have been associated with a larger effect of the lengthy fast on those animals that were less efficient generally. It also supports the observation of others (Hastings *et al.* 1997, Bünger *et al.* 1998) that fasting activity levels do not tend to be well correlated with divergence in feed intake. This is further strengthened in the context of a 24 hour energy balance. Assuming the average level of activity within the calorimetric measurements was representative of the lines in general, then the lines were almost identical in the amount of energy liberated from activity in 24 hours (~26 kJ).

Figure 5.7. Line responses in metabolic rate to activity whilst fasted.

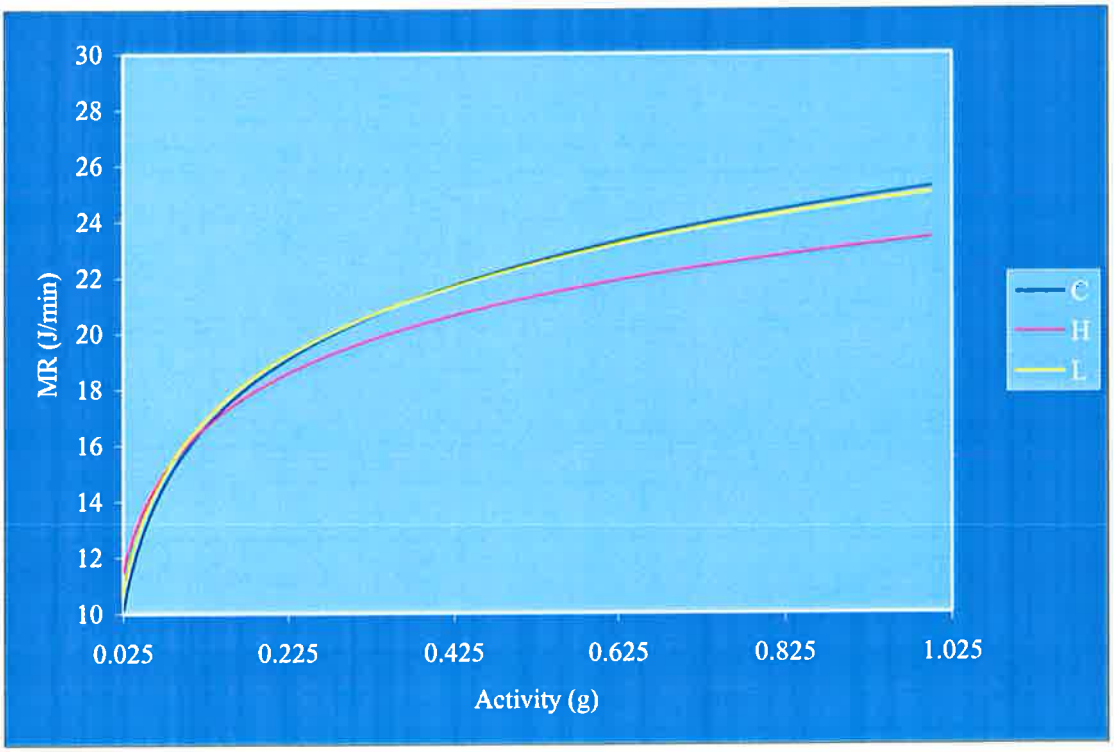
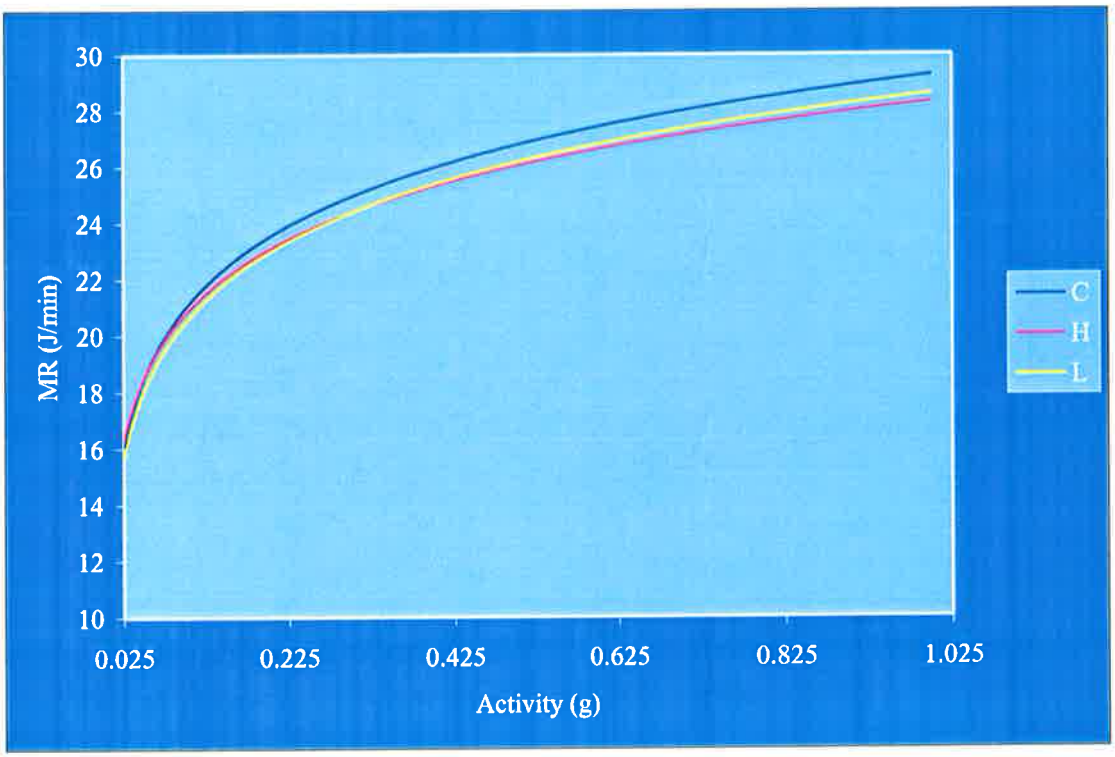


Figure 5.8. Line responses in metabolic rate to activity after re-feeding.



After feeding, line differences in metabolic response to activity disappeared. This was similar to observations made for activity generally, and indicated that selection for net feed intake did not alter the relationship between activity and metabolic rate under maintenance conditions. When average line activity level (Table 5.2) was taken into account, the high line used a lower proportion (28%) of its daily maintenance energy for activity than either the low (36%) or control (35%) lines.

The energy balance revisited

Significant questions were raised by the line-specific mass-energy conversions outlined in Chapters 3 and 4, many of which were addressed by the calorimetric work. It is pertinent to re-illustrate the conversions, including the metabolic rate data. Given most animals measured in the calorimeter were at least 11 weeks of age, it was considered inappropriate to incorporate the data with that of intake during the post-weaning test, and instead it has been applied to those diagrams outlined in the chapter on mature response, which are re-presented in Figures 5.9-5.11. These diagrams required the same assumptions as those in Chapters 3 and 4. Additional assumptions were also made:

1. The mean line activity in the calorimetry experiment was representative of line activity generally. The minimum activity level was 0.001 g.

2. Daily maintenance heat production, basal metabolic rate and the heat increment of feeding were estimated from the line-specific daily intakes and body weights presented in Chapter 4.

To illustrate, an example calculation is presented for the control line (NB MS = metabolic status, i.e. fed vs. fasted):

Body weight = 33.2 g
Daily feed intake = 4.5 g
Mean activity = Intercept + Weight + Line
= -0.016 + 0.002 x 33.2 + 0.010
[Appendix 3, Table A3.1]
= 0.062 g

Daily basal heat production (minimal activity)
= ((Intercept + MS + Weight + ln(Activity) + Line + MS x Intake + MS x Weight + MS x ln(Activity) + MS x Line + ln(Activity) x Line + MS x Intake x Line + MS x ln(Activity) x Line) x 60 x 24) / 1000

= ((28.59 – 3.59 + 0.29 x 33.2 + 3.51 x ln(0.001) + 0.67 – 0.33 x (4.5 / 24) + 0.16 x 33.2 + 0.38 x ln(0.001) – 0.46 + 0.07 x ln(0.001) + 0.86 x (4.5 / 24) + 0.14 x ln(0.001)) x 60 x 24) / 1000
[Appendix 3, Table A3.2]

= 17.12 kJ

Daily maintenance heat production (minimal activity)
= ((Intercept + MS + Weight + ln(Activity) + Line + MS x Intake + MS x Weight + MS x ln(Activity) + MS x Line + ln(Activity) x Line + MS x Intake x Line + MS x ln(Activity) x Line) x 60 x 24) / 1000

= ((28.59 – 0.00 + 0.29 x 33.2 + 3.51 x ln(0.001) + 0.67 + 3.05 x (4.5 / 24) + 0.00 x 33.2 + 0.00 x ln(0.001) – 0.00 + 0.07 x ln(0.001) + 0.52 x (4.5 / 24) + 0.00 x ln(0.001)) x 60 x 24) / 1000
[Appendix 3, Table A3.2]

= 21.35 kJ

Daily maintenance heat production (average activity)
= ((Intercept + MS + Weight + ln(Activity) + Line + MS x Intake + MS x Weight + MS x ln(Activity) + MS x Line + ln(Activity) x Line + MS x Intake x Line + MS x ln(Activity) x Line) x 60 x 24) / 1000

= ((28.59 – 0.00 + 0.29 x 33.2 + 3.51 x ln(0.062) + 0.67 + 3.05 x (4.5 / 24) + 0.00 x 33.2 + 0.00 x ln(0.062) – 0.00 + 0.07 x ln(0.062) + 0.52 x (4.5 / 24) + 0.00 x ln(0.062)) x 60 x 24) / 1000
[Appendix 3, Table A3.2]

= 42.63 kJ

Daily heat increment of feeding
= 21.34 – 17.12
= 4.23 kJ

Daily activity
= 42.63 – 21.35
= 21.28 kJ

Figure 5.9. Control line mass-energy balance.

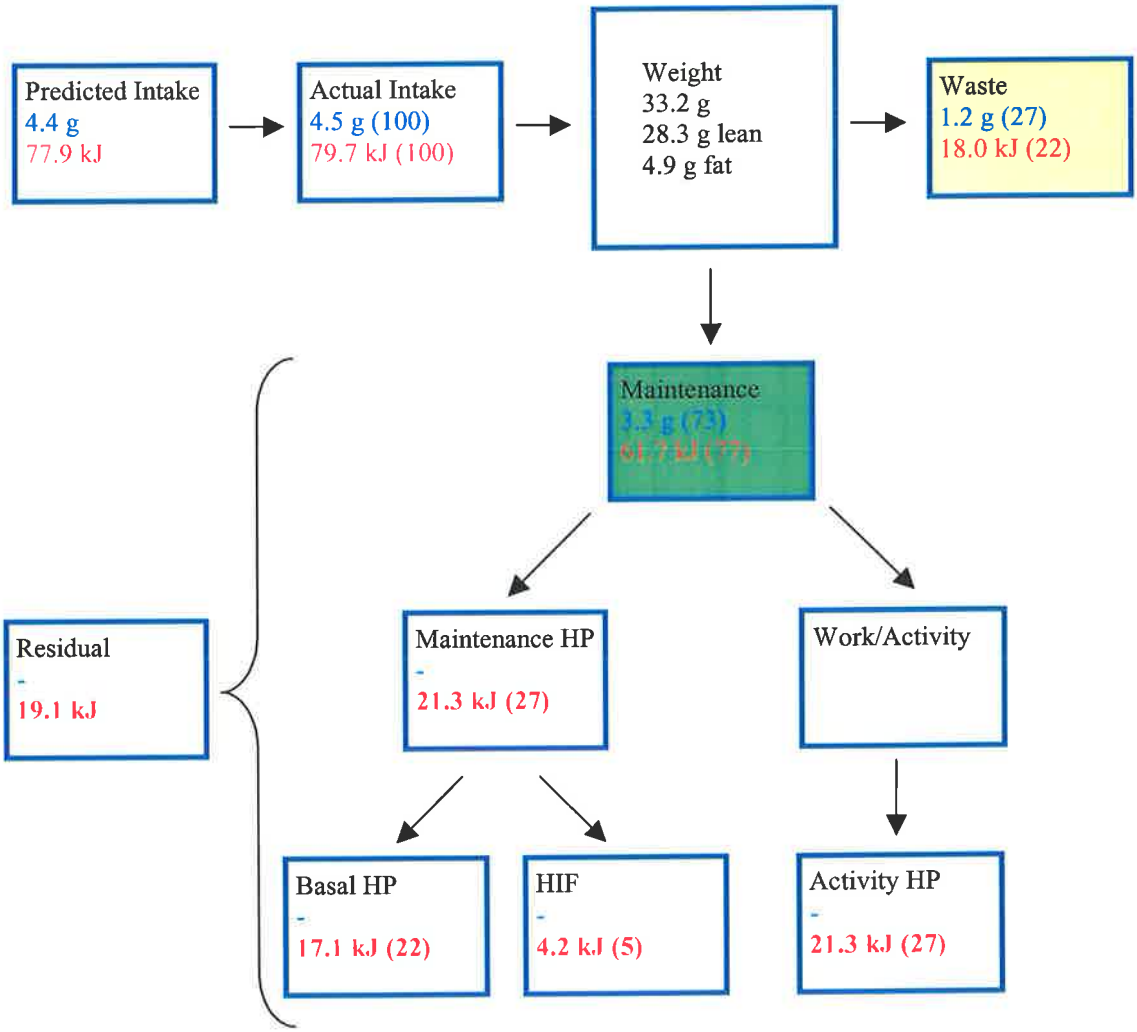


Figure 5.10. High line mass-energy balance.

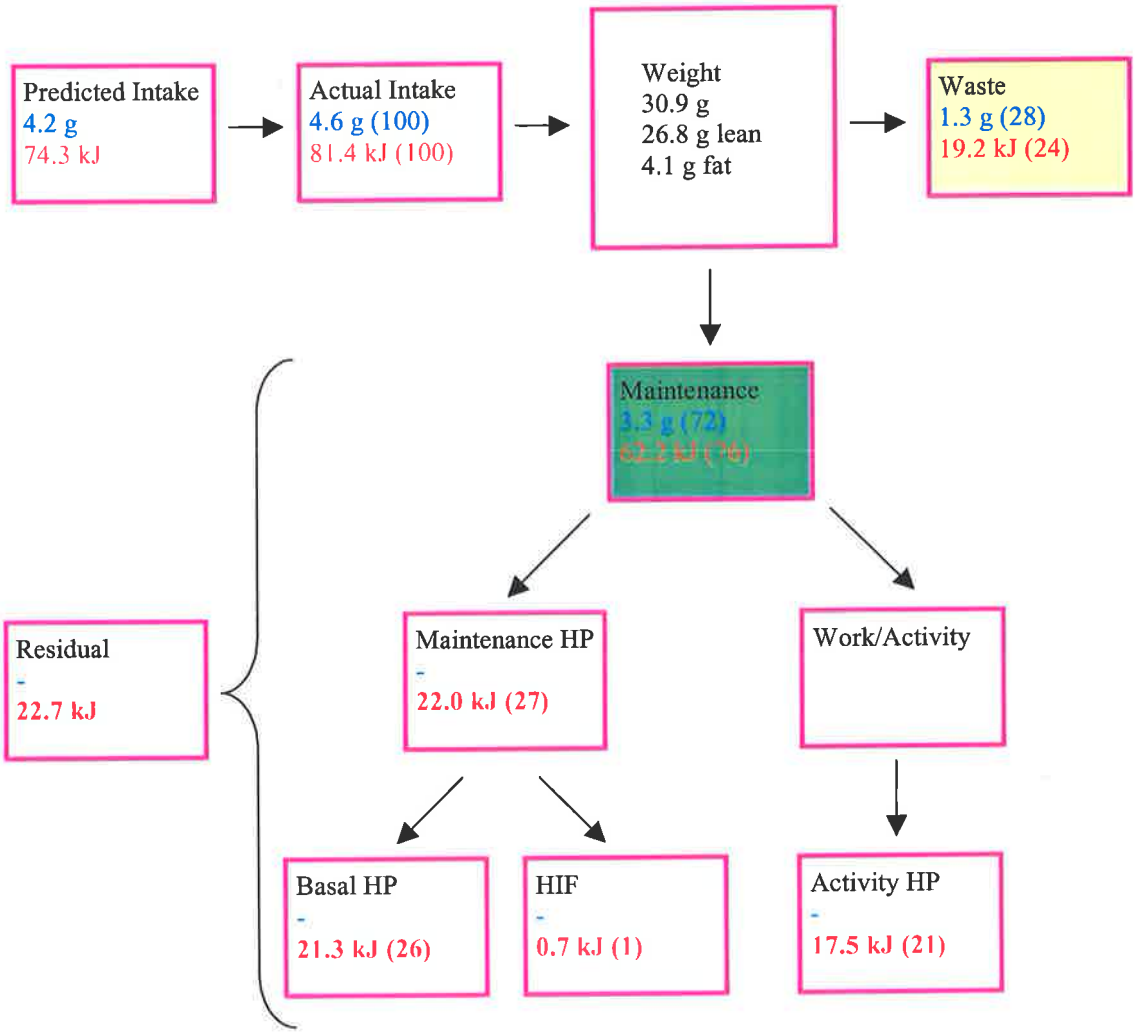
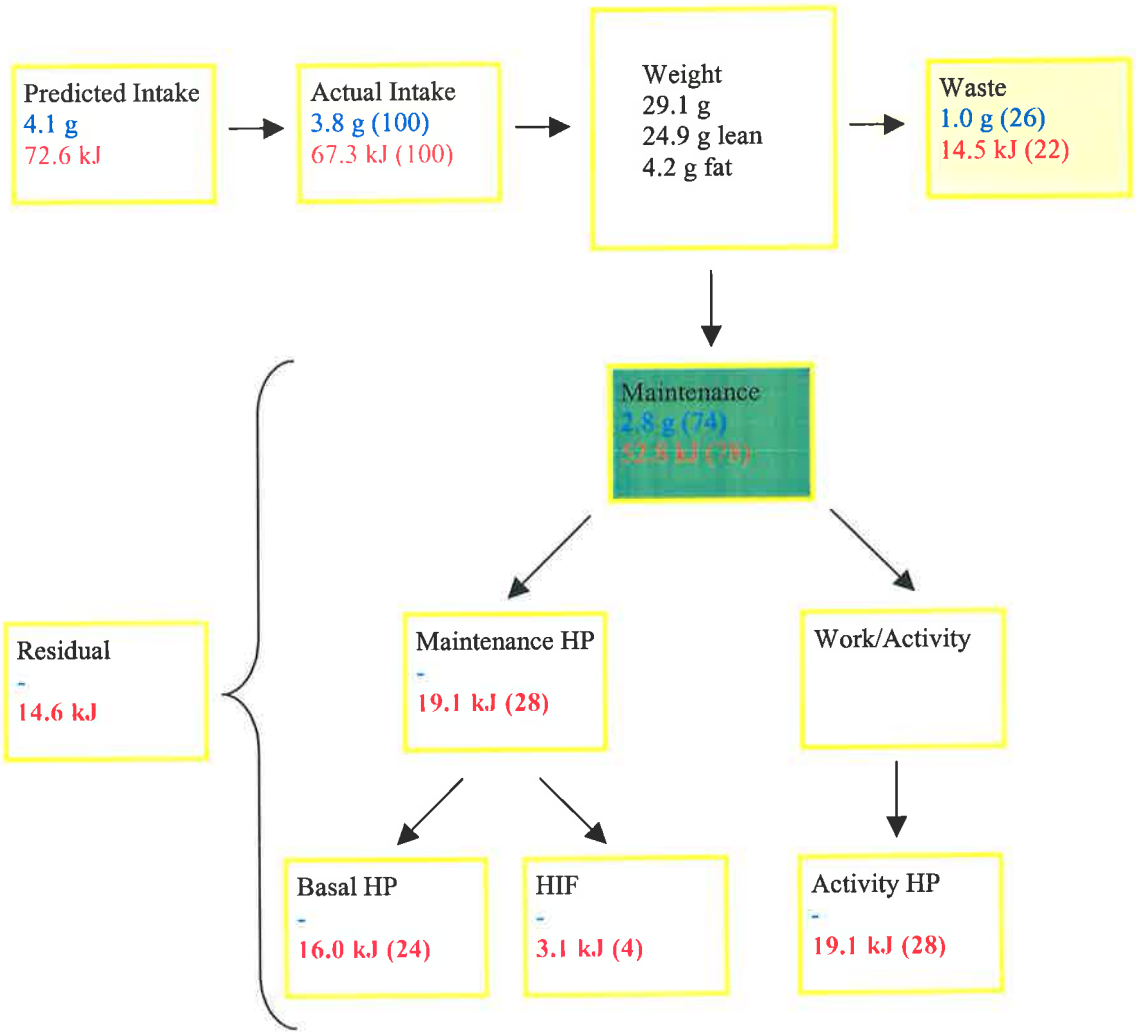


Figure 5.11. Low line mass energy balance.



The diagrams can be used to outline broad differences between the selection lines in their energetic efficiencies of metabolism. What is most evident is that there was still a significant residual energy sink in both lines that was unaccounted for by either faecal waste or the heat by-products of metabolism and activity. Clearly, in a measurement system such as this, there are many areas in which systematic error can lead to substantial discrepancies between different experimental 'compartments'. Nevertheless, on the whole the data sets were quite consistent. Whilst it was not possible to quantify every aspect of the energy balance, other factors that may have contributed to the observed residual include:

1. The energy associated with the transformation of kinetic energy (movement) to useful work. This is independent of the heat produced by the musculature in carrying out the activity.

2. The energy associated with the nitrogenous end-products of protein metabolism.

3. Food wastage from the measurement hoppers.

It is interesting to note that the high line had a significantly higher (55%) residual than the low line, both in terms of absolute energy, and in the relative contribution to overall intake. In absolute terms, the line difference in the residual component (8.1 kJ) was approximately 60% of the line difference in intake, energetically. This suggests that factors other than metabolic rate were more important in determining line differences in intake. Subsequent work on the selection lines by others (Oddy, *pers. comm.*; Fenton, *pers. comm.*) has indicated that the observed residual values could be attributed to large correlated responses in 24-hour activity levels, and particularly nocturnal activity. This again highlights the difficulty in extrapolating the

current activity data – the data itself was ideal for adjusting metabolic rate to a baseline, but was inappropriate for making broad conclusions about line differences in activity itself. Mousel *et al.* (2001) have detailed the difficulties associated with measuring daytime activity levels in a primarily nocturnal species.

Of the accurately observed variables in the current study, it would appear that the line differences were primarily a function of differences in appetite and/or rates of gut passage rather than gross digestibility, with little if any significant changes in the efficiency of post-absorptive processes or the relationship between activity and energy expenditure. The small difference in the heat increment of feeding in favour of the high line supported this observation, possibly indicating that selection for net feed intake was acting directly on gut efficiency (i.e. the low line had a higher heat increment due to catabolising more nutrients per unit intake). If so there may be also have been line differences in organ weights, which will be examined in a later chapter. However, there appeared to be no concommitant increase in basal metabolism to maintain larger organs. Despite the large residual mass and energy values, the relationship between the heat increment of feeding and basal metabolism appeared sufficiently flexible that selective improvements in overall efficiency were still possible. Direct selection on an index of these two parameters may prove a useful means of improving selection response.

Conclusions

Selection for post-weaning net feed intake has resulted in a small but significant effect on a number of metabolic parameters, most notably the heat increment of feeding, but also the relationship between activity and metabolic rate. There was some evidence, too, of line differences in overall activity levels, although these require further

examination. Whilst none of these changes could be termed definitive, it was abundantly clear that there was substantial genetic variation in the relationships of the various parameters. More specific selection criteria targeting these relationships may offer a more effective means of improving overall efficiency, although the increased costs associated with measurement of these parameters may prove to be prohibitive in the real-world environment of the animal production system.

Chapter 6.

Correlated responses in visceral organ size.

Introduction

Selection for post-weaning net feed intake has produced substantial correlated responses in a range of intake and growth traits, both at the age of selection (Chapter 3), and at maturity (Chapter 4). It also resulted in small, but significant, correlated responses in a range of metabolic parameters (Chapter 5), the heat increment of feeding and basal metabolism being but two. Whilst measures of heat production can give an overall estimate of the metabolic efficiency of an individual, it was also considered pertinent to examine some of the more discrete physiological responses to selection. Whilst it was not possible to conduct a comprehensive study of the specific biochemistries unique to each line, it has been well documented (Brody, 1945; Koong *et al.*, 1985; Burrin *et al.*, 1990; Perry *et al.*, 1997; Nyachoti *et al.*, 2000) that organ size itself may also play an important role in determining the relative efficiencies of specific biological processes. This is often a function of the allometric scaling of organ size with body size, and is important to account for when studying the relationships between growth, intake, maintenance and metabolism (Brody, 1945; Reiss, 1989).

Several studies have indicated that a decreased plane of nutrition results in a decrease in metabolic rate (Marston, 1948; Ledger and Sayers, 1977; Gray and McCracken, 1979). In a series of experiments with pigs, rats and sheep, a decreased plane of nutrition consistently resulted in decreased relative sizes of visceral organs, such as liver, kidney, stomach and intestines (Koong, *et al.*, 1982; Ferrell and Koong, 1985; Ferrell *et al.*, 1986). Regression analysis of data from these studies indicates that a

good relationship exists between weights of liver and gut tissues and estimates of maintenance energy requirements. Furthermore, the observed differences in maintenance energy requirements between breeds of animal and stage or level of production has also been attributed to the relative weights of visceral organs (Smith and Baldwin, 1974; Jenkins and Ferrell, 1983; Jenkins *et al.*, 1986).

Although the visceral organs represent approximately 6-10% of body-weight, estimates indicate that visceral tissues account for 40-50% of whole-body cardiac output, protein synthesis and heat production (Davis *et al.*, 1981; Webster, 1981). Metabolic activity of an organ is the product of organ size and metabolic activity per unit tissue. Conceivably, changes in organ size alone can account for observed differences in whole-body metabolism (Smith and Baldwin, 1974; Canas *et al.*, 1982); however, changes in total organ metabolic activity could result from differences in metabolic activity per unit tissue as well.

The estimation of the efficiency of growth, as well as of fat and protein deposition, has generally been based on the partition of energy intake into portions used for maintenance and growth. With this type of partition, estimation of efficiency of energy utilization for growth must be based on an accurate estimate of the energy requirement of maintenance. The maintenance requirement is generally expressed in relation to metabolic body size ($weight^{0.75}$; Kleiber, 1961). However, metabolic body size refers, in its original conception, only to comparisons among mature animals of different species. In recent years, energy requirement for maintenance when expressed on the basis of metabolic body size has been observed to differ due to age, sex, or different physiological stages or status. For example, estimates of maintenance energy requirements in growing pigs range from 100 to 200 $kcal/Wt^{0.75}$

(Kielanowski and Kotarkinska, 1970). Maintenance requirement is directly related to fasting heat production. Based on data on blood flow rate and O_2 consumption, Webster (1981) estimated that heat production from the liver, gut, skin and kidneys accounted for 45% of total heat production of rats at rest. Most of this heat is associated with protein synthesis, and the greatest rates of synthesis occur in tissues such as the liver and gut rather than in the muscle. In fact, almost half of the total protein synthesis occurred in these two tissues in lean Zucker rats at 200-350 g body weight (Webster, 1980).

To enhance and extend the information derived from both the EM-Scan and the indirect calorimeter, it was decided to undertake a series of dissections on individuals from generations 10 and 11 to compare visceral organ sizes between the selection lines.

Materials and Methods

Animals

Measurements were conducted on generations 10 and 11 (7 and 8 for the control line), which were deemed to have diverged significantly for the trait undergoing selection. 176 individuals were sampled from the population after calorimetric analysis, and excluding those individuals that had been selected as parents to produce the next generation of progeny. As animals were first required to undergo a three week intake test for future selection, coupled with a limited throughput of animals per day in the calorimeter, age at measurement was not truly post-weaning, and showed a broad range. Age was instead fitted as a covariate in subsequent analyses to examine age related differences in organ size response to selection for post-weaning net feed intake

Animals were fasted for 24 hours prior to euthanasia. All selected animals were killed by cervical dislocation. Viscera were extracted and separated into the principle components of heart, liver, stomach, caecum and intestinal tract. Any peritoneal fat and/or gut fill was removed, and the samples were snap frozen and then freeze dried overnight. Dry weights were then recorded for all samples. The samples remained in storage at –80°C for further analysis.

Analyses

A series of linear models (PROC GLM, SAS 1989) were used to analyse organ weights. A general model was first fitted to all organs. A number of main effects and interactions did not account for a significant proportion of the variance for any trait and were subsequently excluded from the model. The final model included:

parity (PAR 1, 2, 3)

age at measurement (AGE 119-308 days)

litter size (LIT 3-16 pups)

sex (SEX M, F)

line (LIN C, H, L)

sex by line

inbreeding coefficient (INC 0-0.37) by line

A summary of the numbers of mice measured for each organ is presented in Appendix 1, Table A1.3.

Results

Correlated responses to selection for post-weaning net feed intake in organ size were examined for generations 10 and 11 (7 and 8 for the control line), and results from linear models are presented for type III sums of squares in Table 6.1.

Table 6.1. Source mean squares from analysis of organ variance.

Source[a]	Heart	Liver	Caecum	Stomach	Intestine
R^2 (%)	53	50	26	51	41
CV (%)	13	19	23	12	18
Error DF	112	103	102	106	90
Error MS	0.26	70.98	0.31	0.32	31.19
PAR	0.00	331.41*	0.02	1.29*	28.75
AGE	4.91**	1720.42**	1.06	14.72**	283.82**
LIT	0.16	754.79**	1.87*	2.43**	142.98*
SEX	15.00**	2699.57**	3.03**	0.83	318.42**
LIN	1.24*	11.17	0.63	1.24*	149.86*
SEXxLIN	0.93*	202.44	0.38	0.30	55.49
INCxLIN	1.05**	3.48	0.68	2.00**	155.27**

[a] All source and error mean squares reported $x10^{-4}$
* $p<0.05$
** $p<0.01$

Litter parity effects

Parity accounted for a significant proportion of the variation in the liver and the stomach. Animals from second and third parity litters had 17% larger livers than animals from first parity litters. Animals from third parity litters had 10% larger stomachs than animals from first and second parity litters.

Inbreeding effects

The main effect of inbreeding was not included in the model. However, there were a number of significant interactions with line, which are outlined in a later section on line effects.

Age effects

Organ size tended to increase with age in all cases except the caecum, where the effect of age was insignificant. The regression coefficients in mg/week were as follows: heart 0.3±0.1; liver 5.3±1.1; stomach 0.5±0.1; intestine 2.4±0.8.

Litter size effects

Litter size accounted for a substantial proportion of the variation for all organs measured except the heart. In all cases where the effect was significant, mice from larger litters had smaller internal organs. The regression coefficients in mg/animal were as follows: liver –14.2±4.3; caecum -0.7±0.3; stomach -0.8±0.3; intestine -6.6±3.1.

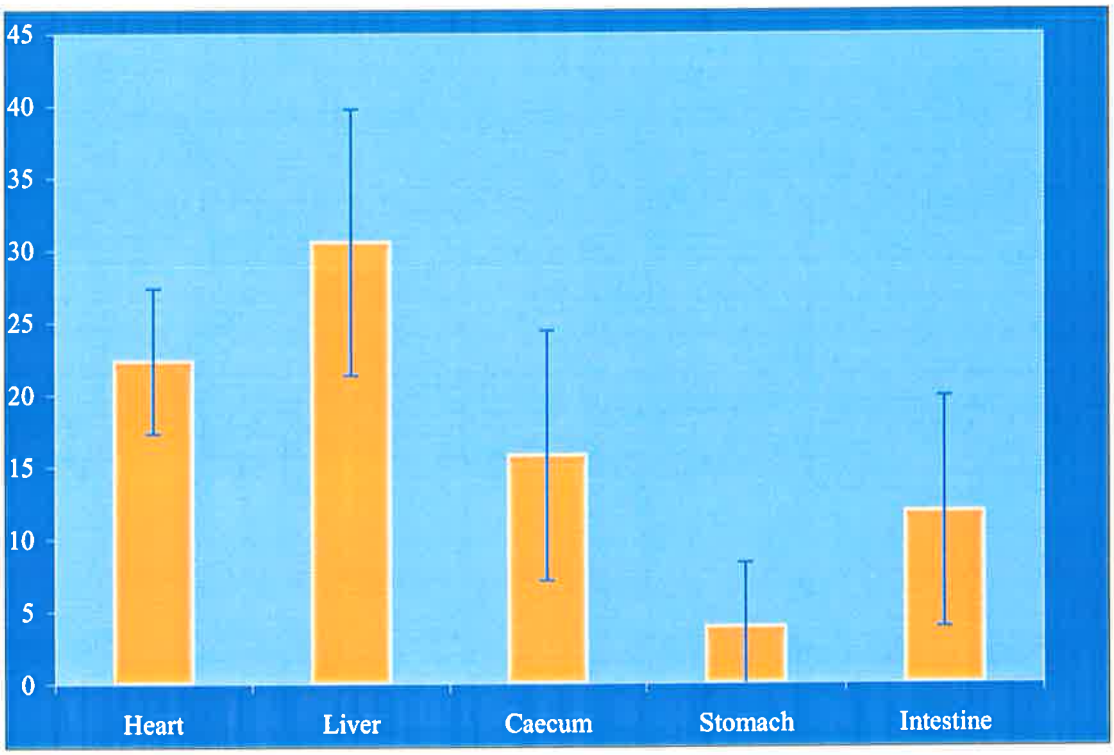
Sex Effects

There was distinct heterogeneity between sexes for all organs except the stomach (Table 6.2, Figure 6.1). Male organ weights were consistently heavier than female organ weights (16% on average), although the effect was moderated by line in the case of the heart: males had 26% bigger hearts in the control and high lines, but were only 13% bigger in the low line.

Table 6.2. Least squares means for males and females for organ weight in milligrams dry matter.

	Heart	Liver	Caecum	Stomach	Intestine
♂	45.7	502.5	29.1	54.0	400.1
SE	1.4	28.5	1.6	1.6	20.5
♀	37.4	384.8	25.1	51.9	357.6
SE	1.5	29.4	1.7	1.8	22.4

Figure 6.1. Percentage deviation of males from females for organ size (±SE).



Line Effects

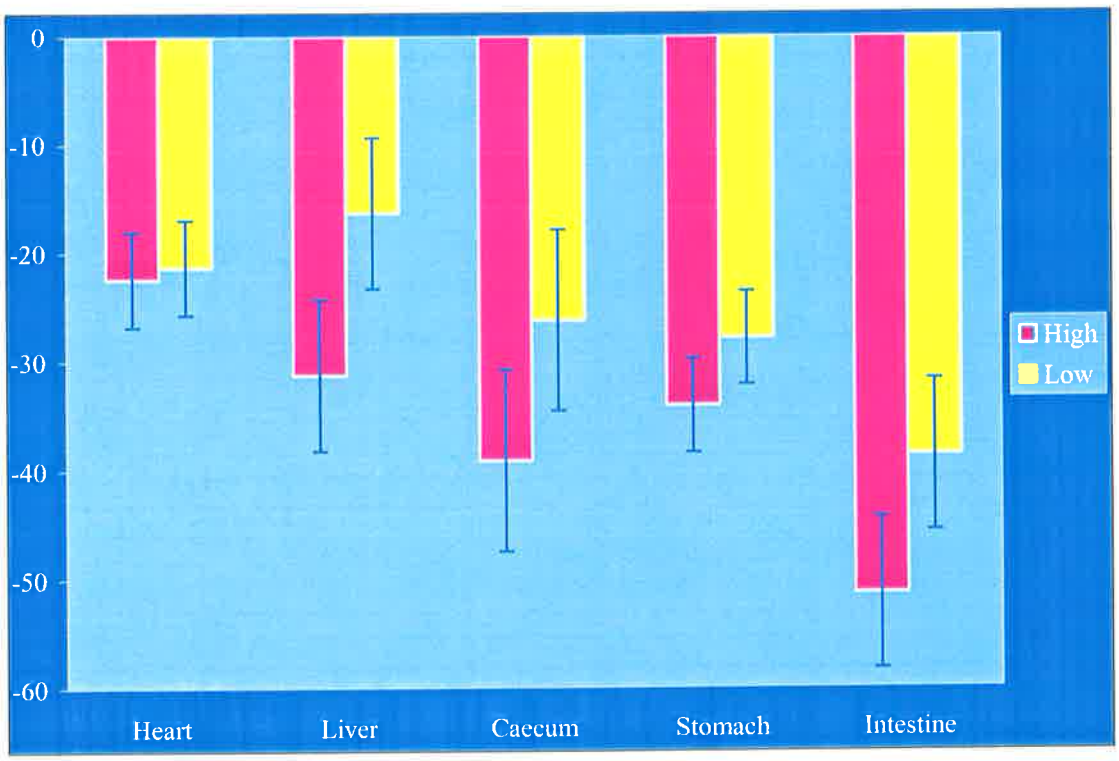
The main effect of line was significant for the weights of the heart, stomach and intestine, and least squares means for lines are presented in Table 6.3. Discrete differences between the high and low lines are also presented graphically in Figure 6.2 as percentage deviations from the control line. It should be noted that the pooled

standard errors in Figure 6.2 are inflated by the significantly larger standard errors of the control line, which was under-represented in the sample. For pairwise comparisons between the high and low selection lines, it is more appropriate to examine the standard errors from table 6.3.

Table 6.3. Least squares means for selection and control lines for organ weight in milligrams dry matter.

	Heart	Liver	Caecum	Stomach	Intestine
Control	48.7	527.3	34.6	66.7	541.0
SE	3.8	76.4	4.3	4.1	57.2
High	37.8	362.5	21.1	44.0	263.4
SE	1.0	17.4	1.2	1.2	13.6
Low	38.3	441.2	25.6	48.2	332.1
SE	1.3	21.9	1.6	1.6	15.6

Figure 6.2. Percentage deviation of high and low net feed intake selection lines from the control line for organ size (±SE).



The control line had hearts that were 28% heavier than the selection lines, however both sex and inbreeding moderated the effect of line. In females, line rankings were C=L, L=H, C>H whereas in males the line rankings were C>H>L. Inbreeding was associated with an increase in heart weights in the control line (regression coefficient 94±34 mg.unit^{-1}).

Although there was no main effect of line on liver weight, pair-wise comparisons indicated that the control and low lines had livers that were 45% and 22% bigger respectively than the high line. The difference between the low and control lines was not significant. A similar result was observed for the caecum. Caeca from the control and low lines were 64% and 21% heavier respectively than the high lines (Table 6.3)

Control line animals had 39% heavier stomachs than the low line, which were in turn 9% heavier than the high line. Inbreeding moderated the effect of line: stomach weights tended to increase with inbreeding in the control (regression coefficient 124±36 mg.unit^{-1}) and high (regression coefficient 89±37 mg.unit^{-1}) lines. The low line was unaffected by the level of inbreeding.

Similar line rankings were observed for intestinal weights: control line animals had 63% heavier intestines than the low line, which were in turn 26% heavier than the high line. The line effect was again moderated by the level of inbreeding: intestinal weights tended to increase with inbreeding in the control line (regression coefficient 1783±504 mg.unit^{-1}).

Discussion

The weights of many organs scale both intraspecifically and interspecifically on body weight with exponents of close to one (Reiss, 1989). However, although the concept

of metabolic body size (weight$^{0.75}$) has gained widespread use in the field of energy metabolism, and was even used in the current experiment to adjust measures of intake, its application to the growing animal has been questioned (Reiss, 1989). Fasting heat production, or maintenance, rather than being a constant function of body size, has been shown to vary because of breed, sex, condition, physiological state, production level, nutrition level and environmental conditions. It is little wonder then, that selection for a trait such as net feed intake may have substantial effects on organ size.

The sexual dimorphism observed for most organs was generally consistent with that observed for mature body weight (Chapter 4), provided a linear relationship between organ weight and body weight was assumed. However, if organs truly scale to some power of body weight (e.g weight$^{0.75}$), then the differences observed were generally higher than expected.

Although the scaling effects of body weight also complicated comparisons of organ weights between the two selection lines and the significantly larger control line, comparisons between the high and low lines specifically were considerably more robust due to their similar mature weights. In the current experiment, it would appear that a decrease in net feed intake was generally associated with an increase in the size of metabolically active visceral organs such as the liver and gut. Indeed, when the sum of organ weights was analysed, the low line was on average 20% heavier than the high line. How might this difference be associated with the observed divergence in efficiency between lines? With specific reference to gut weights (i.e. caecum, stomach and intestines), and assuming no line differences in metabolic activity per unit organ weight, then an increase in gut size may have increased the efficiency of absorption by increasing the available surface area. However, this was not supported

by line differences in gross efficiency (Chapter 4). Alternatively, larger organs such as the liver and the stomach may have been more efficient at extracting metabolically useful energy from a given weight of food. Unfortunately, the results from the preceding chapters are equivocal in this regard: the high line tended to retain more energy on an absolute basis, with the lines identical on an energy per unit mass basis (Chapters 3, 4).

Although visceral organs represent only about 10% of body weight, they are reported to account for a large proportion of total body expenditure in farm animals (Gill *et al.*, 1989; Yen *et al.*, 1989; Huntington, 1990; Yen, 1997). For instance, Yen (1997) estimated the contribution of portal vein-drained organs and the liver to whole-animal oxygen consumption to be about 25% and 20% respectively in the growing pig. Furthermore, there is a great deal of evidence in the literature emphasizing the strong relationship between organ size and fasting heat production. Much of this evidence has been derived from studies examining the relationship between nutritional status and organ size. Koong *et al.* (1985) demonstrated that previous level of nutrition and its associated change in average daily gain had a significant effect on maintenance requirement, fasting heat production and weights of metabolically active organs. They further demonstrated that changes in weight of metabolically active organs was responsible for a substantial portion of the differences observed in fasting heat production.

In the current experiment, the dry-weight of visceral organs measured accounted for only 2% and 3% of body weight in the high and low lines respectively. However, they were assumed to have a substantial affect on fasting heat production, and this was substantiated in the results presented in the previous chapter, where the low line

had a higher basal metabolic rate than the high line. Two processes, namely Na^+/K^+ pumping and protein turnover, are known to be responsible for the high energy expenditure by gut tissues and the liver (Lobley, 1988; Kelly and McBride, 1989; McBride and Kelly, 1990), and these specific processes warrant further investigation in the selection lines. Furthermore, dry tissue samples were initially retained for subsequent analysis of chemical composition but this did not prove possible due to financial constraints – the samples remain available.

Potentially of greater interest was a possible relationship between the heat increment of feeding and organ size. The heat increment is assumed related to the specific metabolic output of the those organs associated with absorption and utilisation of foodstuffs. It would appear that the greater organ mass of the low line was in fact more efficient at these underlying processes, as the low line had a lower heat increment of feeding in absolute terms. Selection appears to have acted on the intrinsic efficiency of gut metabolism in this instance.

There is little doubt that in real terms, the response to selection probably affected both the rate of absorption and the efficiency of nutrient utilisation. To strengthen the study, it may have been useful to examine specific rates of metabolism in individual organs, but again the restrictions of time and economics did not permit such an experiment.

Conclusion

There were clear responses in organ weight to selection for post-weaning net feed intake. More efficient animals tended to have larger viscera, and this appeared to be associated with an increase in both the rate and quality of nutrient uptake from the gut, although results were far from definitive. Based on the results from calorimetric

studies, it would appear that the larger organs of the low line were themselves more efficient at carrying out absorption and utilisation on a per unit organ basis, but were still responsible for a significant increase in basal metabolic rate due to their associated energetic costs.

There is some concern that selecting for net feed intake will tend to increase visceral organ mass at the expense of economically important tissues, and this may be of particular importance in larger species. However, it would appear that, at least in the mouse, the relatively large contribution of the viscera to total body metabolism was such that the effect of selection on the ratio of viscera to liveweight was negligible (i.e. large changes in metabolism were effected through small changes in organ size).

Chapter 7.

Correlated responses in reproductive rate.

Introduction

Reproductive performance is an important determinant of profitability in many animal production systems, so its genetic determination and interrelationships with other major traits, namely growth rate, body composition and food intake are important to the animal breeder.

The mouse has been used extensively as a model to help understand the basic genetic and physiological mechanisms involved in traits of importance in larger mammalian species. Reproductive performance has been investigated in outbred populations of mice either by studying lines selected for litter size, or its components, ovulation rate and embryonic survival, or by studying it as a correlated trait to selection for other traits. In almost all published reports of reproductive performance as a correlated trait in mice, selection has been practiced for body weight or growth rate (Roberts, 1965; Roberts, 1979; McCarthy, 1982). In these published studies, litter size has been used as a measure of reproductive performance, and has usually changed in the direction of selection (MacArthur, 1949; Falconer, 1953; Rhanefeld *et al.*, 1966), but not in all cases (Bradford, 1971). Changes in ovulation rate in the same direction as changes in body weight have been shown to be the primary reason for associated responses in litter size (MacArthur, 1944; Fowler and Edwards, 1960; Land, 1970), although the biological mechanisms involved in these relationships are not yet fully understood.

The current study examined the correlated response in litter size, as an indicator for reproductive performance, to selection for post-weaning net feed intake.

Materials & Methods

Analysis

Litter size data for generations 10 and 11 was used in the analysis (196 litters from 32 sires and 80 dams). Litter size was analysed using a sire-dam model (PROC MIXED, SAS, 1989) fitting litter parity, line and parity by line as fixed effects, with the litter's sire id and dam id fitted as a random effects nested within line. Effectively, the analysis examined the reproductive performance of sires and dams from generations 9 and 10. The average ages of sires and dams were: 16 weeks at parity 1, 28.5 weeks at parity 2, and 42 weeks at parity 3. A summary of the numbers of litters recorded is presented in Appendix 1, Table A1.4.

Results

Correlated responses to selection for post-weaning net feed intake in litter size were examined for generations 10 and 11 (7 and 8 for the control line). The variance of sire nested within line converged to 0, that of dam nested within line was 1.12, and the residual variance was 4.54. Type III tests of fixed effects in the final model are presented in Table 7.1, and least squares means are presented in Table 7.2. The least squares means for parity by line are also presented graphically in Figure 7.1.

Table 7.1. Type III tests of fixed effects for litter size data.

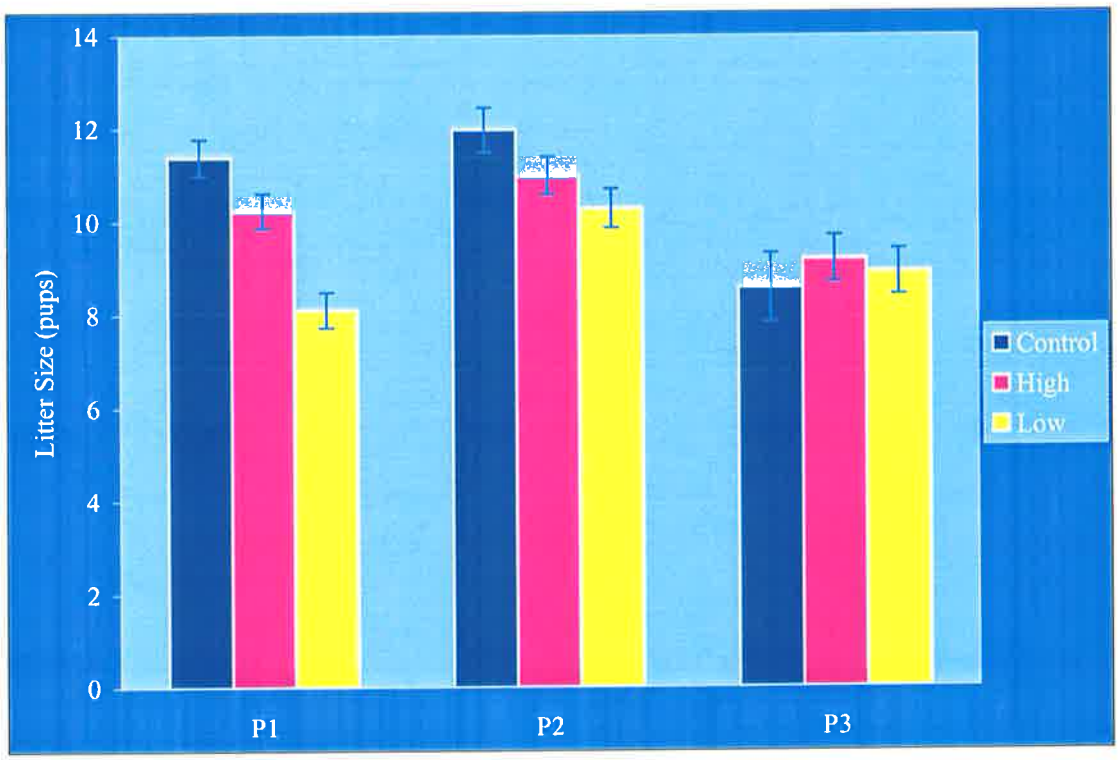
Effect	Numerator DF	F Value	F Prob.
Parity	2	16.69	**
Line	2	6.54	**
Parity x Line	4	3.93	**

** Pr < 0.01

Table 7.2. Least squares means from the analysis of litter size data (pups).

Effect			LS Mean	SE
Parity	1		9.9	0.2
	2		11.1	0.3
	3		8.9	0.3
Line	Control		10.6	0.4
	High		10.1	0.3
	Low		9.1	0.3
Parity x Line	1	Control	11.4	0.4
	2	Control	12.0	0.4
	3	Control	8.6	0.4
	1	High	10.2	0.5
	2	High	11.0	0.4
	3	High	9.2	0.4
	1	Low	8.1	0.7
	2	Low	10.3	0.5
	3	Low	8.9	0.5

Figure 7.1. Interaction between parity and line for litter size (±SE).



Overall, there was a significant effect of parity on litter size, with second parity litters being 12% and 24% bigger than first and third parity litters respectively. Line was also significant. Control and high line animals had similar litter sizes, and had 17% and 11% bigger litters respectively than low line animals. The main effects were moderated by an interaction between parity and line. With respect to parity, line rankings were C>H>L for first parity litters, C=H, C>L, H=L for second parity litters, and C=H=L for third parity litters. With respect to line, parity rankings were 1=2>3 for the control line, 2=1, 2>3, 1=3 for the high line, and 2>1=3 in the low line.

Discussion

For the past four decades there has been substantial interest in the litter size of multiparous species, particularly for those species of economic importance to the livestock industry. There is a wealth of data on a host of variables that influence litter size, and there have been many successful selection experiments using mice as a model. Realised litter size at birth depends on several components. Litter size has been successfully increased through direct selection on increased number born (Falconer and Roberts, 1960; Joakimsen and Baker, 1977; Bakker *et al.*, 1978; Eisen, 1978; Kirby and Nielsen, 1993) or on its components: ovulation rate (Land and Falconer; 1969), prenatal survival (Bradford, 1969, 1979), uterine capacity (Kirby and Nielsen, 1993), and an index of components (Kirby and Nielsen, 1993).

Response to selection for increased litter size is realised in part through an increase in number of ova shed (Falconer and Roberts, 1960; Joakimsen and Baker, 1977; Bakker *et al.*, 1978). Bakker *et al.* (1978) also observed an improvement in survival before and after implantation. Direct selection for ovulation rate usually has increased number of ova shed, but litter size does not increase proportionally and has not always

increased (Riberio *et al.*, 1996). This is due to a concomitant increase in prenatal mortality (Bradford, 1969; Land and Falconer, 1969). This may result from uterine capacity not increasing along with ovulation rate, and hence a lower proportion of embryos survive (Riberio *et al.*, 1996).

It must be emphasised that first parity litter size is only one component of lifetime reproductive performance. Until recently, there has been little published information on the correlated responses of litter size in subsequent parities and on other components such as total number of parities and post-natal maternal performance, and also on the related genetic and phenotypic parameters. Luxford *et al.* (1990) reported heritability estimates for cumulative reproductive performance measured over one, two and three parities, and over lifetime, for lines of mice selected for first parity litter size over approximately 50 generations. All estimates were low when compared with traits such as growth and intake, and were accompanied by large standard errors. Many fell outside the normal range of zero to one, indicating a low additive genetic component to the observed variation. In general, estimates for most traits were higher in the control line than in either of the selected lines.

In the absence of other factors, such as selection for reproductive capacity (Luxford *et* al., 1990; Wallinga and Bakker, 1978; Eisen and Saxton; 1984) or advanced age, a general increase in litter size from first to second parities has been documented in nearly all multiparous species, and particularly in mice (e.g. Newman *et al.*, 1985; Brien and Hill, 1986; Krackow and Gruber, 1990). The results for parities 1 and 2 of the current study conform to these observations. The reduction in litter size in parity 3 was attributed to the age of both sires and dams in all lines (~42 weeks). The effect of age on litter size has also been thoroughly researched elsewhere and there is little

new in the current findings. Comparative lifetime performance studies with virgin and mated mice (Lamb, 1977) have shown that the general stress involved in the reproductive process accelerates senescence. The decrease in litter size associated with normal chronological ageing has been attributed to degeneration in uterine function (Biggers *et al.*, 1962).

There was a clear correlated response in litter size to selection for net feed intake. Ignoring the effects of age (parity 3), it would appear that in the mice in the current study, the response was predominantly in first parity litters, which were significantly smaller in the low net feed intake line. Nielsen *et al.* (1997a, b) divergently selected mice for heat loss over fifteen generations, total heat loss being used as an indicator trait of maintenance energy. Significant differences between the high heat loss (equivalent to the high net feed intake) and low heat loss (equivalent to low net feed intake) lines for number born ranged between 1.1 and 2.0 during generations 10 and 15, similar in magnitude to the results for first parity litters in the current study. However, in the heat loss study, differences between the lines were larger when measured in the second parity. The authors ascribe this to a scaling effect, as the means were also greater. They found the line differences were primarily due to ovulation rate rather than ova success.

The positive correlated responses in number born to selection for net feed intake was considered to be undesirable. Animals that were more efficient at maintaining body weight had lower rates of reproduction. This same relationship was reported earlier by Brien *et al.* (1984). They reported a difference of 2.6 pups at first parity between high and low lines of mice selected over 10 generations for feed intake adjusted for 4

week body mass. The differences were attributed to ovulation rate, similar to the results of the heat loss study (Nielsen, 1997b).

The physiological explanation of why selection for higher or lower maintenance energy causes a correlated change in ovulation rate is unclear. It may be that the variation in intake, net of primary functions such as growth and maintenance, observed in growing and mature animals in a non-pregnant state is purely an expression of a latent physiological buffer that is up-regulated and drawn upon during pregnancy. Rauw *et al.* (1999) conducted an experiment reciprocal to that of the current study – 2 lines of mice, a high litter size line and a randomly mated control line, were developed, and the correlated responses in net feed intake and other growth and intake traits between 3 and 10 weeks of age were examined. Selection for high litter size produced proportionately identical increases in both mature weight and mature daily intake. After size-scaling (Taylor, 1980), small but significant line-differences remained. Rauw *et al.* (1999) attributed these differences to specific genetic factors that were independent of mature size and associated with net feed intake. In growing mice the line differences between females were insignificant, however at maturity the high litter size females were substantially less efficient at maintaining body weight under non-reproductive conditions. Rauw *et al.* (1999) suggested that the higher mature net feed intake in the high litter size line indicated that these animals had more resources available as a response to environmental stress that the control line animals. However, when compared to the results of the present study, the results from immature mice suggest that reproductive capacity is not the sole physiological basis for differences in net feed intake throughout an animal's lifetime. Rauw *et al.* (1999) extended their study to examine the effects of selection on feed resource allocation in reproductive females and its consequences for pup

development, and this, together with the response of the net feed intake selection lines to reproductive stresses in the current study, is examined in more detail in the next chapter.

Attention must again be drawn to the fact that the first, and indeed even the second parity litter size, is only a component of lifetime reproductive performance, which is as much dependent on reproductive longevity. Finn (1963) postulated that overcrowding in the uterus, i.e. high numbers of embryos, can lead to premature ageing and reduced fertility by hastening degeneration of uterine function. This may prove to be a selective advantage for low net feed intake animals, particularly after long-term selection, where a reduced litter size may be associated with a longer reproductive life.

The results observed in mice are not consistent with the relationship observed following selection in chickens. Bordas *et al.* (1992) and Schulman *et al.* (1994) found no changes in egg number or egg weight with selection for residual feed consumption. However, across breeds (heavy meat lines and egg laying lines) of chickens, Hocking *et al.* (1985) found that egg laying lines had the higher feed intake per metabolic size in addition to their higher egg laying rate.

Clearly, the influences of the various factors that comprise reproductive efficiency are very dependent on the species under study. Selection to decrease energy demands for maintenance in a livestock species, if faced with the same genetic relationship between these characteristics as observed here in mice, would require attention also for number born in an index. One would expect this antagonism between traits as they relate to overall economic value in a selection index to be greater in a species in which reproductive rate is more limiting. Nevertheless, it is much too early to write

off net feed intake as a valuable selection tool, based purely on the response in reproductive rate in the mouse.

Conclusion

Although selection for reduced net feed intake in mice was associated with a reduction in litter size of first parity litters, the effect was not sustained in subsequent parities. The results from the current study are only directly applicable to other multi-parous species such as pigs and chickens – they are difficult to extrapolate to species with lower reproductive rates such as sheep and cattle. Furthermore, results from other multi-parous are inconclusive and tend to conflict with those observed in mice. The results from the current study may implicate a range of biological factors associated with a reduction in reproductive rate, but other studies in mice have tended to postulate ovulation rate as the primary mechanism for reduced fertility in high efficiency animals.

Chapter 8.

Net feed intake during pregnancy and lactation.

Introduction

Results from previous studies have clearly demonstrated significant direct and indirect correlated responses to selection for post-weaning net feed intake in mice (Hastings *et al.*, 1997; Bünger *et al.*, 1998; Hughes *et al.*, 1998) and other species (Bordas *et al.*, 1992; Arthur *et al.*, 2001). Many of these responses may have implications for improving efficiency in livestock production systems. However, extrapolating results from laboratory species to the production environment must be undertaken with a degree of caution. One issue that was most apparent in the current study was that the feeding regime was essentially *ad-libitum* and, perhaps more importantly, there were no intake energy demands placed on individuals equivalent to those that would normally occur in a production environment, aside from those associated with normal growth and development. It could be argued that, particularly for females, it is unlikely that individuals from a breeding herd would be in a non-productive state for any length of time in many agricultural systems (Pitchford, *pers. comm.*). Furthermore, there is substantial evidence that maintenance intake varies substantially with both potential and actual level of lactation, egg production, etc… (e.g. Taylor *et al.*, 1986) It was considered pertinent to examine the effects of both pregnancy and lactation on net feed intake and other associated traits in the final generations of selection.

The initial hypothesis was that the significant line differences observed for net feed intake and raw intake under ideal (*ad-libitum* feed, maintenance conditions) would converge throughout pregnancy and lactation, rendering the efficiency gains obtained

through selection redundant. It was considered that as the low net feed intake (high efficiency) line had a lower intake net of that required for maintaining body-weight at maturity, females from that line would have a limited ability to re-partition 'non-core' intake to the requirements of litter growth and/or lactation, resulting in an increase in raw intake and/or a decrease in litter weight, and hence a decrease in efficiency. This was in part demonstrated in the previous chapter where low net feed intake was associated with significantly lower litter sizes, although it must be noted that average litter weight was similar between lines. In effect, selection for low net feed intake may have acted on specific components of intake associated with production (i.e. reproductive and lactation) that were a) not expressed at selection age, and b) not included in the phenotypic model for intake.

Materials & Methods

Animals

Approximately 40 males and 40 females per line were randomly sourced at maturity from generations 9 and 10 of selection. Female intake and body weight was measured for 3 weeks using the same methodology described in chapters 3 and 4. Females were then randomly mated to males from the same line on a 1:1 basis. Males were removed from mating cages after 4 days. Pregnancies resulted from approximately 25 matings from each line. Female intake and body weight was again measured on a weekly basis through pregnancy and for three weeks post-natally, during which time total litter weight was also recorded.

Analysis

As with many experimental studies, the analysis of this data set evolved significantly over time. Four different approaches are presented to represent this evolution and to illustrate the comparisons and contrasts in interpretation that the different approaches allow. It is important to note that matings were within line. This confounded the effect of litter line with that of dam line, which made the data set simpler to analyse, whilst reducing the power of the analysis slightly.

Approach 1

Initially, these data were analysed in a manner analogous to that used to estimate net feed intake in Chapters 3 and 4. The data set was divided into three discrete time-periods of approximately three weeks in duration: 1. pre-pregnancy, 2. pregnancy and 3. lactation. For each period, net feed intake was estimated using the GLM procedure (SAS, 1990) fitting a model to daily intake that included the covariables average daily gain and metabolic mid-weight. Net feed intake represented the error or residual term of the model. During lactation, average daily gain and mid-weight were calculated using the sum of dam and litter weights. The first week of the pre-pregnancy test period was treated as an adjustment phase and hence was excluded from the model.

The GLM procedure was then used to fit a general model to all growth and intake traits within successive periods, including net feed intake, daily feed intake, average daily gain, mid-weight, maintenance requirement/maintenance efficiency (pre-pregnancy) and food conversion ratio/gross efficiency (pregnancy and lactation). The final model included management group (1, 2, 3, 4) and line (control, high, low).

Management group was actually a composite of generation and replicate and hence was confounded with age. It was not possible to fit a management group by line interaction as the high line was not fully represented in all management groups and would therefore have been inestimable.

Approach 2

To examine the responses of growth and intake during pregnancy and lactation with greater detail, the data was further partitioned into individual weeks. The same model for intake was used to estimate net feed intake on a weekly basis.

The general model for all traits was also identical with one important exception. As data was not collected on an exact seven day cycle, and there was some variation in gestation length, average days from birth was fitted as a covariate to data from 'weeks' 5-11 to adjust for differences in gestation length/litter age. Week 4 was excluded from the data set as this week incorporated 4 days for mating.

For comparison, a general model was also fitted to weight data for dams and pups (litter weight/litter size) for the final 4 weeks. Several new trait definitions were introduced using this data (Table 8.1).

Table 8.1. Summary of additional traits used in the analyses with their abbreviations and units.

Abbreviation	Trait	Units
DMWT	Dam mid-weight	g(bodyweight)
DADG	Dam average daily gain	g(bodyweight)
PMWT	Average pup mid-weight	g(bodyweight)
PADG	Average pup average daily gain	g(bodyweight)

Approach 3

Net feed intake was again estimated weekly using the same model as Approach 2. A repeated measures analysis of variance was then fitted to all data from weeks 1-11 using GLM to account for correlations between weeks. The model fitted included:

week (WEK 1-11)

management group (MGP 1, 2, 3, 4)

line (LIN control, high, low)

time by management group

time by line

where week was fitted as the repeated measure.

It should be noted that when time (in this case, week) is the repeated measure, the model fitted assumes approximately equally-spaced time intervals between repeats (although this can be adjusted in the case of polynomial contrasts). This obviously was not the case between weeks 3 and 5 (data from week 3-4 was excluded due to mating; data from week 4-5 was excluded due to low numbers measured), and it was important to be cautious when interpreting contrasts between successive weeks in these circumstances.

Approach 4

The three previous approaches were effectively longitudinal studies of line response to pregnancy and lactation. An alternative parameterisation was considered that examined the relationship between net feed intake or daily feed intake with body weight and/or daily gain (i.e. 'longitudinal' in weight or gain or both, rather than time).

Linear mixed models were fitted to both net feed intake and daily feed intake using PROC MIXED (SAS, 1989). The models fitted included:

management group (MGP 1, 2, 3, 4)

line (LIN control, high, low)

mid-weight and/or average daily gain (MWT 25.0-181.25 grams/ADG -3.7-5.7 grams.day^{-1})

management group by mid-weight and/or average daily gain

line by mid-weight and/or average daily gain

with animal ID fitted as a random effect nested within line.

Results

Approach 1

The percentage variance accounted for (R^2) by the initial model of daily feed intake used to estimate net feed intake is illustrated in Figure 8.1. The model accounted for progressively more variation in intake as production levels increased.

The variation in daily feed intake showed a similar response in the first two periods, but increased substantially during lactation. The variation in net feed intake increased slightly over the course of the experiment (Figure 8.2). The percentage variance accounted for by the model (R^2), residual coefficient of variation (CV), error degrees of freedom, error mean square and source type III mean squares for the analysis of intake and growth over the three successive measurement periods are presented in Table 8.2. There was no clear trend in the amount of variation explained by the model across traits.

Figure 8.1. Percentage variance accounted for by the models of daily feed intake over the three successive periods.

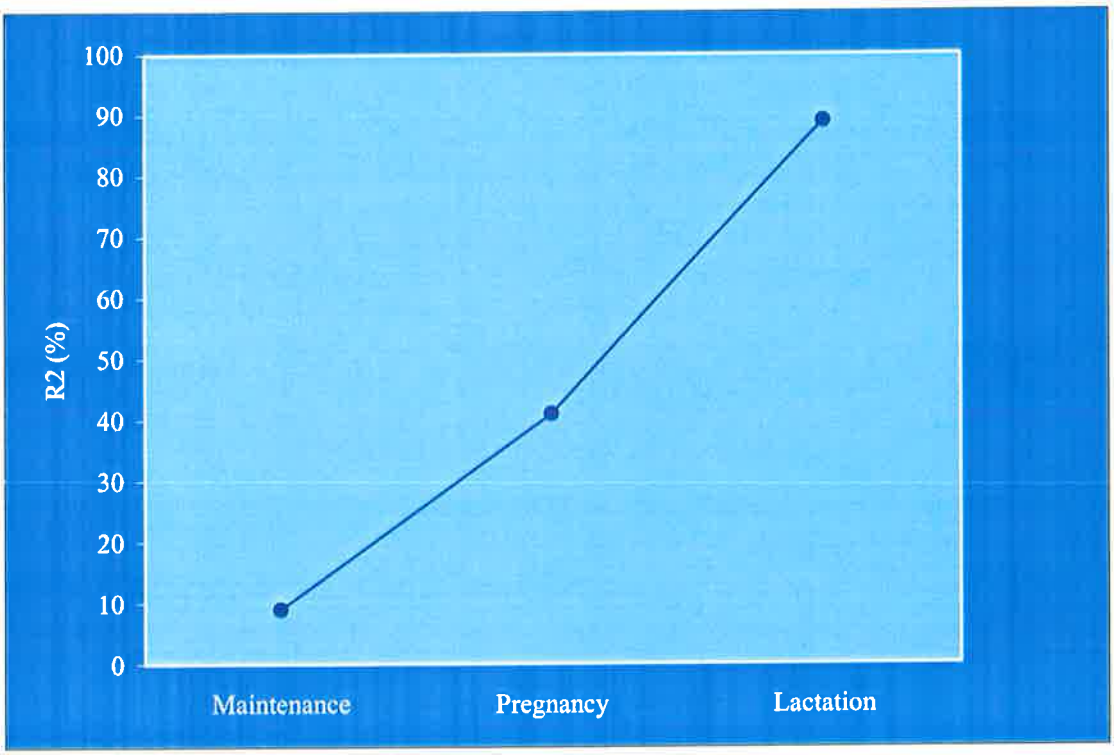


Figure 8.2. Raw standard deviations for net feed intake and daily feed intake over the three successive periods.

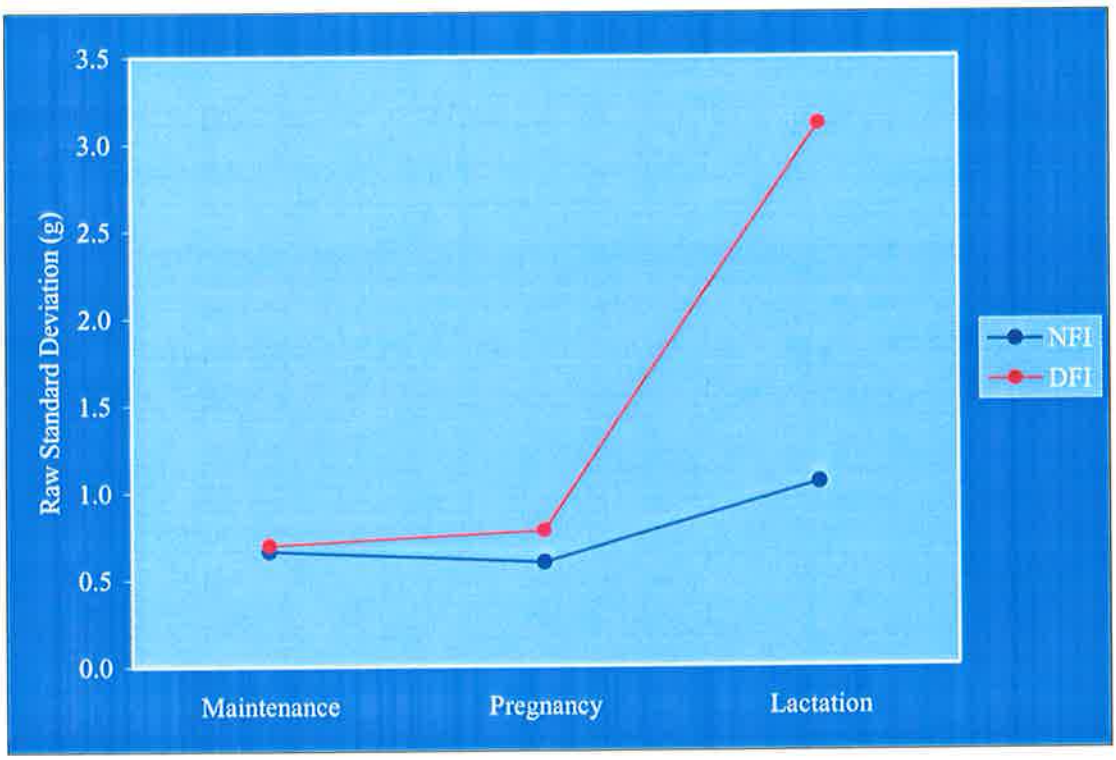


Table 8.2. Variation in pre-pregnancy and pre- and post-natal growth and intake traits.

Source	NFI	DFI	ADG	MW	FCR	GE	MR	ME
Pre-preg.								
R^2 (%)	33	28	3	27	4	4	39	40
CV	-	14.6	-556.7	12.4	580.9	-422.4	16.4	15.9
Error DF	64	64	65	65	64	64	64	64
Error MS	0.3	0.4	0.0	17.1	33270	0.001	0.000	1.66
Group	0.5	0.5	0.0	110**	3485	0.000	0.002*	7.80**
Line	3.6**	3.6**	0.0	35*	37547	0.000	0.004**	14.38**
Pre-nat.								
R^2 (%)	41	60	24	14	12	8	57	57
CV	-	10.7	35.0	10.3	33.8	31.7	11.8	12.9
Error DF	33	33	34	34	33	33	33	33
Error MS	0.2	0.3	0.1	19	6	0.002	0.000	1.24
Group	0.7*	1.8**	0.1	30	8	0.002	0.001**	10.13**
Line	1.2*	2.7**	0.1	23	2	0.001	0.001**	6.32*
Post-nat.								
R^2 (%)	18	20	25	11	18	21	29	29
CV	-	22.6	37.1	20.5	30.7	22.2	8.6	9.0
Error DF	60	60	60	60	60	60	60	60
Error MS	0.98	8.4	0.9	243	3	0.002	0.000	0.29
Group	2.85*	35.2	4.9**	542	8*	0.007	0.001**	2.05**
Line	0.39	5.1	1.9	135	9	0.005	0.000	0.05

* $p < 0.05$
** $p < 0.01$

Table 8.3 presents least squares means for the successive periods. The results are also presented graphically in Figures 8.3 through 8.8 (includes only efficiency measures for ratio traits). Prior to discussion of individual traits, it should be noted that the estimates for the ratio traits (i.e. maintenance requirement, maintenance efficiency, food conversion ratio and gross efficiency) were stage-dependent: clearly gross efficiency and food conversion ratio were of little value under maintenance conditions at maturity, as they were based on units of gain. They were really only of interest during pregnancy and lactation. Similarly, all four measures were somewhat confounded during the latter stages, as measures of intake incorporated both a gain

and maintenance component which could not be separated. These traits have been included for interest.

Table 8.3. Net feed intake selection lines' least squares means for pre-pregnancy and pre- and post-natal growth and intake traits.

		NFI	DFI	ADG	MW	FCR	GE	MR	ME
Pre-	C	0.04	4.3	-0.04	35.6	5.5	-0.01	0.124	8.3
pregnancy	SE	0.12	0.1	0.02	0.9	38.3	0.01	0.004	0.3
	H	0.44	4.6	-0.02	31.3	81.7	-0.00	0.143	7.2
	SE	0.13	0.1	0.03	1.1	43.3	0.01	0.005	0.3
	L	-0.40	3.8	-0.02	33.3	11.1	-0.01	0.116	8.9
	SE	0.12	0.1	0.02	0.9	37.7	0.01	0.004	0.3
Pre-natal	C	0.13	5.1	0.78	42.5	7.5	0.15	0.121	8.5
	SE	0.14	0.1	0.07	1.2	0.7	0.01	0.004	0.3
	H	0.26	5.4	0.93	39.9	6.7	0.17	0.136	7.8
	SE	0.20	0.2	0.11	1.7	1.0	0.02	0.006	0.4
	L	-0.32	4.5	0.71	40.3	6.9	0.16	0.112	9.2
	SE	0.13	0.1	0.07	1.2	0.6	0.01	0.004	0.3
Post-natal	C	-0.04	12.8	2.60	76.3	5.4	0.20	0.167	6.1
	SE	0.22	0.6	0.21	3.4	0.4	0.01	0.003	0.1
	H	0.12	11.8	1.97	71.0	6.7	0.17	0.164	6.2
	SE	0.27	0.8	0.26	4.2	0.5	0.01	0.004	0.1
	L	-0.18	12.4	2.46	74.7	5.4	0.19	0.165	6.1
	SE	0.20	0.6	0.20	3.2	0.3	0.01	0.003	0.1

Prior to pregnancy, the observed results were very similar to those obtained for mature mice detailed in Chapter 4: the selection lines were significantly different for net feed intake (~20%) and daily feed intake (~20%), but not for gain or mid-weight. The high line had a 17% higher maintenance requirement and was 18% less efficient at maintaining body weight than the low line (Table 8.3). The control line was intermediate for all traits that showed substantial selection line differences. There was a significant management group effect for mid-weight and measures of efficiency incorporating mid-weight, due to a significantly lower mid-weight in the oldest of the four groups.

Figure 8.3. Daily feed intake before, during and after pregnancy (±SE).

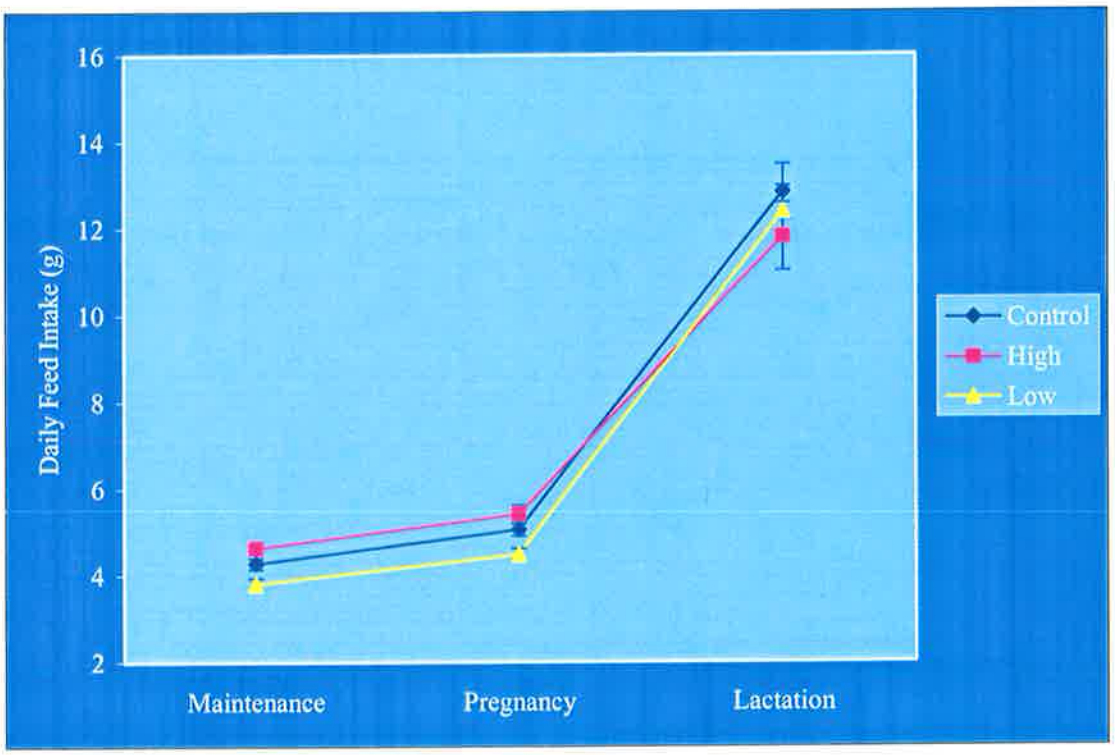


Figure 8.4. Net feed intake before, during and after pregnancy (±SE).

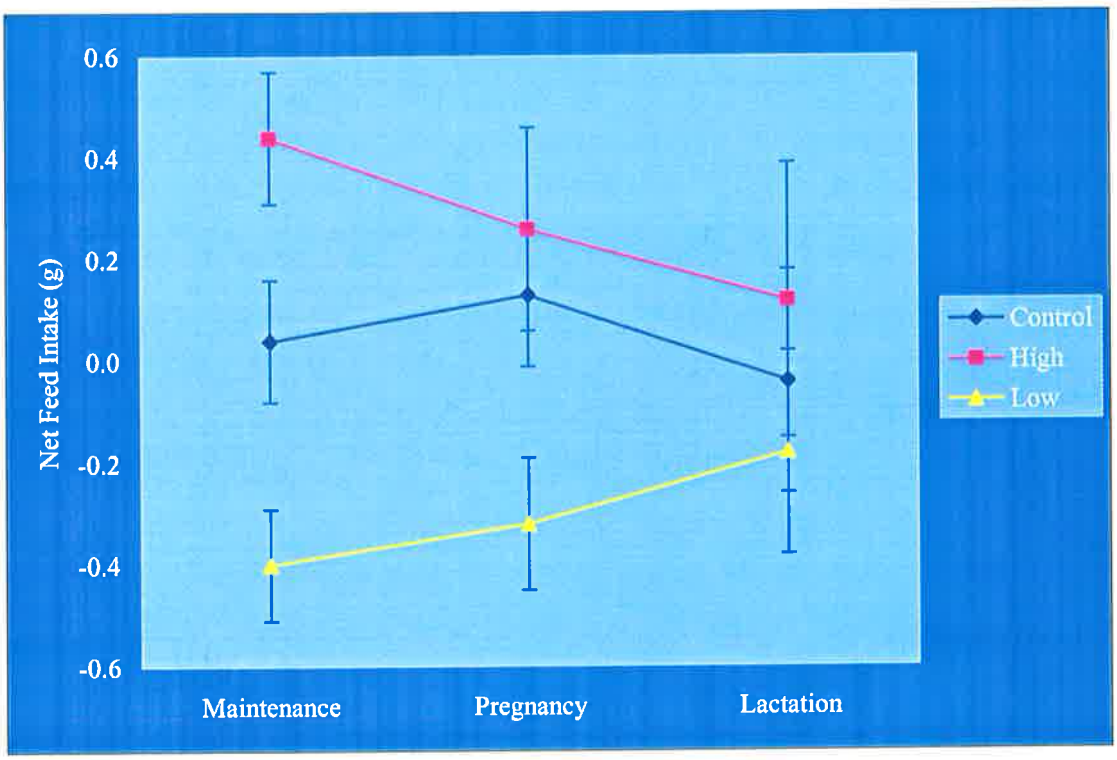


Figure 8.5. Average daily gain before, during and after pregnancy (±SE).

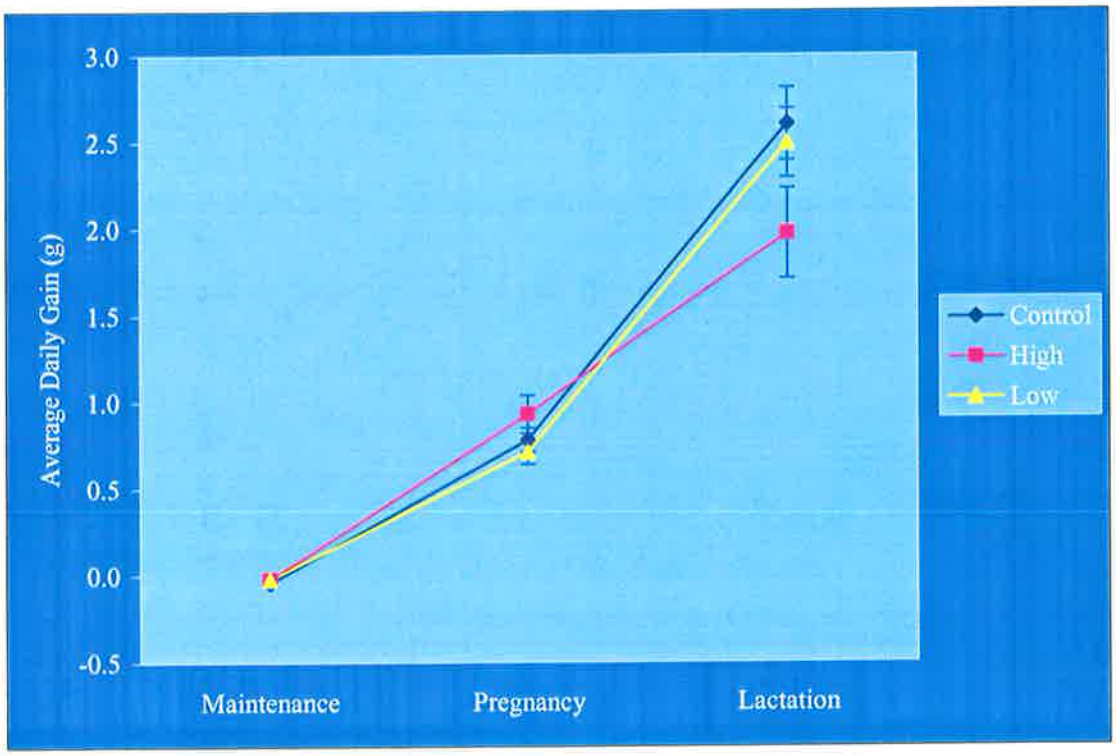


Figure 8.6. Mid-weight before, during and after pregnancy (±SE).

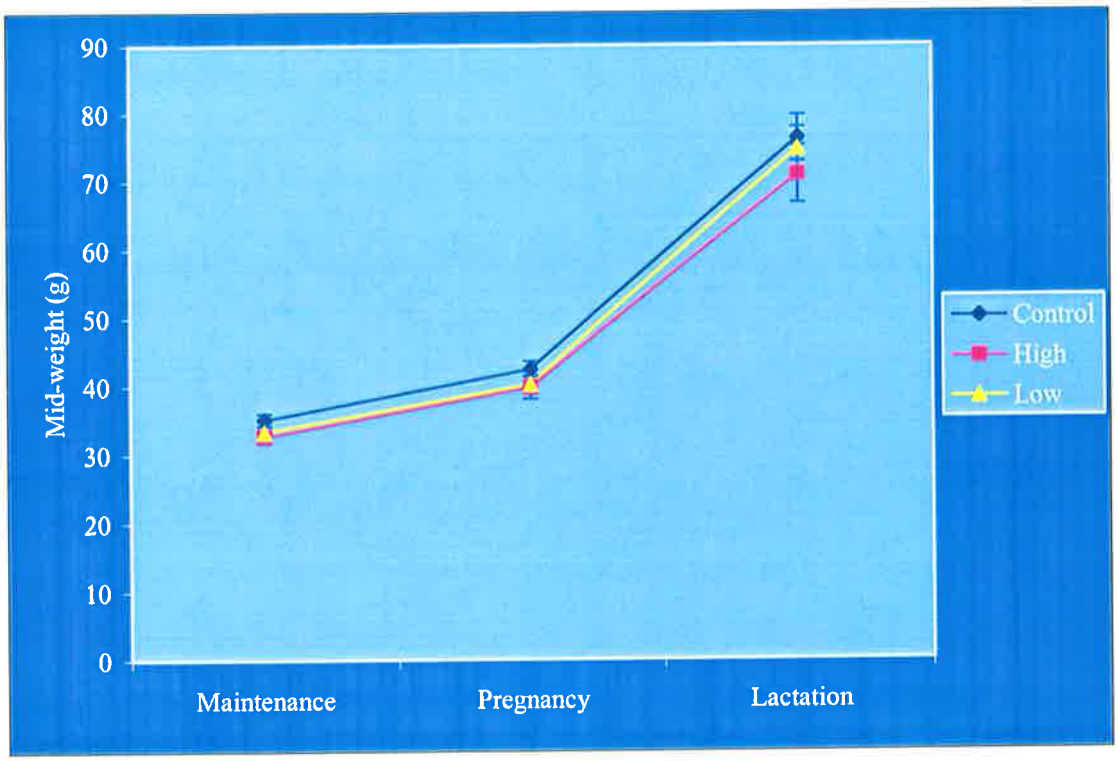


Figure 8.7. Maintenance efficiency before, during and after pregnancy (±SE).

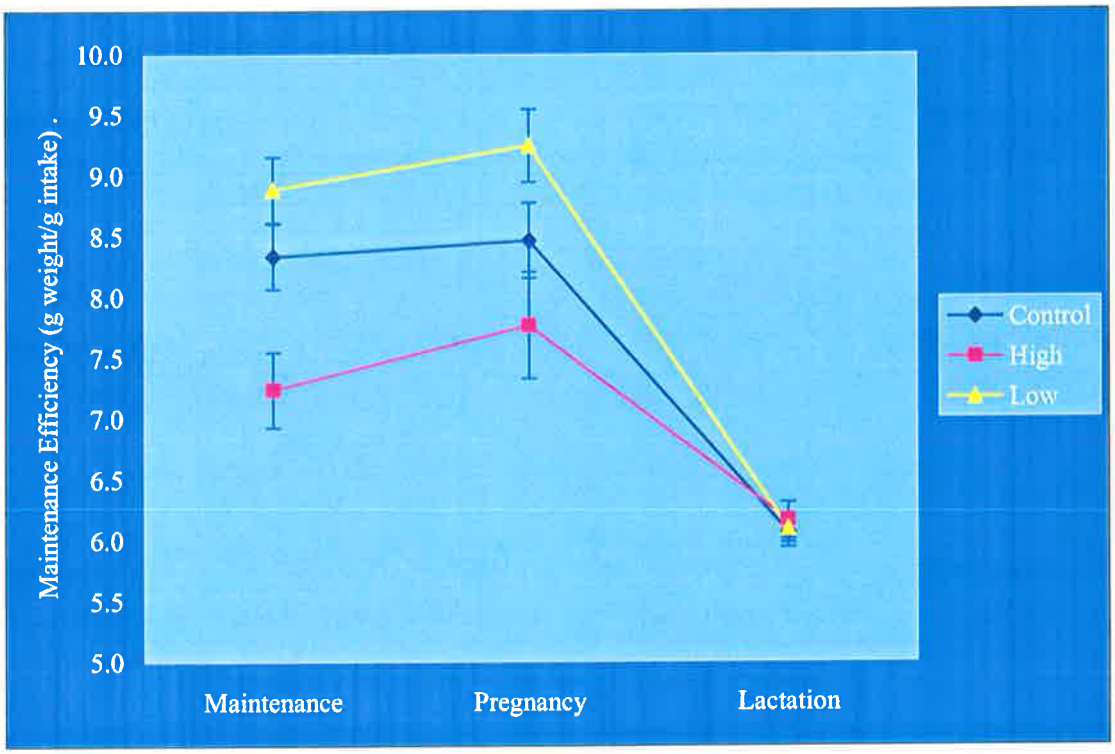
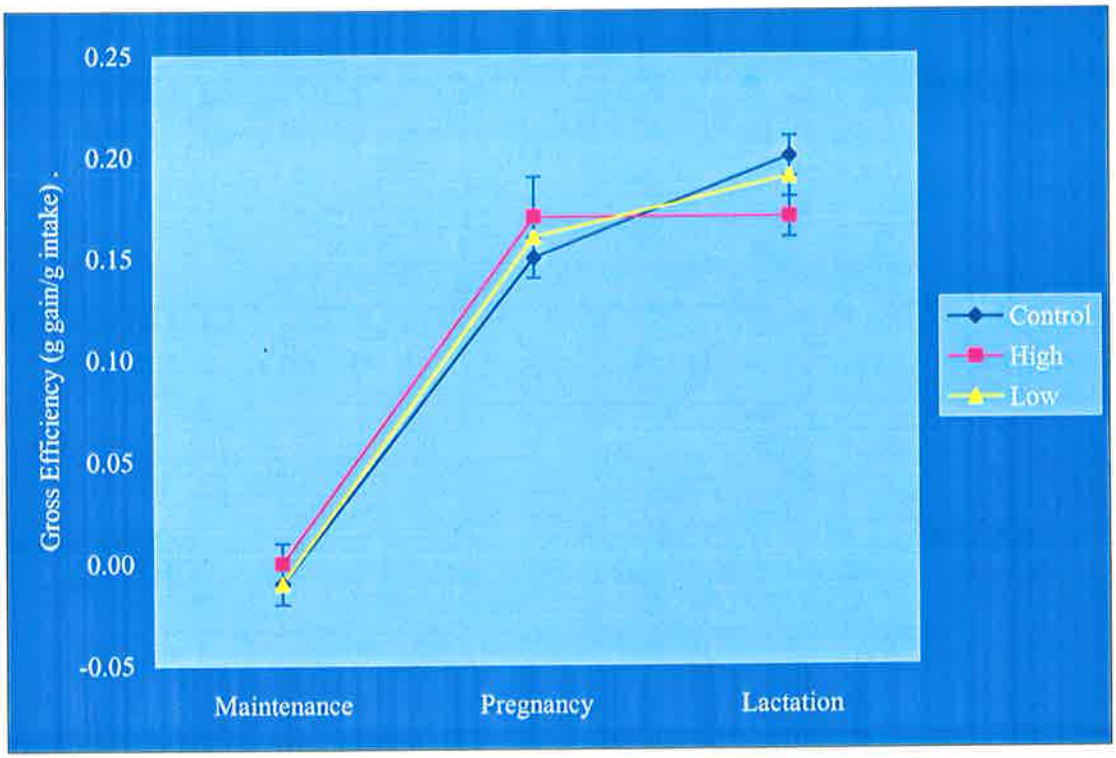


Figure 8.8. Gross efficiency before, during and after pregnancy (±SE).



During pregnancy, the lines were still significantly different for net feed intake, although the difference was smaller (~12%) than during maintenance (~20%). Both lines showed a small increase in daily feed intake of approximately 1 gram per day. Not surprisingly, both lines had similarly higher rates of gain (~0.8 $g.day^{-1}$) during pregnancy and were heavier on average. The lines also had similar rates of conversion and neither line was superior with respect to gross efficiency. As expected, the high line had a higher maintenance requirement (27%) and a lower maintenance efficiency (16%) than the low line. The control line mice were again intermediate for traits that differed significantly between the selection lines. There was a significant group effect on intake and maintenance, which again stemmed from a single group of pregnant females, this time the youngest, that had a substantially lower intake and net feed intake than the others.

Post-natally, none of the lines were significantly different from zero for net feed intake, nor were there any line differences in daily intake. All lines showed a substantial increase in intake from pregnancy through to lactation of approximately 7 grams per day, due almost exclusively to a large increase in body mass (dam and offspring) associated with a growing litter. This was reflected in faster rates of gain in all lines and higher mid-weights, neither of which showed significant line differences. There were no longer any differences between the lines in maintenance efficiency or maintenance requirement. Management group was significant for net feed intake, daily gain and maintenance requirement, all of which tended to increase with the age of the group, and for food conversion ratio and maintenance efficiency, which tended to decrease with age.

Approach 2

The percentage variance accounted for (R^2) by the initial model of daily feed intake, used to estimate net feed intake, is illustrated in Figure 8.9. The amount of variation accounted for by the model dropped initially as females lost a small amount of weight when re-introduced to the feeders. There was a substantial increase during pregnancy, which plateaued sharply during lactation.

The raw standard deviations for both net feed intake and daily feed intake are presented in Figure 8.10. The variation in net feed intake was roughly constant from maintenance through pregnancy, increasing slightly during lactation. The variation in daily feed intake showed a much greater increase throughout lactation.

The percentage variance accounted for by the model (R^2), residual coefficient of variation (CV), error degrees of freedom, error mean square and source type III mean squares for the analysis of intake and growth over the 11 successive measurement periods are presented in Appendix 4, Table A4.1. Although there was no clear systematic trend, the model tended to explain the most variation in individual traits during pregnancy for most traits.

The least squares means for the 11 successive periods are presented in Appendix 4, Table A4.2. The results are also presented graphically in Figures 8.11 through 8.16 (includes only efficiency measures for ratio traits). To emphasise similarities and differences in the responses of the selection lines, the control line was excluded from the graphs. The issues outlined for the ratio traits in the section on 3-weekly averages also applies to the weekly data in the current and subsequent sections.

Figure 8.9. Percentage variance accounted for by the models of daily feed intake over the eleven weeks of measurement.

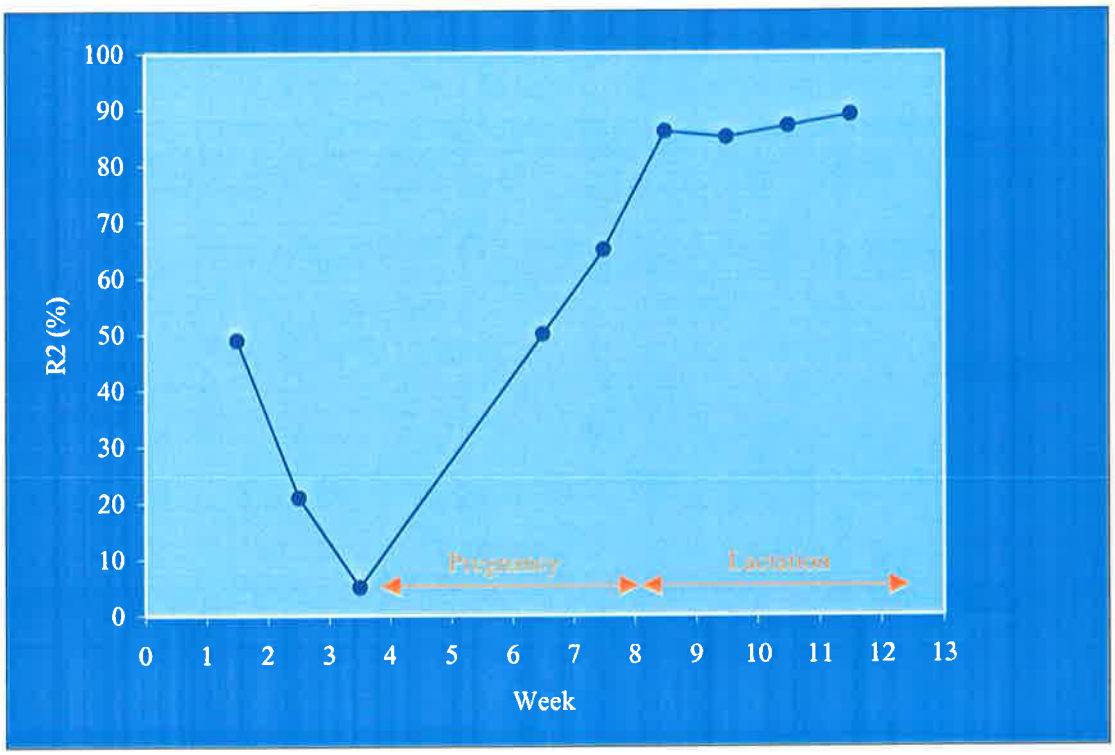


Figure 8.10. Raw standard deviations for net feed intake and daily feed intake over the eleven weeks of measurement.

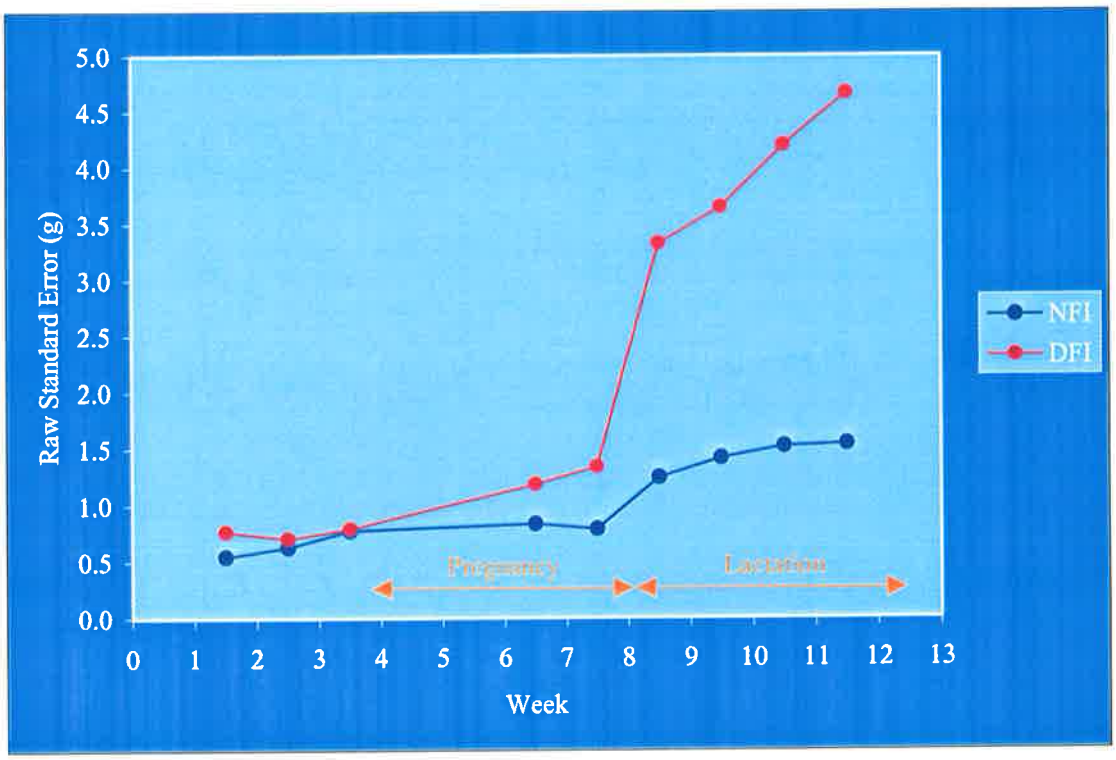


Figure 8.11. Daily feed intake over 11 weeks of measurement (±SE).

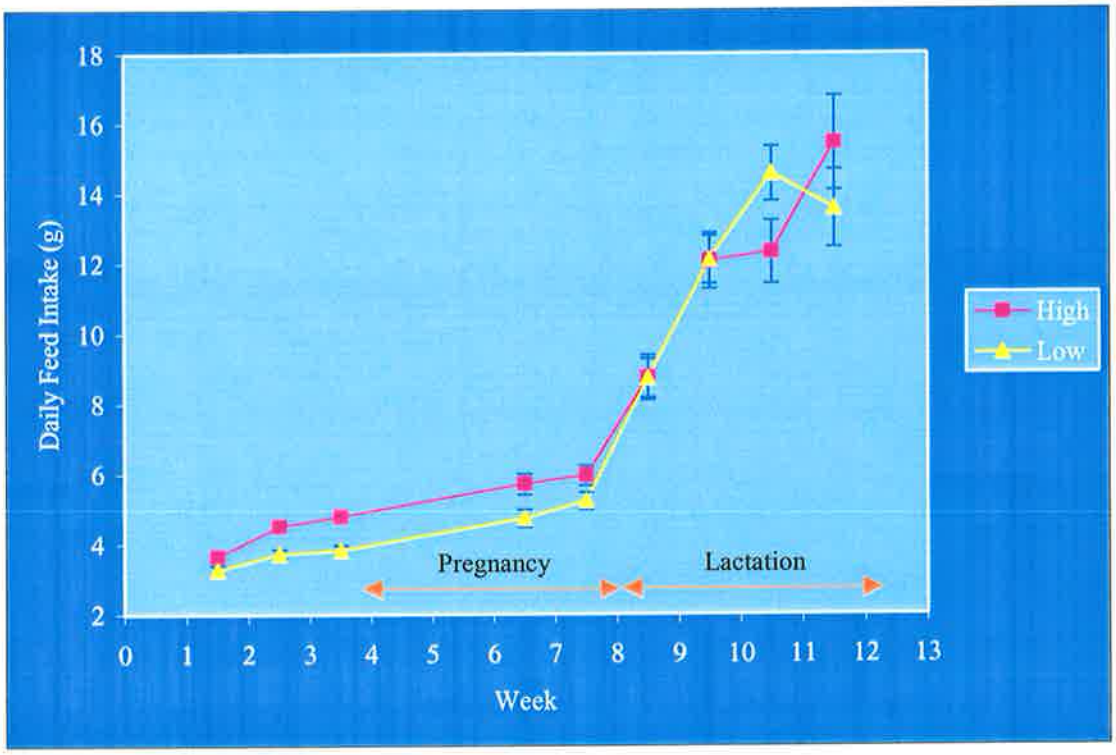


Figure 8.12. Net feed intake over 11 weeks of measurement (±SE).

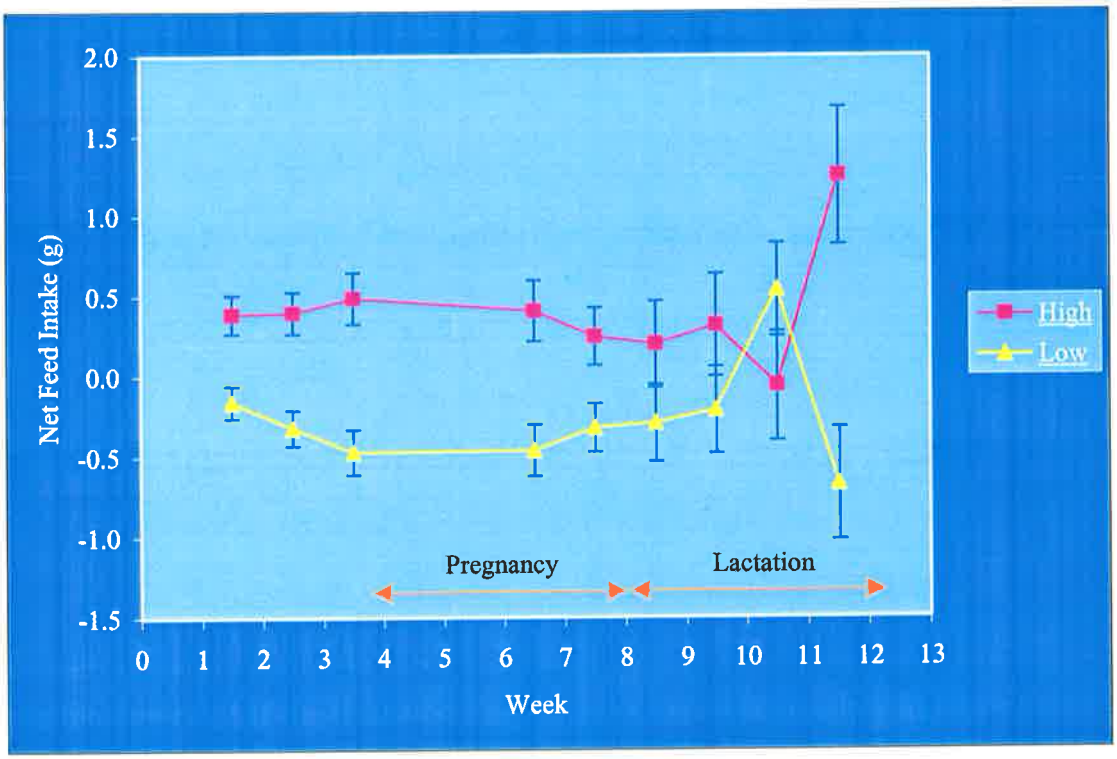


Figure 8.13. Average daily gain over 11 weeks of measurement (±SE).

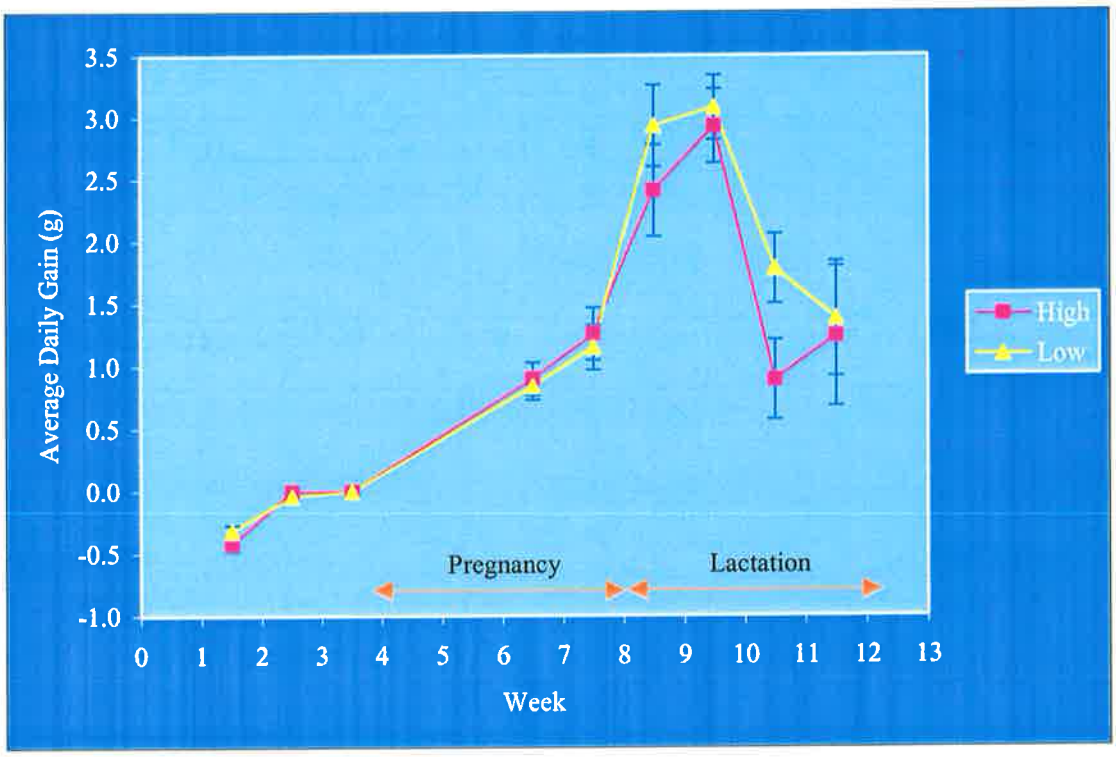


Figure 8.14. Mid-weight over 11 weeks of measurement (±SE).

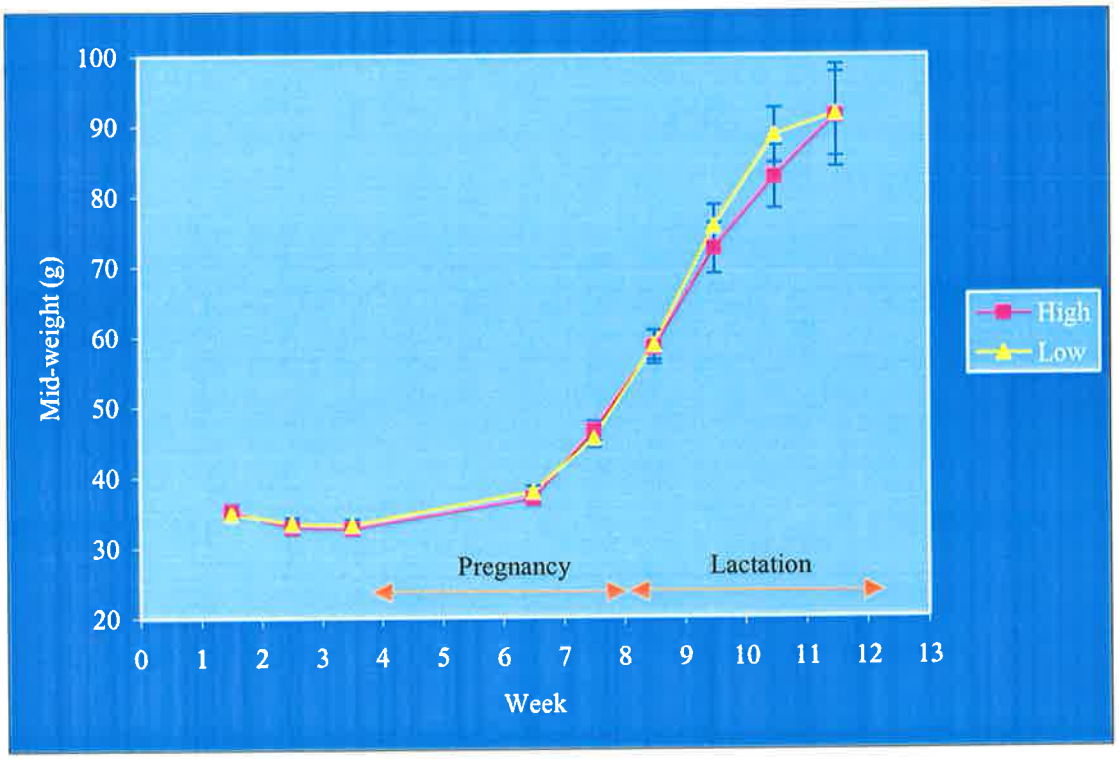


Figure 8.15. Maintenance efficiency over 11 weeks of measurement (±SE).

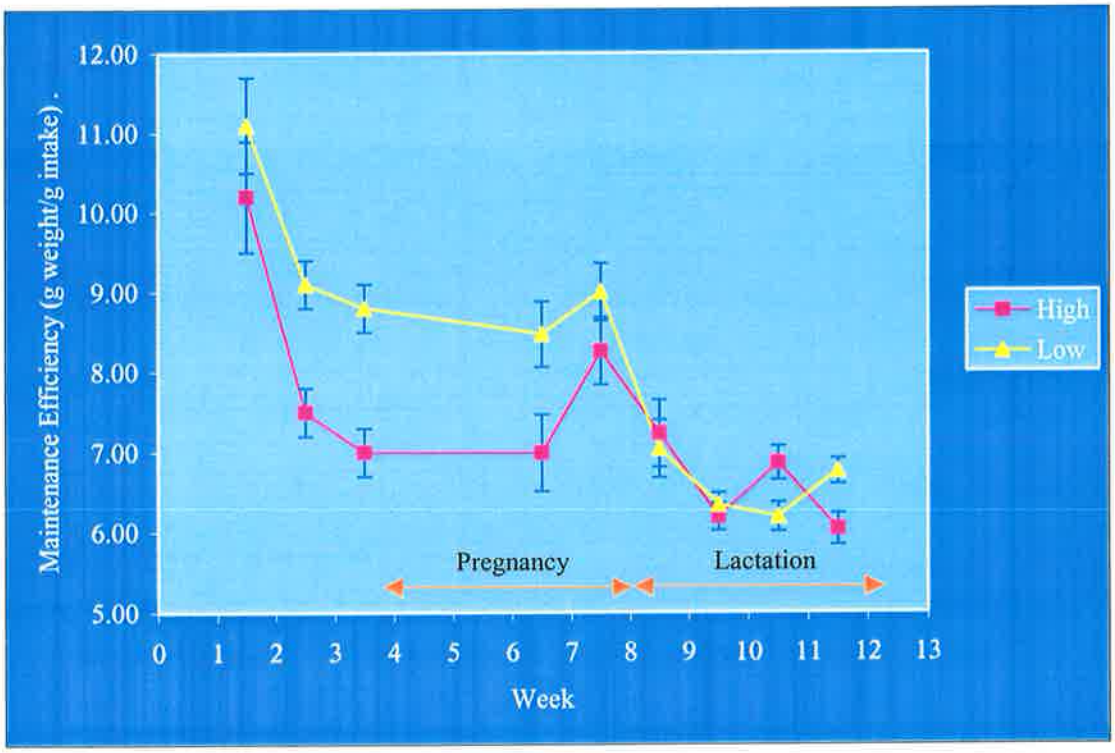
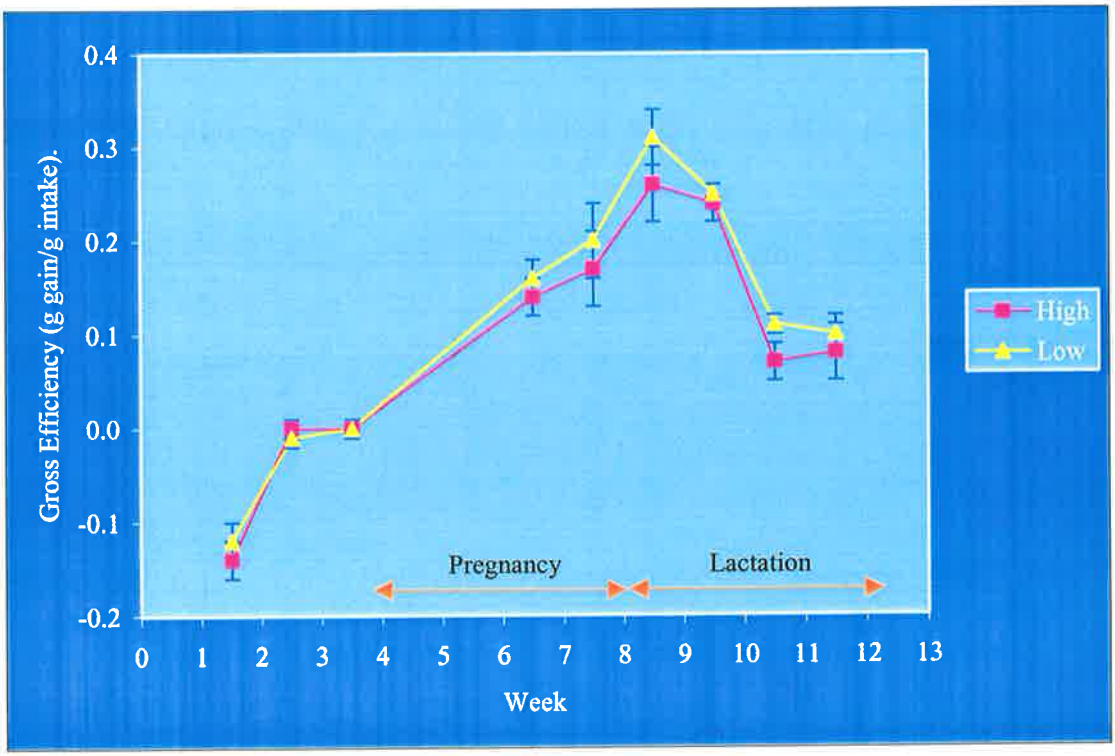


Figure 8.16. Gross efficiency over 11 weeks of measurement (±SE).



The line differences in net feed intake remained relatively constant throughout the maintenance and pregnancy periods, only converging during early to mid-lactation, and then diverging substantially again in the final week of measurement. Overall, the response in daily feed intake was similar to the results observed for the 3-week averages: intake tended to increase gradually from maintenance through pregnancy, and then increased sharply during early lactation before beginning to plateau. The high line tended to have a higher daily intake during the first two periods, but there was no systematic difference between the lines during lactation.

The early results for daily gain reflected those in intake: rates of gain tended to accelerate steadily during pregnancy, with a much faster rate of growth for the dam/litter unit during early lactation. However, rates of gain decreased sharply from mid- to late-lactation. The lines responded similarly throughout the measurement period. These responses in gain produced the traditional sigmoidal shape for the response in mid-weight, and again there were no observed line differences in mid-weight throughout the period.

Maintenance requirement tended to increase asymptotically from maintenance through early pregnancy, before dropping sharply in late pregnancy and then rising again during lactation. The inverse was observed for maintenance efficiency. The high line had a higher maintenance requirement and lower maintenance efficiency earlier, which tended to increase as pregnancy progressed, but the line differences disappeared in late pregnancy and remained similar throughout lactation. Food conversion ratio did not show any specific trends, and was similar between lines throughout the course of the experiment, but this was largely due to large standard

errors on the results. Gross efficiency showed a pattern analogous to that for daily gain, and there were no significant line differences in any week.

Approach 3

Multivariate and univariate test statistics with their associated significance levels, together with contrast mean squares, for the analysis of intake and growth over the 11 successive measurement periods, are presented in Appendix 4, Table A4.3. Least squares means for the 11 successive periods are presented in Appendix 4, Table A4.4, and the results are also presented graphically in Figures 8.17 through 8.22 (includes only efficiency measures for ratio traits). To emphasise similarities and differences in the responses of the selection lines, the control line was excluded from the graphs.

Clearly, although allowing for the correlation structure between successive measurements produced some small but significant changes in specific data points, the majority of the substantial changes occurred in weeks 3-4 and 6-7, where it is already known that the correlation is inappropriate due to missing data. To all intents and purposes, the repeated measures model produced substantially similar results to those observed for the general linear models presented in the previous section.

Figure 8.17. Daily feed intake over 11 weeks of measurement (±SE).

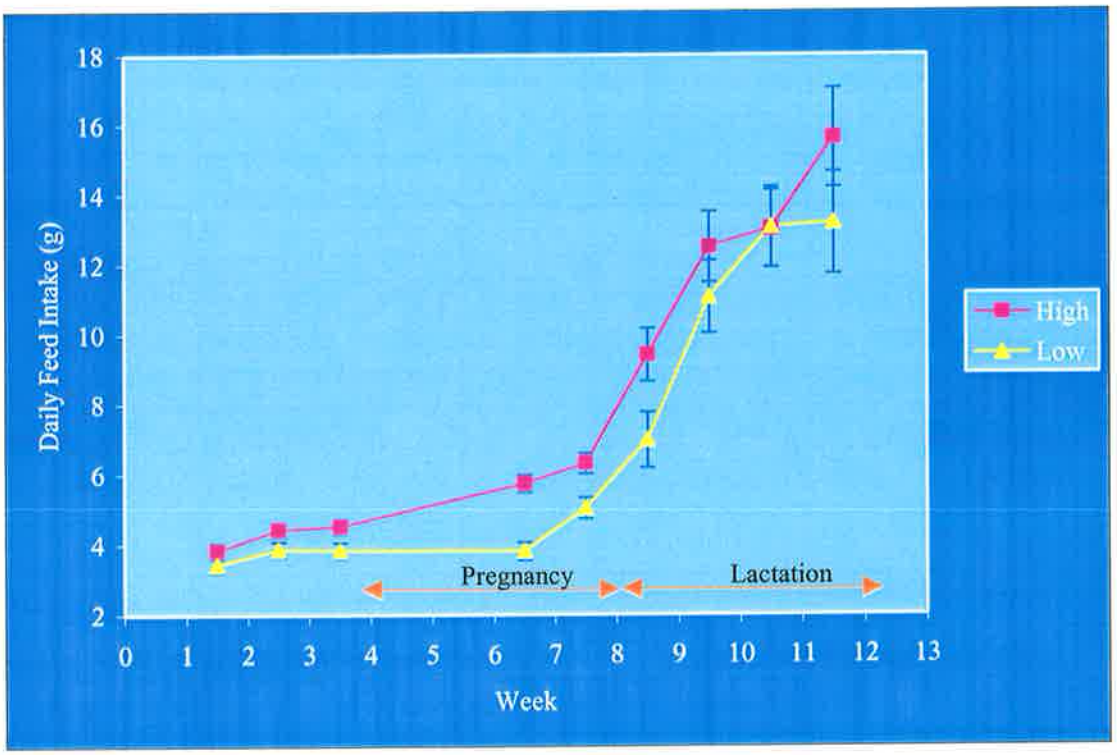


Figure 8.18. Net feed intake over 11 weeks of measurement (±SE).

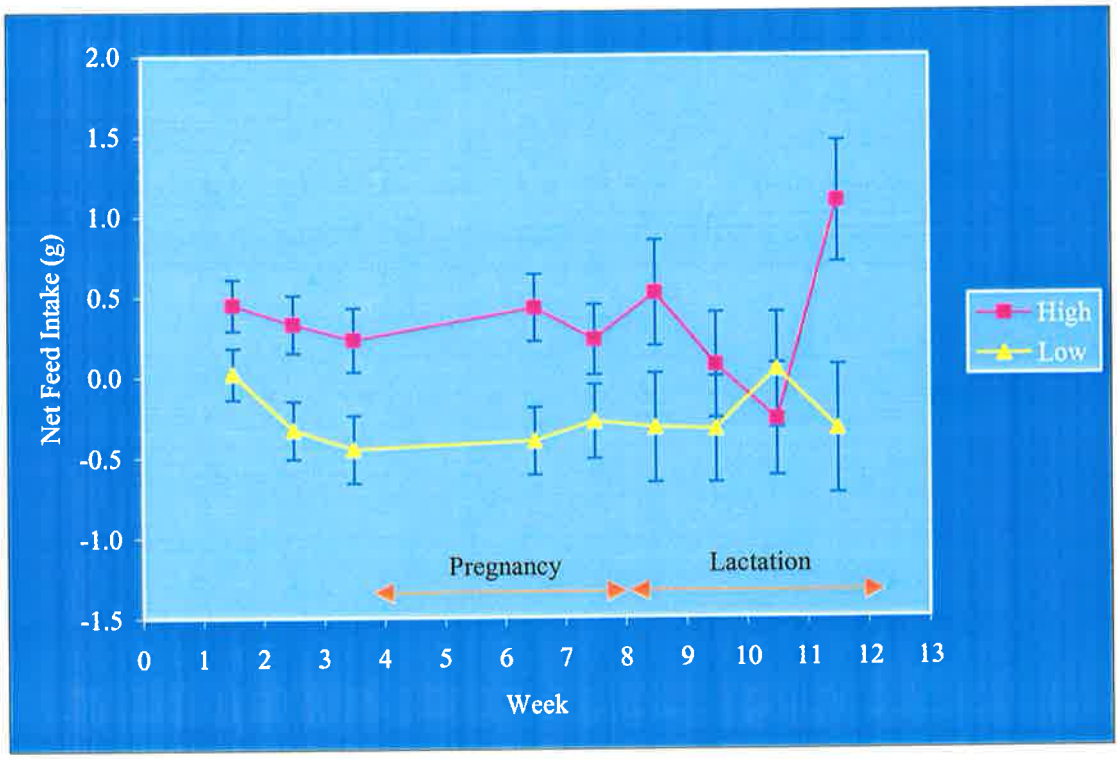


Figure 8.19. Average daily gain over 11 weeks of measurement (±SE).

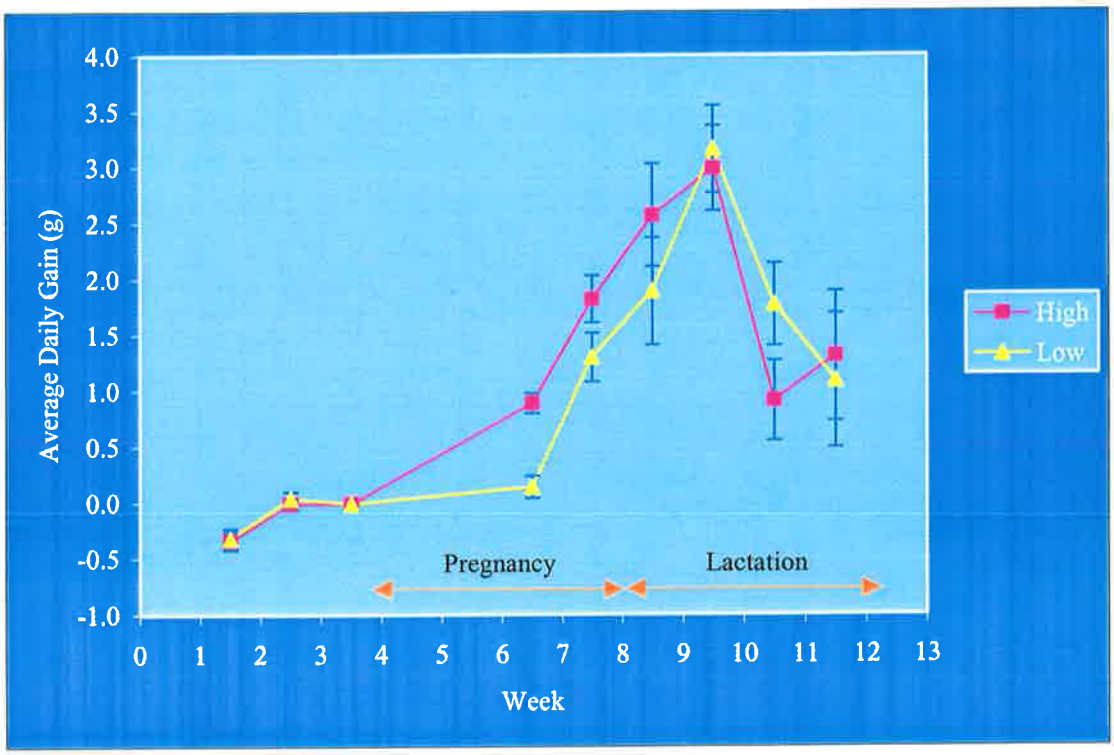


Figure 8.20. Mid-weight over 11 weeks of measurement (±SE).

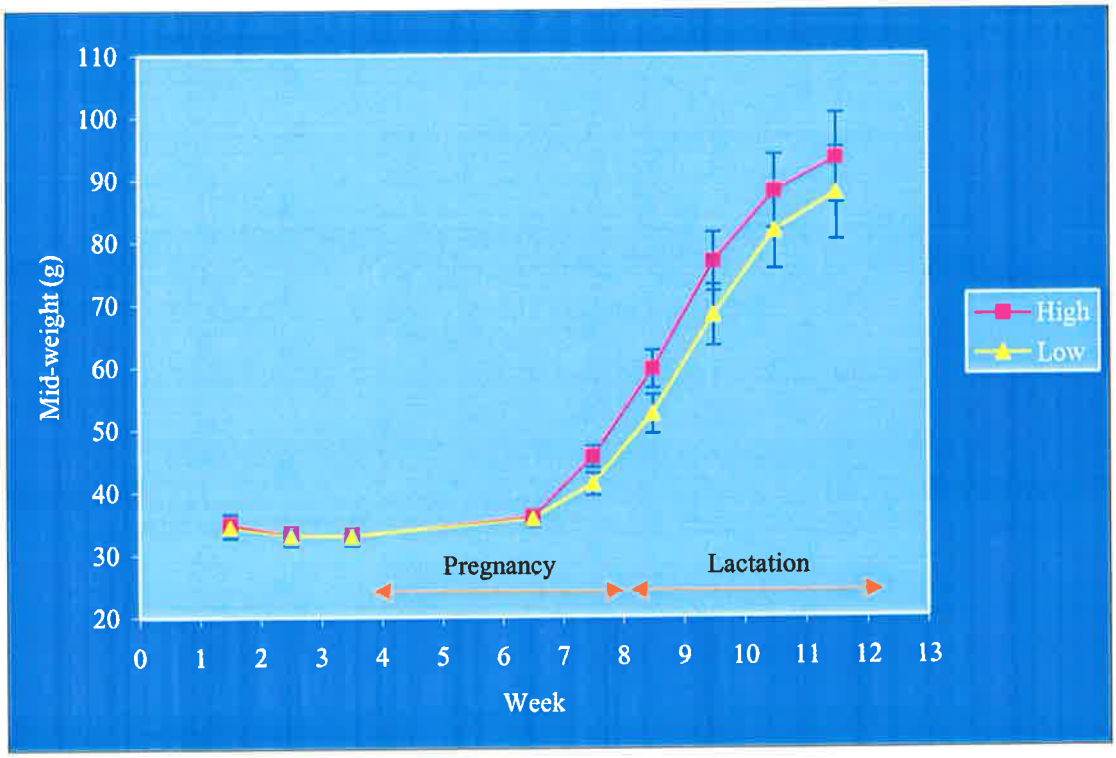


Figure 8.21. Maintenance efficiency over 11 weeks of measurement (±SE).

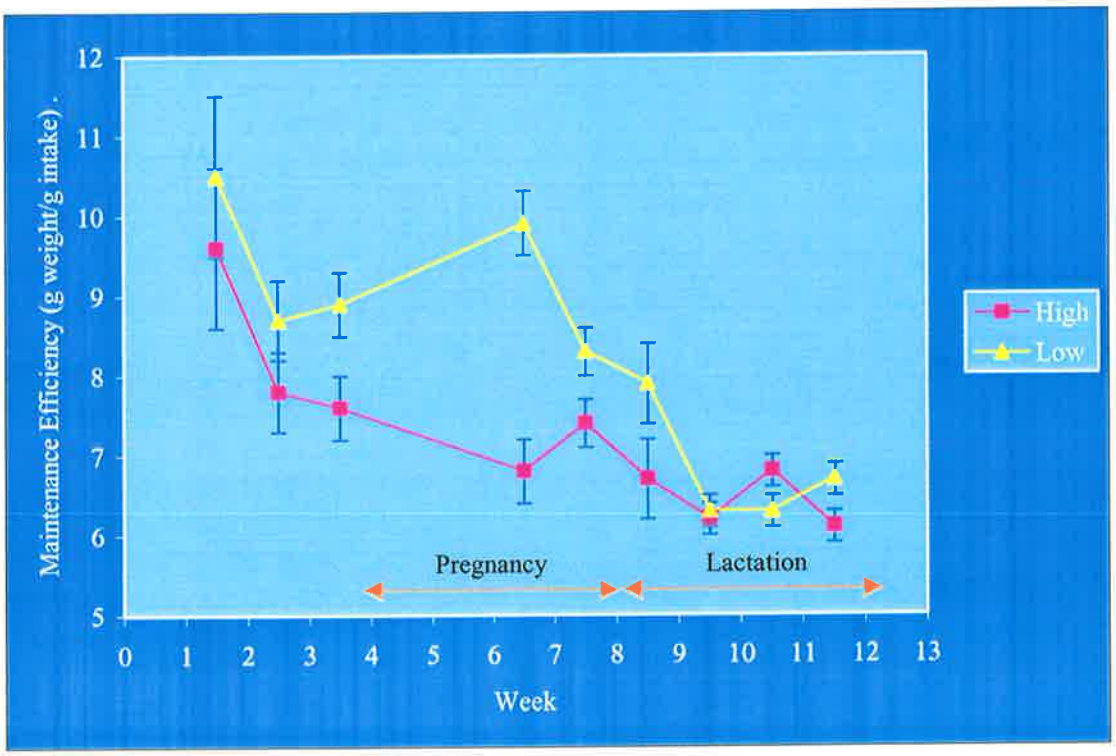
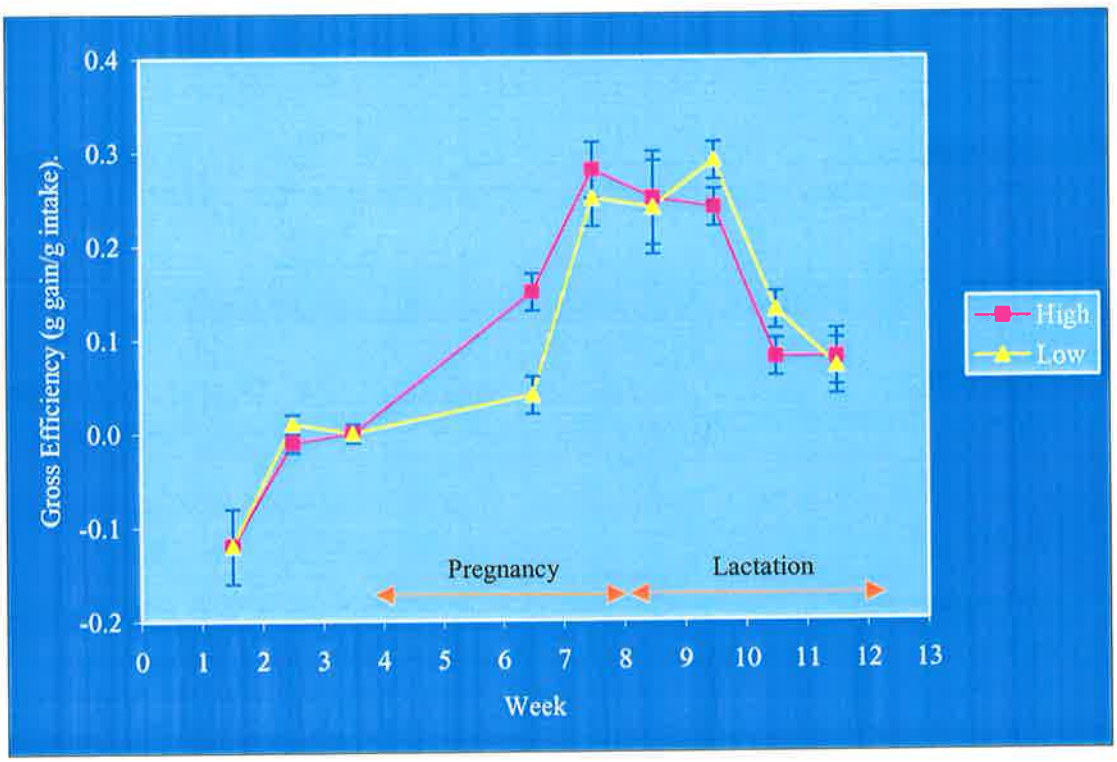


Figure 8.22. Gross efficiency over 11 weeks of measurement (±SE).



Approach 4

Type III tests of fixed effects used in the models for intake are presented in Table 8.4. Least squares means, regression coefficients and variance components for the mixed models are presented in Table 8.5.

Table 8.4. Type III tests of fixed effects for intake data.

	NFI			DFI		
Effect	Num. DF	F	Prob.	Num. DF	F	Prob.
MWT model						
MGP	4	1.09	NS	4	4.35	**
LIN	2	1.90	NS	2	2.37	NS
MWT	1	0.15	NS	1	2594.26	**
MGPxMWT	4	0.41	NS	4	0.84	NS
LINxMWT	2	1.98	NS	2	0.59	NS
ADG model						
MGP	4	2.88	*	4	0.62	NS
LIN	2	9.96	**	2	2.85	NS
ADG	1	0.17	NS	1	276.57	**
MGPxADG	4	1.70	NS	4	0.31	NS
LINxADG	2	0.02	NS	2	1.76	NS
MWT + ADG model						
MGP	4	1.59	NS	4	4.59	**
LIN	2	2.30	NS	2	1.07	NS
MWT	1	0.91	NS	1	1698.69	**
ADG	1	0.85	NS	1	147.09	**
MGPxMWT	4	1.31	NS	4	2.01	NS
MGPxADG	4	2.85	*	4	4.01	**
LINxMWT	2	3.50	*	2	2.23	NS
LINxADG	2	1.31	NS	2	3.75	*

* $p < 0.05$
** $p < 0.01$

Table 8.5. Least squares means for fixed effects, regression coefficients, and variance components from the mixed model analysis of net feed intake and daily feed intake (grams).

Model		NFI				DFI			
MWT									
Effect		*LS Mean*	*SE*	*Coeff.*	*SE*	*LS Mean*	*SE*	*Coeff.*	*SE*
MGP	15	-0.07	0.18			8.24	0.51		
	18	-0.18	0.14			7.21	0.54		
	19	-0.15	0.07			7.57	0.55		
	20	0.23	0.10			8.00	0.61		
	21	0.18	0.07			8.58	0.70		
Line	C	-0.07	0.08			6.81	0.81		
	H	0.33	0.09			8.81	0.80		
	L	-0.26	0.08			8.14	0.81		
MWT				0.003	0.004			0.178	0.006
MGPxMWT	15			0.000	0.008			0.176	0.012
	18			0.000	0.008			0.189	0.012
	19			0.001	0.004			0.187	0.006
	20			-0.004	0.006			0.175	0.008
	21			0.003	0.004			0.178	0.006
Line*MWT	C			-0.002	0.005			0.181	0.007
	H			0.005	0.005			0.176	0.007
	L			0.003	0.004			0.178	0.006
Var. Comp.		*Estim.*				*Estim.*			
ID(Line)		0.00				0.00			
Residual		1.06				2.21			
ADG									
Effect		*LS Mean*	*SE*	*Coeff.*	*SE*	*LS Mean*	*SE*	*Coeff.*	*SE*
MGP	15	-0.05	0.19			8.08	0.62		
	18	-0.08	0.15			7.64	0.50		
	19	-0.15	0.07			7.83	0.25		
	20	0.23	0.10			8.02	0.35		
	21	0.17	0.07			8.24	0.25		
Line	C	-0.06	0.08			7.83	0.27		
	H	0.36	0.09			8.37	0.31		
	L	-0.24	0.08			7.69	0.27		
ADG				0.02	0.07			2.18	0.24
MGPxADG	15			-0.01	0.14			2.23	0.47
	18			0.18	0.12			2.45	0.42
	19			0.00	0.07			2.06	0.23
	20			-0.06	0.09			2.04	0.29
	21			0.02	0.07			2.18	0.24
LinexADG	C			0.02	0.07			2.45	0.24
	H			0.03	0.07			2.06	0.25
	L			0.02	0.07			2.18	0.24
Var. Comp.		*Estim.*				*Estim.*			
ID(Line)		0.00				0.00			
Residual		1.05				11.92			

MWT+ADG									
Effect		*LS Mean*	*SE*	*Coeff.*	*SE*	*LS Mean*	*SE*	*Coeff.*	*SE*
MGP	15	-0.04	0.18			8.05	0.30		
	18	-0.09	0.15			7.69	0.28		
	19	-0.15	0.07			7.65	0.25		
	20	0.24	0.10			8.13	0.28		
	21	0.16	0.07			8.44	0.31		
Line	C	-0.06	0.08			7.59	0.40		
	H	0.37	0.09			8.64	0.41		
	L	-0.24	0.08			7.75	0.40		
MWT				0.002	0.006			0.16	0.01
ADG				0.016	0.087			0.49	0.11
MGPxMWT	15			0.001	0.011			0.16	0.01
	18			-0.012	0.010			0.15	0.01
	19			0.007	0.005			0.17	0.01
	20			-0.002	0.007			0.15	0.01
	21			0.002	0.006			0.16	0.01
MGPxADG	15			0.005	0.185			0.50	0.22
	18			0.302	0.154			1.10	0.19
	19			-0.153	0.085			0.45	0.10
	20			-0.043	0.106			0.72	0.13
	21			0.016	0.087			0.49	0.11
LinexMWT	C			-0.008	0.006			0.15	0.01
	H			0.005	0.006			0.16	0.01
	L			0.002	0.006			0.16	0.01
LinexADG	C			0.112	0.091			0.74	0.11
	H			-0.016	0.090			0.51	0.11
	L			0.016	0.087			0.49	0.11
Var. Comp.		*Estim.*				*Estim.*			
ID(Line)		0.00				0.00			
Residual		1.04				1.51			

The primary tests of interest in this analysis were the interactions of weight or gain with line. In the first model, the overall interaction of line with mid-weight was not significant for either net feed intake or daily feed intake. The same results were observed for daily gain by itself in the second model. In the third model, which incorporated both body weight and gain, there was a significant interaction between line and mid-weight for net feed intake, and between line and average daily gain for

daily feed intake. In both cases, the selection lines were quite similar, and when the control line was removed from the analysis, the interactions with line disappeared.

Discussion

The 3-week average data (Approach 1) demonstrated a marked improvement in the fit of the model used to estimate net feed intake from daily feed intake. This suggests that the up-regulated physiological load imposed by pregnancy and lactation on intake acted primarily through growth rate and weight maintained, with little impact on those physiological parameters that were independent of growth or body weight (i.e. components of net feed intake). Examination of the standard deviations for net feed intake and daily feed intake confirmed these findings. As expected, the variation in daily feed intake increased substantially (by approximately 450% between pre-pregnancy and lactation) throughout the course of the experiment, and particularly during lactation, when the mean daily feed intake for the dam/litter unit was approaching its maximum. However, the variation in net feed intake remained relatively constant throughout the experiment, indicating that the increase in variance in daily feed intake was almost exclusively due to increases in gain or body weight. This was further substantiated by the least-squares means for the two intake traits: prior to pregnancy, the absolute (in terms of mass) contribution of net feed intake to total daily feed intake was approximately 10% in the selection lines, whereas during lactation, it was closer to 1% in both lines.

The much greater contribution of gain and body weight to the observed variation in daily intake during pregnancy and lactation is illustrated in the responses in maintenance efficiency and gross efficiency. In the early stages of the experiment, when production was low, the low intake line had a superior maintenance efficiency

directly attributable to net feed intake. Later, as rates of gain increased and the relative contribution of maintenance to intake was reduced, the lines converged despite retaining significant divergence in net feed intake. This was most evident during lactation, when the lines were no longer significantly different for maintenance efficiency, and was attributed to a faster rate of gain of the litters in the low line during lactation, and a concomitant increase in gain-related intake rather than net feed intake. Although there were no differences between the lines in gross efficiency at any stage, the trend was for the low line to be more efficient during lactation, again due to higher rates of gain rather than as a consequence of their underlying net feed intake.

Another important observation was that the line differences in net fed intake tended to converge over the course of the experiment. This suggests that, independent of the relationship between net feed intake and daily feed intake mediated by the changes in growth and body weight outlined above, the increase in level of production tended to have a small but significant direct effect on the physiological causes of net feed intake itself. It is not possible to outline the specific effects of pregnancy and lactation, but inferences can be drawn from the work in previous chapters. It may be that the high line was better able to compensate for the morphological and physiological changes that occurred during pregnancy and lactation by re-allocating previously 'wasted' intake to growth and development of a litter, thereby reducing their intake net of growth and body weight, whereas the low line, already closer to a 'biological' limit of efficiency, was required to increase overall intake to support the relative inefficiencies of the reproductive 'machinery'. The work of Rauw *et al.* (1999) on litter size selection lines (see Chapter 7) drew some important corollaries with the current work in this respect. The authors examined the responses in net feed intake and associated

traits in both high litter size and control lines during pregnancy and lactation. The premise of their investigations was that high litter size females, that normally exhibit substantially higher mature net feed intake than their control counterparts under non-productive conditions, were in effect anticipating, physiologically, the metabolically stressful periods of pregnancy and lactation associated with larger litters.

Conceptually, the high litter size line was equivalent to the high intake line of the present study – both exhibited an improved efficiency during pregnancy and lactation due to their greater 'buffering' capacity. However, Rauw *et al.* (1999) noted that according to the Resource Allocation Theory of Beilharz *et al.* (1993), greatly increasing litter sizes by means of artificial selection may drastically change the resource allocation pattern. In a resource-limited environment this may result in the situation where buffer resources and resources for processes other than reproduction are reallocated towards pregnancy and lactation. The first law of thermodynamics, which recognises conservation of energy, prevents a female from producing and sustaining larger litters than she can energetically support. Increasing litter size beyond the point that can be supported by intake of feed must then result in reallocation of maternal requirements to offspring, or in diminished offspring development.

It is this point that highlights the fundamental difference between the results of Rauw *et al.* (1999) selecting on litter size and the current study selecting on net feed intake. In the case of the litter size selection line, although females became more efficient under the physiological stress of pregnancy and lactation, the greater demands of a larger litter caused a loss of condition that was maintained through to weaning. Furthermore, the greater efficiency was insufficient to supply offspring with adequate

resources, resulting in reduced pup development and increased pre-weaning mortality rates. Reallocation towards reproductive performance of buffer resources that were otherwise available for processes such as physical activity, responses to pathogens and stress put the animals more at risk of behavioural, physiological and immunological problems (Rauw *et al.*, 1998) and may have compromised future reproductive potential (Rogowitz, 1998). In the current experiment, although there was a small positive correlation between net feed intake and litter size (Chapter 7), the resulting differences were not large enough to create a substantially higher physiological load on the high net feed intake females during pregnancy and lactation – in effect, their greater buffering capacity was under-exploited and they proved to be more efficient than their low line counterparts without any loss in reproductive capacity. Physiologically, the low net feed intake line was equivalent to the high litter size line during pregnancy and lactation – they both were at the limits of their physiological capacity to supply elevated levels of production, necessitating either a decrease in production (large litter size line) or a decrease in efficiency (low net feed intake line). Overall, the results from the 3 week averages suggested that selection for post-weaning net feed intake acted upon underlying physiological processes associated with mature maintenance that, while remaining present during up-regulated phases of production, are of far less significance to overall efficiency.

The greater resolution of the weekly data produced a number of results that improved substantially on those of the 3-week averages. The convergence in net feed intake of the selection lines over the entire period observed earlier appears to be due almost entirely to the events occurring during early- to mid-lactation. Energy intake from farrowing to peak lactation increases greatly to acquire sufficient energy for maternal maintenance and milk production (Rauw *et. al.*, 1999). In addition, to accommodate

this large increase in feed demands, lactating mice experience an increase in liver, heart, lung and gut size (Speakman and McQueenie, 1996).

From the results for gain and mid-weight, mid-lactation would appear to have been the point at which litters reached the point of inflection on the traditional growth curve, again underlying it's significance in the morphological and physiological development of mammalian species. The gain component of intake appears paramount in determining the relative contribution of net feed intake (at least when it has been selected upon post-weaning) to overall efficiency. It may be that there is a threshold in the rate of gain above which the contribution of net feed intake is biologically (and therefore economically) unimportant. This is particularly damning in light of the fact that the point of inflection on the growth curve, when growth rate is at its maximum, is generally also close to point at which animals reach their maximum daily intake (Parks, 1982). However, it should be noted that, in the current experiment, the selection lines appeared to re-diverge towards the end of lactation. Indeed, the divergence was actually greater in the final week than in the beginning week. Given that the convergence in efficiency was for only 2 weeks out of 11, it would appear that the overall effect of the response to selection was still of significant value in breeding females, which was the original aim of the selection program.

The substantial divergence in the final week was probably due a range of factors:

1. Pups were approaching weaning age and hence were probably progressing on to dry food. The inefficiencies associated with the conversion of dry matter to milk, and then from milk to body mass, which would tend to dilute line differences in net feed intake, were thus being progressively reduced.

2. The pups were also approaching the age at which selection for net feed intake had been undertaken in the past. Although high, the correlation between post-weaning and mature net feed intake was less than one (r_G=0.6), and hence the measure of mature net feed intake for the dams observed at the beginning of the test would have tended to result in smaller line differences than measures from a combination of the dams' mature net feed intakes and pups' weaning net feed intakes.

3. Pups became more active in late lactation – any line differences due to differing levels of activity would consequently have become more pronounced.

The results from the fourth approach are less conclusive. Given that net feed intake is a trait which already accounts for the effects of gain and body weight, it was not surprising that the models incorporating these individual traits tended to show no significant differences between the selection lines in their response. However, similar results for daily feed intake tend to echo the results obtained by the previous approaches during lactation: although there were significant line differences in intake at low levels of production, these tended to be swamped by the effects of gain and body weight maintenance when taken over the course of the whole experiment. Within this approach, it would appear that selection for net feed intake had little effect on the overall efficiency of the system, regardless of production level.

Overall, the results from this experiment suggested that the underlying physiological basis for net feed intake was process-dependant. Although measures of net feed intake at different ages have previously been demonstrated to be positively correlated, it would appear that measures of net feed intake under different production environments (e.g. individual growth vs. dam/litter growth during lactation) were substantially different. Clearly, the effects of selection for net feed intake on system-

wide efficiency in a given population would be heavily dependant on the length of time breeding individuals were required to remain at specific levels of production (e.g. maintenance vs. lactation).

A number of previous studies in both dairy cattle (van Arendonk *et al.*, 1991; Ngwerume and Mao, 1992; Svendsen *et al.*, 1993; Veerkamp *et* al., 1995) and laying hens (Luiting and Urff, 1991a; Bordas *et al.*, 1992; Luiting, 1991) have clearly demonstrated that significant variation in net feed intake remains during elevated levels of production. It must be noted that both of these species represent highly productive breeds, and the same may not be true of breeds that are primarily bred for the production of meat only. Furthermore, and possibly of greater importance, in these studies level of production itself was incorporated in the model of intake used to estimate net feed intake. Clearly, one could add variables to the model indefinitely until all biologically significant variation in intake was explained, and net feed intake was effectively zero. However, one of the aims of selection is to provide a trait that is relatively easy to measure, and that accounts for a large proportion of the variation in the underlying trait of interest without coming up against the law of diminishing returns. Furthermore, including production tends to assume that selection decisions will be based on mature, productive individuals, when the basic premise of selection for net feed intake in the current study was to conduct it at a young age so as to allow a more rapid response and a shorter generation interval. It may be useful to estimate net feed intake from a limited number of variables in the first instance (e.g. growth and body weight), but to measure these variables under a range of production environments, so as to produce a better understanding of the correlations between successive measures of net feed intake. Extrapolating this to its logical extreme, further study may be warranted to determine whether it is possible to select on an

index of net feed intake under different production conditions, and the most cost-effective method for doing so in an animal production environment. It may yet prove that there is enough variation in feed intake net of growth and maintenance in specific environments (both production-type and geographical-type), particularly in species where progeny numbers are high, or in breeds with high levels of production, to make it both biologically and economically feasible to select upon.

Conclusion.

Although selection for reduced post-weaning net feed intake produced significant improvements in efficiency during early growth and development, and at maturity under maintenance conditions, these improvements were not maintained during pregnancy and lactation. However, it must be noted that at no time did the low net feed intake line become less efficient than the high net feed intake. Clearly, the use of net feed intake as a means to improve overall production efficiency, and particularly with respect to the productive capacity of dams, will be heavily dependent on the production environment – the age at which selection is carried out, the breeding system (ratio of progeny to parents, sires to dams, etc…), the length of time spent in a productive status versus a maintenance status, etc…

In conclusion, it must be emphasised that the efficiency of the lines in the present study converged for only a short period during peak lactation, possibly during a period of negative energy balance, and re-diverged shortly afterwards. In a production environment, where peak lactation often coincides with peak pasture supply, this would have little impact on overall production efficiency. Provided periods of limited feed supply coincide with periods of low production, selection for

post-weaning net feed intake based on growth/weight components alone remains an attractive means to improve overall production efficiency.

Appendix 1.

Experimental animal numbers.

Table A1.1. Numbers of animals measured post-weaning by sex, generation and replicate (Chapter 2).

		NFI_{PW}		Wt_{21}		DFI_{PW}		DFO_{PW}		ADG_{PW}		MWT_{PW}		FCR_{PW}		GE_{PW}		$\%F_{PW}$	
G	R	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1[a]	1	50	61	58	67	*50*	*61*	0	0	*53*	*66*	*53*	*66*	50	61	50	61	0	0
2[a]	1	38	52	44	54	*38*	*52*	0	0	*43*	*53*	*43*	*53*	38	52	38	52	15	0
2[a]	2	59	47	61	48	*59*	*47*	0	0	*60*	*48*	*60*	*48*	59	47	59	47	51	0
3[a]	1	87	77	93	92	*87*	*77*	0	0	*89*	*89*	*89*	*89*	87	77	87	77	89	89
3[a]	2	93	88	100	94	*93*	*88*	0	0	*100*	*94*	*100*	*94*	93	88	93	88	99	94
4[a]	1	98	88	147	137	*98*	*88*	0	0	*105*	*93*	*105*	*93*	98	88	98	88	105	93
4[a]	2	97	90	168	149	*97*	*90*	0	0	*99*	*93*	*99*	*93*	97	90	97	90	99	93
4[a]	3	92	96	158	148	*92*	*96*	0	0	*98*	*98*	*98*	*98*	92	96	92	96	98	98
5[b]	1	83	95	134	149	*83*	*95*	0	0	*87*	*104*	*87*	*104*	83	95	83	95	0	0
5[c]	2	0	0	142	145	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
5[c]	3	0	0	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
6[d]	1	77	76	97	98	*77*	*76*	0	0	*92*	*94*	*92*	*94*	77	76	77	76	0	0
6[c]	2	0	0	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
7[e]	1	79/**26**	83/**28**	87/**31**	95/**29**	*79/**26***	*83/**28***	0/**25**	0/**25**	*82/**26***	*90/**28***	*82/**26***	*90/**28***	79/**26**	83/**28**	79/**26**	83/**28**	0/**24**	0/**24**
7[c]	2	**49**	**47**	**53**	**52**	***49***	***47***	**45**	**47**	***50***	***49***	***50***	***49***	**49**	**47**	**49**	**47**	**1**	**9**
7[c]	3	**14**	**24**	**32**	**39**	***14***	***24***	**12**	**25**	***17***	***25***	***17***	***25***	**14**	**24**	**14**	**24**	**0**	**0**
7[c]	4	**0**	**0**	**0**	**0**	***0***	***0***	**0**	**0**	***0***	***0***	***0***	***0***	**0**	**0**	**0**	**0**	**0**	**0**
8[d]	1	84	81	98	102	*84*	*81*	0	0	*97*	*101*	*97*	*101*	84	81	84	81	0	0
8[c]	2	29	30	46	52	*29*	*30*	0	0	*29*	*31*	*29*	*31*	29	30	29	30	0	0
8[c]	3	18	26	47	54	*18*	*26*	0	0	*18*	*26*	*18*	*26*	18	26	18	26	0	0
9[d]	1	80	75	96	91	*80*	*75*	0	0	*93*	*89*	*93*	*89*	80	75	80	75	0	0
10[d]	1	**87**	**85**	**126**	**119**	***87***	***85***	**84**	**82**	***88***	***85***	***88***	***85***	**87**	**85**	**87**	**85**	**79**	**70**
10[d]	2	**100**	**130**	**107**	**133**	***100***	***130***	**98**	**128**	***101***	***131***	***101***	***131***	**100**	**130**	**100**	**130**	**16**	**33**
10[d]	3	**83**	**71**	**108**	**96**	***83***	***71***	**86**	**72**	***89***	***79***	***89***	***79***	**83**	**71**	**83**	**71**	**0**	**0**
11[d]	1	55	58	87	85	*55*	*58*	0	0	*58*	*58*	*58*	*58*	55	57	55	58	0	0
11[d]	2	31	40	58	79	*31*	*40*	0	0	*31*	*40*	*31*	*40*	31	40	31	40	0	0
	Σ	1509	1548	2178	2207	*1509*	*1548*	350	379	*1605*	*1664*	*1605*	*1664*	1509	1547	1509	1548	676	603

[a] *Unselected individuals*
[b] High, low and control lines
[c] Control line only
[d] High and low line only
[e] High and low lines/Control line

Italicised numbers represent individuals used to estimate NFI_{PW} on accumulated data set.

Bold numbers represent individuals used to analyse correlated responses in generation 10 (high and low lines) and generation 7 (control line).

Table A1.2. Numbers of animals measured at maturity by sex, generation and replicate (Chapter 3).

		NFI_{MAT}		DFI_{MAT}		DFO_{MAT}		ADG_{MAT}		MWT_{MAT}		MR_{MAT}		ME_{MAT}		$\%F_{MAT}$	
G	R	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
1[a]	1	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
2[a]	1	0	51	*0*	*51*	0	0	*0*	*52*	*0*	*52*	0	51	0	51	0	0
2[a]	2	0	45	*0*	*45*	0	0	*0*	*47*	*0*	*47*	0	45	0	45	0	0
3[a]	1	0	86	*0*	*86*	0	0	*0*	*87*	*0*	*87*	0	86	0	86	0	0
3[a]	2	0	89	*0*	*89*	0	0	*0*	*89*	*0*	*89*	0	89	0	89	0	0
4[a]	1	0	89	*0*	*89*	0	0	*0*	*90*	*0*	*90*	0	89	0	89	0	0
4[a]	2	0	86	*0*	*86*	0	0	*0*	*86*	*0*	*86*	0	86	0	86	0	0
4[a]	3	0	58	*0*	*58*	0	0	*0*	*59*	*0*	*59*	0	58	0	58	0	0
5[b]	1	0	99	*0*	*99*	0	0	*0*	*102*	*0*	*102*	0	99	0	99	0	0
5[c]	2	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
5[c]	3	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
6[d]	1	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
6[c]	2	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
7[e]	1	**0**	**0**	***0***	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
7[c]	2	**19**	**0**	***19***	***0***	**19**	**0**	***19***	***0***	***19***	***0***	**19**	**0**	**19**	**0**	**2**	**4**
7[c]	3	**24**	**7**	***24***	***7***	**23**	**6**	***26***	***7***	***26***	***7***	**24**	**7**	**24**	**7**	**16**	**1**
7[c]	4	**0**	**0**	***0***	***0***	**0**	**0**	***0***	***0***	***0***	***0***	**0**	**0**	**0**	**0**	**6**	**3**
8[d]	1	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
8[c]	2	20	38	*20*	*38*	0	0	*20*	*38*	*20*	*38*	20	38	20	38	0	0
8[c]	3	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
9[d]	1	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
10[d]	1	**62**	**0**	***62***	***0***	**62**	**0**	***64***	***0***	***64***	***0***	**62**	**0**	**62**	**0**	**6**	**12**
10[d]	2	**80**	**62**	***80***	***62***	**74**	**63**	***83***	***63***	***83***	***63***	**80**	**62**	**80**	**62**	**18**	**12**
10[d]	3	**0**	**0**	***0***	***0***	**0**	**0**	***0***	***0***	***0***	***0***	**0**	**0**	**0**	**0**	**44**	**10**
11[d]	1	55	57	*55*	*57*	0	0	*55*	*57*	*55*	*57*	55	57	55	57	0	0
11[d]	2	0	0	*0*	*0*	0	0	*0*	*0*	*0*	*0*	0	0	0	0	0	0
	Σ	260	767	*260*	*767*	178	69	*267*	*777*	*267*	*777*	260	767	260	767	92	42

[a] Unselected individuals
[b] *High, low and control lines*
[c] Control line only
[d] High and low lines/Control line
[e] High and low line only

Italicised numbers represent individuals used to estimate NFI_{PW} on accumulated data set.

Bold numbers represent individuals used to analyse correlated responses in generation 10 (high and low lines) and generation 7 (control line).

Table A1.3. Numbers of organs measured by sex and line (Chapter 5).

	Control		High		Low	
Organ	♂	♀	♂	♀	♂	♀
Heart	21	11	38	16	52	26
Liver	18	10	34	18	52	23
Caecum	20	11	37	14	46	25
Stomach	23	11	36	15	47	26
Intestine	17	11	32	12	45	25

Table A1.4. Numbers of litters recorded by generation, line and parity (Chapter 6).

	Control			High			Low		
Parity	1	2	3	1	2	3	1	2	3
Generation 10	23	15	10	28	26	22	24	25	23
Generation 11	15	9	0	12	8	0	16	7	0

Appendix 2.

Development of a semi-automated, open-circuit indirect calorimeter for small animals.

Overview

Calorimetry is the measurement of heat. By means of animal calorimetry we can estimate the energy costs of living. All life processes including growth, work and agricultural production (milk, eggs, wool, etc.) use energy, the source of the energy being food. The energy content of food is metabolised in the body into other forms, only some of which are useful in the sense of growth or production. Much of the wasted energy is given off from the body in the form of heat.

The heat may be measured directly by physical methods (Direct Calorimetry) or it may be inferred from quantitative measurement of some of the chemical by-products of metabolism (Indirect Calorimetry). These alternatives are possible because of the natural constraints imposed on energy transformations by the laws of thermodynamics. Of fundamental importance are the Law of Conservation of Energy (energy cannot be created or destroyed, only changed in form) and Hess' Law of Constant Heat Summation (the heat released by a chain of reactions is independent of the chemical pathways, and dependent only on the end-products). In effect, these laws ensure that the heat evolved in the enormously complex cycle of biochemical reactions that occur in the body is exactly the same as that which is measured when the same food is converted into the same end-products by simple combustion on a laboratory bench or in a calorimeter.

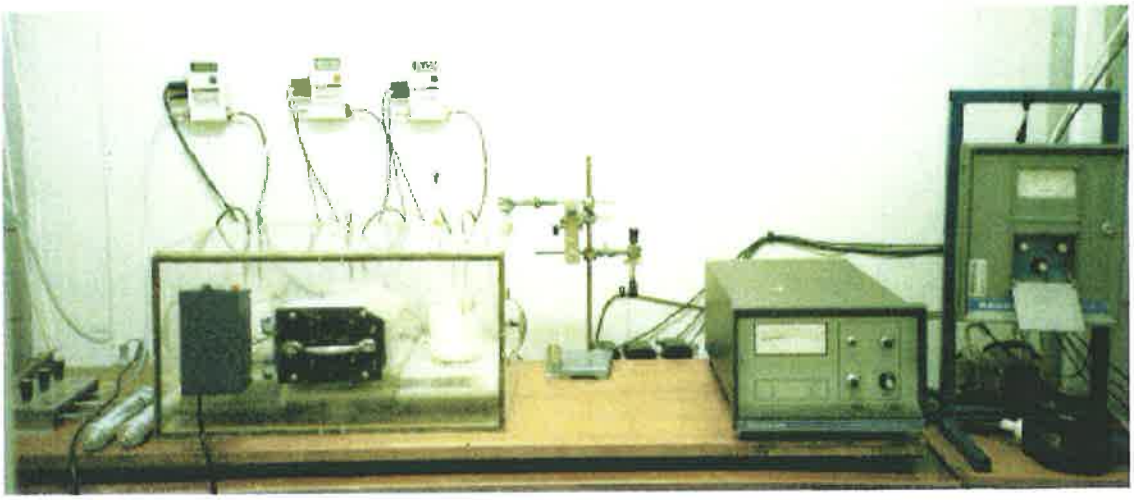
Whilst direct calorimeters measure the rate of heat dissipation of a subject, indirect calorimeters measure the rate of heat generation; averaged over a long period of time the two rates will be equal or very nearly so. Indirect calorimetry estimates heat production from quantitative measurements of materials consumed and produced during metabolism. Most methods involve estimation of respiratory gas exchange and these may be classified according to their operating principles as confinement, closed circuit, total collection and open-circuit systems.

In confinement systems the subject is held in a sealed chamber and the rates of change of gas concentration in the chamber are recorded.

In closed-circuit systems the subject is again held within or breathes into a sealed apparatus; the carbon dioxide and water vapour produced by the subject are measured as the weight gain of appropriate absorbers, and the amount of oxygen consumed is measured by metering the amount required to replenish the system. In total collection systems all the air expired by the subject is accumulated in order to measure subsequently its volume and chemical composition.

There are two major forms of open-circuit calorimetry. In one the subject breathes directly from atmosphere and by means of a non-return valve system expires into a separate outlet line. In the second form, the subject inspires from, and expires to, a stream of air passing, by means of a pump or fan, across the face. In both cases the flow of air is measured either on the inlet or outlet side of the subject. Air from the outlet is either collected continuously or periodically for later analysis, or is sampled continuously for on-line analysis. It is with a particular variant of this form of indirect calorimeter that the following description deals.

Figure A2.1. The Waite Institute small animal calorimeter.



For fast-response measurements and for long-term studies, an open-circuit system employing flow meters, electronic gas analysers and recording equipment is necessary. We built a semi-automated flow-through calorimeter for studies on mice.

The circuit of this simple multi-chamber system is shown in Figure A1.2 It had three perspex chambers (~500 ml) that acted as animal cages. These were painted flat white to remove between-chamber visual contact and to reduce the influence of heat radiated from the animal on temperature within the chamber (see below). Each chamber was lined with animal litter during measurement to simulate the animal's normal environment and to absorb faecal waste and urine. The chambers were kept within a secondary perspex chamber which provided a controlled temperature environment. This chamber was normally maintained at 28°C, which represents the mid-point of a mouse's thermo-neutral zone in which metabolism is neither up-regulated to maintain core temperature nor reduced to prevent over-heating.

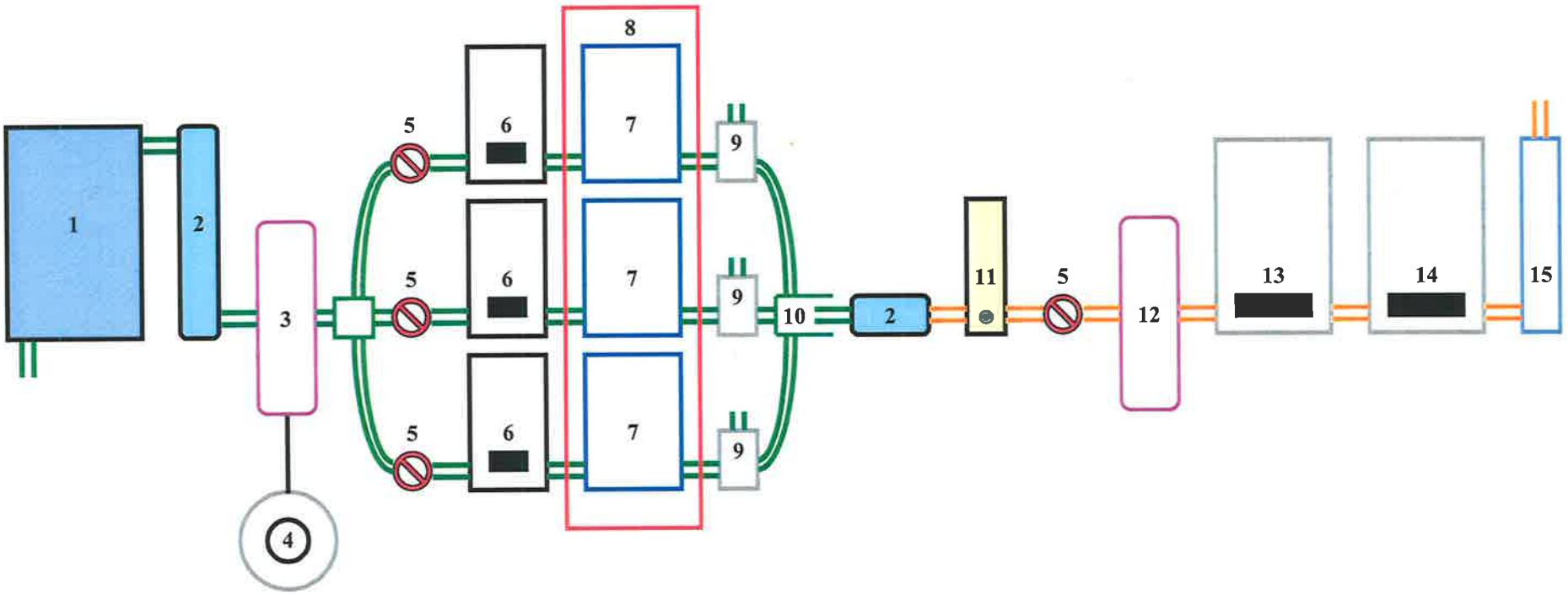
The three modes of sensible heat transfer depend primarily on the difference between the animal's surface temperature and the corresponding environmental temperature (Dauncey, 1991). For radiative, convective and conductive heat transfer, these

temperatures are radiant, air and floor respectively. For determining metabolic rate under standard conditions, it is simplest to ensure that the air and radiant temperatures are the same. This was achieved in the current study by housing the chambers within a temperature controlled, positive airflow perspex box under a constant artificial light source, effectively making use of the concept of a false inner wall. The chambers themselves were internally coated in a radiatively neutral flat white paint. Convective heat loss was reduced by maintaining a low rate of passage of dry air through the chamber. Conductive heat loss was minimised in a similar manner to that of radiation: the chambers were elevated on a small central platform (~1cm diameter) within the temperature controlled box, thus maintaining a thermoneutral surface on which the mice rested, which itself was overlaid with standard bedding, acting as an insulator.

Fresh, atmospheric air was pumped into the chambers through 6 ml tubing by a single serially-wound diaphragm pump whose overall flow rate was coarsely controlled via a rheostat. Needle valves provided fine control over individual chamber flow rates.

Prior to chamber entry, the air was first passed through a cold-water bath (0°C) via a series of coiled water traps to remove moisture from the air. It then passed through a secondary chemical drier (Drierite indicating $CaSO_4$), prior to trifurcating into the three chamber lines. Flow rate was measured on the inlet side of the chambers via three mass flow meters calibrated for flows of up to 500 ml/minute. The advantage of mass flow meters over other forms of flow measurement is their independence from the influence of temperature and pressure variations in the measured air stream. The small volume of the chamber and relatively large flow rates (400-480 ml/min)

Figure A2.2. Diagram of the semi-automated multi-channel calorimeter used at Waite for measurement of the energy expenditure of mice.



1. Water bath
2. Drierite
3. Pump (450 ml/min)
4. Rheostat
5. Needle valve
6. Mass flow meter
7. Chamber
8. Temperature control box
9. 3-way solenoid valve
10. Atmospheric vent
11. Ball flow meter
12. Pump (350 ml/min)
13. O_2 analyser
14. CO_2 analyser
15. Absolute back-pressure regulator

resulted in a relatively small lag time of approximately 3 minutes, whilst the small diameter of inlet and outlet ports and the movement of animal itself resulted in an adequate mixing of chamber air without the aid of a fan.

Outlet air passed through 6 ml tubing to three solenoid valves which regulated chamber flow to the gas analysers. Timing of the solenoid valves was digitally controlled by a Strawberry Tree analogue to digital converter and associated software. Off-line chambers were vented to atmosphere whilst the single on-line chamber flow passed to an open-air vent where it was sub-sampled by a second diaphragm pump running at 300 ml/min. Flow rate was again regulated by needle valve. This sample was passed through a chemical drying agent (Drierite) prior to entry into an Beckmann infra-red Carbon Dioxide analyser and a Beckmann paramagnetic Oxygen analyser. The operation of these analysers is detailed extensively elsewhere (McClean and Tobin, 1987). Finally, flow was directed to atmosphere via a back-pressure regulator which maintained a constant pressure within the analyser system (the Oxygen analyser, essentially measuring partial pressure of oxygen, was particularly susceptible to variation in atmospheric pressure).

Activity was measured as the deviation of successive (0.5 seconds) weight measurements on a fine digital scale placed beneath each metabolic chamber. The theory was as follows: scales measure the action of gravity on mass. For a stationary object, the weight reading should remain constant. However, a moving object such as a mouse tends to exert a force in opposition to gravity to displace its mass. This force is muscular in nature in larger biological organisms, and is indirectly translated as a transient force applied to a fulcrum, in the present case the floor of the metabolic chamber. The magnitude of the force was represented by the deviation of the scale

reading from the previous measure of weight of the animal. Given a strong correlation between measured activity and metabolic rate in 24 hour studies, the theory appeared sound.

The gas analysers were calibrated daily using downscale and upscale calibration gases flowed under the same temperature, pressure and flow conditions as those during measurement.

All analysers, flow meters and scales were data-logged via the A/D converter. The measurement protocol was as follows. Each chamber was on-line for one minute. The first 55 seconds were not recorded and acted as an adjustment phase during which time the dead air-space from the previous chamber was flushed from the system. Ten half-second measurements were then recorded prior to activation of the solenoid switch. After all three chambers had been on-line once, atmospheric air was vented into the system to measure drift occurring in the analyser readings. The cycle then began again. Flow and activity were measured at half-second intervals for each chamber for the entire measurement period.

Metabolic Rate Calculation

In closed-circuit systems the primary measurements are oxygen consumption and carbon dioxide and methane production. These quantities may be substituted directly into an equation of the Brouwer type (see McClean and Tobin, 1987, table 3.8). If the quantities of food intake and excreta are recorded and these materials sampled and analysed for carbon and nitrogen content, heat production may alternatively be calculated from the carbon-nitrogen balance.

For open-circuit systems the primary quantities measured are ventilation rate and the composition of inlet and outlet air; the computation of oxygen consumption (V_{O_2}), respiratory quotient (r) and metabolic rate (M) appears more complex at first. The principle of the calculations is the same regardless of whether the ventilating air stream consists purely of respired air as in a mask, mouthpiece or tracheal cannula fitted with inspiratory and expiratory valves, or whether the subject breathes freely into a moving air stream as in a ventilated hood or respiration chamber.

Inlet air is often fresh air which may contain up to 5% by volume of water vapour. The remaining part (i.e. dried, fresh air) has a constant composition and contains 20.95% oxygen, 0.03% carbon dioxide and 79.02% inert gases, which consist primarily of nitrogen. Thus

$$F_{IO_2} = 0.2095$$

$$F_{ICO_2} = 0.0003$$

$$F_{IN_2} = 0.7902$$

Because these values are constant for dry fresh air and because in most gas analysers the air is dried before analysis, gas concentrations are normally expressed as fractions of dry air. Hence for exhaust air or expired air the gas concentrations are F_{EO_2}, F_{ECO_2} and for ruminant animals which also produce methane, F_{CH_4}.

A major advantage of the whole-body instrument is that the subject is unhampered by close-fitting apparatus. Accurate measurements can therefore be made over periods of 24 hours or more. However, the larger instrument has an inherently slow response time, the duration of which is related to the size of the chamber and its ventilation rate. The oxygen concentration in a calorimeter at time t after a unit step change in gas production is of the form:

$$1 - \exp(-tF/V)$$

where V is the volume of the calorimeter and F the air-flow rate. The time constant V/F is the time for a 63 per cent response to a step change in gas production. Three time constants give the time for a 95 per cent response. With large time constants it is only possible, using conventional analysis, to estimate gas exchange at all accurately in the steady state. In the non-steady state nitrogen is no longer conserved owing to the gases in the calorimeter being alternatively concentrated and diluted over short periods of time. However, this can be accounted for in a more complete theoretical analysis which allows the volume of nitrogen in the calorimeter to change with time. This is covered in great detail in a 1984 paper by Brown *et al.* For the sake of brevity, it is enough to note that Method II of the Brown paper was the method of choice for calculation of the rapid response concentrations of O_2 and CO_2 in the Waite calorimeter. This required minimal computational effort and used the difference between the value of gas concentration at the time point immediately before the point in question and that at the time point immediately afterwards, divided by the time interval between them, and linear interpolation to estimate simultaneous values for chamber and atmospheric gas concentrations. The average of each set of ten consecutive readings at all points of gas measurement was used to improve precision by removing random variability in repeat readings on the same sample.

Values for O_2 consumption and CO_2 production were then substituted into a standard equation for calculation of metabolic rate in kW (McClean and Tobin, 1987).

$$M = \alpha V_{O_2} + \beta V_{CO_2}$$

where α and β are constants derived empirically by Brouwer (16.18 and 5.02 respectively).

The improved transient response of the method detailed by Dauncey *et al.* (1984) accounted for any changes in metabolism that occured during the relatively short periods of measurement undertaken on the Waite calorimeter. It also allowed correlation of metabolic rate with the instantaneous measures of activity derived from the scale-type activity monitor. It came at a cost of increased noise in the estimates during periods of changing gas production or consumption, but this effect was small due to the relatively small time constant for this particular system.

Appendix 3.

ANOVA solutions to analysis of activity and metabolism (Chapter 5).

Table A3.1. ANOVA solutions for selected fixed effects from analysis of activity data.

Effect			Estimate	SE
Intercept			-0.016	0.032
Metabolic status	Fasted		0.065	0.020
	Fed		0.000	.
Time of day	Early morning		0.006	0.011
	Late morning		0.014	0.010
	Early afternoon		0.001	0.011
	Late afternoon		0.000	.
Parity	1		-0.008	0.010
	2		0.000	.
Age[a]			0.001	0.009
Weight			0.002	0.001
Sex	Female		0.005	0.011
	Male		0.000	.
Line	Control		0.010	0.022
	High		-0.006	0.021
	Low		0.000	.
M_status x Intake	Fasted		0.048	0.019
	Fed		0.020	0.019
M_status x Time of day	Fasted	Early morning	0.047	0.007
	Fasted	Late morning	-0.003	0.006
	Fasted	Early afternoon	-0.000	0.007
	Fasted	Late afternoon	0.000	.
	Fed	Early morning	0.000	.
	Fed	Late morning	0.000	.
	Fed	Early afternoon	0.000	.
	Fed	Late afternoon	0.000	.
M_status x Parity	Fasted	1	0.022	0.007
	Fasted	2	0.000	.
	Fed	1	0.000	.
	Fed	2	0.000	.
M_status x Age[a]	Fasted		-0.013	0.005
	Fed		0.000	.
M_status x Weight	Fasted		0.001	0.001
	Fed		0.000	.
M_status x Sex	Fasted	Female	-0.026	0.007
	Fasted	Male	0.000	.
	Fed	Female	0.000	.
	Fed	Male	0.000	.

Effect			Estimate	SE
M_status x Line	Fasted	Control	0.002	0.014
	Fasted	High	0.026	0.013
	Fasted	Low	0.000	.
	Fed	Control	0.000	.
	Fed	High	0.000	.
	Fed	Low	0.000	.
M_stat. x Intake x Line	Fasted	Control	-0.073	0.032
	Fasted	High	-0.053	0.027
	Fasted	Low	0.000	.
	Fed	Control	-0.024	0.032
	Fed	High	0.003	0.027
	Fed	Low	0.000	.

[a] $x10^{-2}$

Table A3.2. ANOVA solutions for selected fixed effects from analysis of metabolic data.

Effect			Estimate	SE
Intercept			28.59	1.68
Metabolic status	Fasted		-3.59	0.84
	Fed		0.00	.
Time of day	Early morning		0.61	0.56
	Late morning		-0.06	0.54
	Early afternoon		-0.28	0.56
	Late afternoon		0.00	.
Parity	1		-0.06	0.54
	2		0.00	.
Age[a]			-0.87	0.45
Weight			0.29	0.04
ln(Activity)			3.51	0.07
Sex	Female		-0.17	0.60
	Male		0.00	.
Line	Control		0.67	1.23
	High		-0.29	1.15
	Low		0.00	.
M_status x Intake	Fasted		-0.33	0.96
	Fed		3.05	0.97
M_status x Time of day	Fasted	Early morning	0.71	0.26
	Fasted	Late morning	0.36	0.24
	Fasted	Early afternoon	0.50	0.26
	Fasted	Late afternoon	0.00	.
	Fed	Early morning	0.00	.
	Fed	Late morning	0.00	.
	Fed	Early afternoon	0.00	.
	Fed	Late afternoon	0.00	.

Effect			Estimate	SE
M_status x Parity	Fasted	1	-0.17	0.25
	Fasted	2	0.00	.
	Fed	1	0.00	.
	Fed	2	0.00	.
M_status x Age[a]	Fasted		-0.14	0.21
	Fed		0.00	.
M_status x Weight	Fasted		0.16	0.02
	Fed		0.00	.
M_status x ln(Activity)	Fasted		0.38	0.09
	Fed		0.00	.
M_status x Sex	Fasted	Female	0.07	0.26
	Fasted	Male	0.00	.
	Fed	Female	0.00	.
	Fed	Male	0.00	.
M_status x Line	Fasted	Control	-0.46	0.71
	Fasted	High	-1.31	0.66
	Fasted	Low	0.00	.
	Fed	Control	0.00	.
	Fed	High	0.00	.
	Fed	Low	0.00	.
ln(Activity) x Sex	Female		0.02	0.06
	Male		0.00	.
ln(Activity) x Line	Control		0.07	0.11
	High		-0.25	0.10
	Low		0.00	.
M_stat. x Intake x Line	Fasted	Control	0.86	1.64
	Fasted	High	0.28	1.39
	Fasted	Low	0.00	.
	Fed	Control	0.52	1.64
	Fed	High	-0.27	1.39
	Fed	Low	0.00	.
M_stat. x ln(Activity) x Line	Fasted	Control	0.14	0.14
	Fasted	High	-0.40	0.13
	Fasted	Low	0.00	.
	Fed	Control	0.00	.
	Fed	High	0.00	.
	Fed	Low	0.00	.

[a] $x10^{-2}$

Appendix 4.

Net feed intake during pregnancy tables (Chapter 8).

Table A4.1. Variation in growth and intake traits for 11 successive weeks, excluding weeks 4-5 (Approach 2).

Week	Source	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
1	R^2 (%)	19	26	40	18	14	39	12	13	-	-	-	-
	CV		20.4	-70.8	13.6	-694.5	-80.1	24.5	25.8	-	-	-	-
	Error DF									-	-	-	-
	Error MS									-	-	-	-
	Group	0.3	3.3**	0.7**	92.3	17303.6	0.121**	0.001	15.0	-	-	-	-
	Line	1.8**	0.8	0.06	41.2	21521.7	0.004	0.001	11.6	-	-	-	-
2	R^2 (%)	33	27	2	27	3	4	35	35	-	-	-	-
	CV		15.3	-1457.9	12.8	-1099.0	-719.7	18.1	17.6	-	-	-	-
	Error DF									-	-	-	-
	Error MS									-	-	-	-
	Group	0.8	0.8	0.0	124.1**	4858.1	0.004	0.002**	9.1**	-	-	-	-
	Line	2.7**	3.3**	0.0	39.5	531.0	0.002	0.003**	14.1**	-	-	-	-
3	R^2 (%)	33	26	11	28	3	11	39	40	-	-	-	-
	CV		16.5	-994.7	11.8	-1003.2	-710.1	17.0	16.6	-	-	-	-
	Error DF									-	-	-	-
	Error MS									-	-	-	-
	Group	0.7	0.4	0.1	108.7**	1643.6	0.003	0.002*	7.4**	-	-	-	-
	Line	4.7**	4.6	0.0	32.0	19163.2	0.001	0.006**	17.2**	-	-	-	-
6	R^2 (%)	31	22	37	56	16	43	49	42	-	-	-	-
	CV		21.2	59.5	10.5	1771.3	49.3	19.1	23.7	-	-	-	-
	Error DF									-	-	-	-
	Error MS									-	-	-	-
	Days	3.3*	2.8	6.5**	148.6**	2649.4	0.193**	0.000	0.1	-	-	-	-
	Group	0.7	1.7	0.1	233.7**	2588.1	0.005	0.007**	27.9**	-	-	-	-
	Line	3.4**	4.2*	0.2	44.6	2011.8	0.005	0.005**	10.8*	-	-	-	-

Week	Source	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
7	R^2 (%)	17	28	36	25	1	33	41	36	-	-	-	-
	CV		20.3	59.6	12.8	851.0	77.5	14.9	21.2	-	-	-	-
	Error DF									-	-	-	-
	Error MS									-	-	-	-
	Days	0.0	0.0	1.0	224.5*	9.5	0.106	0.001	12.4*	-	-	-	-
	Group	0.9	7.9**	8.6**	199.8**	179.2	0.310**	0.010	27.4**	-	-	-	-
	Line	2.2*	4.8*	0.6	17.4	95.9	0.008	0.002	4.9	-	-	-	-
8	R^2 (%)	23	41	35	30	10	26	48	44	34	37	38	31
	CV		29.7	58.8	16.6	1331.4	64.7	18.6	23.7	314.7	7.5	24	37.8
	Error DF												
	Error MS												
	Days	3.0	162.5**	35.4**	1574.0**	210.6	0.185*	0.016**	55.7**	4.3**	3.6	0.0	3.7
	Group	2.4	14.4	3.6	358.1*	440.7	0.023	0.003*	7.9	0.1	43.1**	0.1*	7.8
	Line	1.3	0.1	2.7	11.1	1000.6	0.075	0.000	4.4	0.0	0.5	0.1	0.1
9	R^2 (%)	20	25	34	29	23	31	35	37	15	12	24	31
	CV		26.2	38.3	19.9	49.8	26.6	11.6	11.7	388.1	9.8	31.1	24.0
	Error DF												
	Error MS												
	Days	0.0	41.5	0.4	2568.8**	8.7	0.040**	0.000	0.3	0.0	5.8	0.4**	4.0
	Group	6.4*	42.7*	14.3**	1000.5**	29.8**	0.027**	0.003**	5.0**	1.3*	32.4	0.1	15.3**
	Line	2.0	3.1	0.8	55.3	2.5	0.002	0.000	0.4	0.5	6.1	0.0	7.0

Week	Source	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
10	R^2 (%)	18	25	19	23	5	29	16	18	20	15	27	31
	CV		26.8	78.8	21.2	9886.3	55.3	13.1	13.6	-170.2	10.2	51.2	22.2
	Error DF												
	Error MS												
	Days	3.1	31.6	0.0	682.1	2268.8	0.010	0.000	0.1	0.3	0.0	0.3*	1.0
	Group	5.1	88.2**	4.1	1888.4**	12564.2	0.011*	0.001	2.4*	1.5*	36.6	0.1	32.1**
	Line	7.9*	25.8	7.8*	218.2	801.7	0.026**	0.001*	2.8*	1.5*	48.3	0.1	9.2
11	R^2 (%)	28	21	24	18	16	21	20	19	6	27	23	31
	CV		28.1	91.6	24.1	404.4	75.3	10.3	10.1	-185.7	10.9	63.8	22.8
	Error DF												
	Error MS												
	Days	0.5	5.9	2.6	230.4	32563.2	0.009	0.000	0.0	0.5	7.5	0.3	3.9
	Group	1.8	25.9	7.1	870.4	9162.1	0.014	0.000	0.1	0.2	39.1	0.1	29.0*
	Line	12.8**	24.3	7.6	545.1	24042.9	0.018	0.001*	1.6*	0.2	63.9*	0.4*	14.9

* $p > 0.05$
** $p > 0.01$

Table A4.2. Net feed intake selection lines' least squares means for growth and intake traits for 11 successive weeks, excluding weeks 4-5 (Approach 2).

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
1	C	-0.05	3.39	-0.33	37.4	-49.8	-0.12	0.09	11.7	-	-	-	-
	SE	0.11	0.14	0.05	1.0	20.5	0.02	0.00	0.6				
	H	0.39	3.65	-0.42	35.2	12.4	-0.14	0.11	10.2	-	-	-	-
	SE	0.12	0.16	0.06	1.1	22.5	0.02	0.01	0.7				
	L	-0.16	3.27	-0.32	34.9	-10.5	-0.12	0.09	11.1	-	-	-	-
	SE	0.10	0.14	0.05	1.0	19.3	0.02	0.00	0.6				
2	C	0.09	4.25	-0.01	35.4	-9.8	-0.01	0.12	8.8	-	-	-	-
	SE	0.11	0.13	0.04	0.9	18.9	0.01	0.00	0.3				
	H	0.40	4.52	0.00	32.9	-14.0	-0.00	0.14	7.5	-	-	-	-
	SE	0.13	0.15	0.04	1.0	21.3	0.01	0.01	0.3				
	L	-0.32	3.73	-0.04	33.4	-3.9	-0.01	0.11	9.1	-	-	-	-
	SE	0.11	0.13	0.04	0.9	18.3	0.01	0.00	0.3				
3	C	-0.02	4.29	-0.05	35.0	9.3	-0.01	0.12	8.3	-	-	-	-
	SE	0.14	0.15	0.03	0.8	29.5	0.01	0.00	0.3				
	H	0.49	4.78	0.00	32.7	-48.9	-0.11	0.15	7.0	-	-	-	-
	SE	0.16	0.17	0.04	0.9	33.3	0.01	0.01	0.3				
	L	-0.47	3.83	0.00	33.2	-6.9	-0.00	0.12	8.8	-	-	-	-
	SE	0.14	0.15	0.03	0.8	29.0	0.01	0.00	0.3				
6	C	0.10	5.05	0.70	39.8	6.2	0.13	0.13	8.2	-	-	-	-
	SE	0.15	0.23	0.11	0.8	8.1	0.02	0.01	0.4				
	H	0.41	5.71	0.90	36.9	-0.6	0.14	0.16	7.0	-	-	-	-
	SE	0.19	0.29	0.13	1.0	10.2	0.02	0.01	0.5				
	L	-0.46	4.74	0.84	37.8	-13.5	0.16	0.13	8.5	-	-	-	-
	SE	0.16	0.25	0.11	0.9	8.6	0.02	0.01	0.4				

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
7	C	0.23	6.11	1.48	47.2	4.3	0.20	0.13	8.1	-	-	-	-
	SE	0.16	0.26	0.19	1.2	6.3	0.04	0.00	0.4				
	H	0.25	5.96	1.26	46.5	5.2	0.17	0.13	8.3	-	-	-	-
	SE	0.18	0.28	0.21	1.4	7.0	0.04	0.00	0.4				
	L	-0.32	5.23	1.15	45.4	1.1	0.20	0.12	9.0	-	-	-	-
	SE	0.15	0.24	0.18	1.2	6.0	0.04	0.00	0.4				
8	C	0.11	8.63	2.22	59.5	-4.7	0.19	0.14	7.9	0.12	38.5	0.58	4.5
	SE	0.25	0.58	0.34	2.2	4.9	0.04	0.01	0.4	0.18	0.9	0.05	0.5
	H	0.20	8.75	2.41	58.4	7.1	0.26	0.15	7.2	0.09	38.7	0.53	4.3
	SE	0.27	0.64	0.37	2.4	5.4	0.04	0.01	0.4	0.20	1.0	0.05	0.6
	L	-0.29	8.73	2.93	58.7	6.9	0.31	0.15	7.0	0.20	38.2	0.69	4.3
	SE	0.24	0.55	0.33	2.1	4.7	0.03	0.01	0.4	0.18	0.9	0.04	0.5
9	C	0.32	12.73	3.30	74.9	4.8	0.25	0.17	6.1	0.20	40.2	0.65	7.0
	SE	0.28	0.71	0.27	3.3	0.5	0.01	0.00	0.2	0.14	0.8	0.04	0.4
	H	0.32	12.07	2.93	72.5	5.1	0.24	0.16	6.2	-0.08	39.9	0.60	6.6
	SE	0.32	0.79	0.30	3.6	0.5	0.02	0.00	0.2	0.16	0.9	0.05	0.4
	L	-0.21	12.10	3.08	75.6	4.4	0.25	0.16	6.3	0.20	39.2	0.67	7.7
	SE	0.27	0.68	0.26	3.1	0.5	0.01	0.00	0.1	0.14	0.8	0.04	0.3
10	C	-0.67	13.51	2.07	88.2	11.2	0.14	0.15	6.8	-0.07	40.5	0.48	10.4
	SE	0.32	0.84	0.30	4.2	25.1	0.01	0.00	0.2	0.14	0.9	0.05	0.5
	H	-0.06	12.32	0.89	82.7	11.8	0.07	0.15	6.9	-0.54	38.2	0.38	9.5
	SE	0.34	0.90	0.32	4.5	27.0	0.02	0.00	0.2	0.15	0.9	0.05	0.5
	L	0.54	14.56	1.78	88.6	0.8	0.11	0.16	6.2	-0.53	37.8	0.52	10.8
	SE	0.29	0.78	0.28	3.9	23.2	0.01	0.00	0.2	0.13	0.8	0.05	0.5

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
11	C	-0.29	15.94	2.42	100.9	14.6	0.14	0.16	6.4	-0.55	38.4	0.62	13.1
	SE	0.32	1.02	0.42	5.5	27.1	0.02	0.00	0.2	0.17	0.9	0.07	0.6
	H	1.25	15.44	1.24	91.4	-3.0	0.08	0.17	6.0	-0.33	34.8	0.34	11.2
	SE	0.43	1.35	0.56	7.3	36.0	0.03	0.00	0.2	0.23	1.2	0.09	0.8
	L	-0.67	13.57	1.38	91.6	78.5	0.10	0.15	6.8	-0.38	35.7	0.41	13.0
	SE	0.35	1.11	0.46	6.0	29.5	0.02	0.00	0.2	0.19	1.0	0.07	0.7

Table A4.3. Test statistics, significance levels and pair-wise contrast mean squares for all traits analysed using repeated measures (Approach 3).

	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
Within Subject												
Multivariate[a]												
Time (T)	0.77	0.09**	0.11**	39.2**	0.54**	0.04**	0.12**	0.15**	0.68*	0.25**	11.8**	0.05**
T*Group	0.17**	0.10**	0.14**	2.7**	0.50	0.18**	0.08**	0.06**	0.68	0.64	4.2**	0.38*
T*Line	0.48*	0.42**	0.36**	2.0*	0.62	0.30**	0.30**	0.40**	0.71	0.57	1.3	0.61
Univariate												
Time (T)	0.72	632**	45.2**	18740**	3963	4.48**	0.012**	60.2**	1.61	53.9**	0.22**	185**
T*Group	2.45**	16**	4.2**	382**	5177	0.97**	0.002**	11.8**	0.70	6.9	0.05**	3**
T*Line	1.37**	4	2.0**	69	6942	0.21*	0.001*	4.8**	0.87	8.9*	0.03	2**
Between Subject												
Group	3.44	62.4	9.37*	316	9716	0.024	0.023**	24.5*	0.09	24.4	0.19*	61.0*
Line	10.77*	40.5	3.04	985	2030	0.004	0.017**	31.5*	0.43	33.2	0.22*	7.5
Contrast Variable												
1-2												
Mean	0.38	11.46**	3.29**	92.2**	13286	0.391**	0.017**	151.9**	-	-	-	-
Group	0.82*	4.46**	0.66**	6.5	28620	0.100**	0.006**	50.5**	-	-	-	-
Line	0.80*	0.30	0.04	1.6	25458	0.002	0.000	8.9	-	-	-	-
2-3												
Mean	0.50	0.04	0.01	3.8	4743	0.000	0.000	0.2	-	-	-	-
Group	1.40**	0.50	0.12	2.5	3691	0.008	0.000	1.6	-	-	-	-
Line	0.01	0.05	0.01	1.1	50373	0.001	0.000	0.4	-	-	-	-

	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
Contrast Variable												
3-6												
Mean	0.75	13.12**	10.68**	377.5**	1058	0.364**	0.002	0.0	-	-	-	-
Group	1.15	3.73**	0.65**	9.5	3690	0.016*	0.003**	15.2**	-	-	-	-
Line	0.09	4.07**	1.45**	12.6	24422	0.034**	0.003**	9.0*	-	-	-	-
6-7												
Mean	0.02	37.38**	39.10**	1883.2**	3991	0.909**	0.000	12.4**	-	-	-	-
Group	0.18	3.63**	1.80*	55.9**	2506	0.020	0.006**	25.1**	-	-	-	-
Line	0.57	3.19**	0.28	45.7*	2203	0.012	0.006**	15.0**	-	-	-	-
7-8												
Mean	0.02	173.04**	7.71	4945.6**	375	0.075	0.003	0.7	-	-	-	-
Group	6.18*	77.56**	27.64**	336.1**	619	0.167**	0.013**	44.6**	-	-	-	-
Line	0.91	6.56	1.98	30.4	697	0.037	0.002	7.6	-	-	-	-
8-9												
Mean	0.36	498.61**	38.07**	8931.5**	258	0.069	0.017**	62.6**	0.40	4.6	0.00	165.8**
Group	1.02	1.73	10.22**	219.6**	679	0.196**	0.004**	20.5**	2.00	6.1	0.13**	1.2
Line	1.56	9.58*	5.41	4.7	857	0.048	0.003*	10.1*	0.43	14.3	0.01	0.4
9-10												
Mean	3.39	44.84**	85.34**	5778.2**	685	0.682**	0.004**	6.1**	2.66	34.0*	0.46**	99.8**
Group	3.36*	5.60	10.59**	48.0	160	0.023**	0.001	0.6	3.29	7.8	0.06	2.0**
Line	6.69**	5.82	2.27	62.8	285	0.008	0.001*	1.4	2.02	11.6	0.01	1.7*
10-11												
Mean	7.74*	80.48**	0.00	1779.7**	1849	0.022	0.001	1.3	0.14	114.5**	0.04	78.0**
Group	18.61**	4.31	12.49*	34.9	708	0.066**	0.001	2.4*	1.21	1.4	0.17*	2.1
Line	8.04*	17.32	3.67	151.9*	52	0.010	0.002*	2.9*	3.28	0.10	0.09	2.5

[a] Test statistic presented is Wilks' Lambda.

Table A4.4. Net feed intake selection lines' least squares means for growth and intake traits for 11 successive weeks, excluding weeks 4-5 (Approach 3).

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
1	C	-0.14	3.36	-0.31	37.6	-67.7	-0.11	0.093	11.8	-	-	-	-
	SE	0.12	0.16	0.06	1.2	26.4	0.03	0.005	0.7				
	H	0.45	3.83	-0.34	34.9	-5.6	-0.12	0.112	9.6	-	-	-	-
	SE	0.16	0.21	0.08	1.7	33.4	0.04	0.007	1.0				
	L	0.02	3.44	-0.32	34.4	9.1	-0.12	0.099	10.5	-	-	-	-
	SE	0.16	0.22	0.09	1.7	33.8	0.04	0.007	1.0				
2	C	-0.01	4.10	-0.05	35.6	-6.6	-0.01	0.118	8.8	-	-	-	-
	SE	0.13	0.15	0.04	1.1	16.7	0.01	0.006	0.4				
	H	0.33	4.43	-0.01	33.2	25.2	-0.01	0.136	7.8	-	-	-	-
	SE	0.18	0.20	0.06	1.4	21.1	0.01	0.008	0.5				
	L	-0.33	3.88	0.04	33.1	-19.3	0.01	0.119	8.7	-	-	-	-
	SE	0.18	0.21	0.06	1.5	21.3	0.01	0.008	0.5				
3	C	-0.15	4.16	-0.05	35.0	8.6	-0.01	0.120	8.6	-	-	-	-
	SE	0.15	0.16	0.04	1.0	39.0	0.01	0.005	0.3				
	H	0.23	4.52	-0.01	32.9	-59.5	-0.00	0.139	7.6	-	-	-	-
	SE	0.20	0.21	0.05	1.3	49.3	0.01	0.006	0.4				
	L	-0.45	3.84	-0.01	33.0	12.2	-0.00	0.117	8.9	-	-	-	-
	SE	0.21	0.22	0.05	1.3	49.8	0.01	0.007	0.4				
6	C	0.05	4.87	0.61	39.4	3.6	0.12	0.125	8.4	-	-	-	-
	SE	0.16	0.18	0.07	0.9	11.3	0.01	0.005	0.3				
	H	0.43	5.74	0.89	36.0	-3.1	0.15	0.163	6.8	-	-	-	-
	SE	0.21	0.24	0.09	1.2	14.3	0.02	0.006	0.4				
	L	-0.40	3.82	0.14	35.7	-21.4	0.04	0.109	9.9	-	-	-	-
	SE	0.21	0.25	0.10	1.3	14.5	0.02	0.006	0.4				

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
7	C	0.21	6.31	1.79	47.0	3.9	0.28	0.135	7.5	-	-	-	-
	SE	0.17	0.22	0.16	1.3	0.9	0.02	0.004	0.2				
	H	0.23	6.31	1.82	45.6	4.8	0.28	0.138	7.4	-	-	-	-
	SE	0.22	0.29	0.21	1.7	1.2	0.03	0.005	0.3				
	L	-0.28	5.04	1.30	41.2	5.3	0.25	0.123	8.3	-	-	-	-
	SE	0.23	0.30	0.22	1.8	1.2	0.03	0.005	0.3				
8	C	0.03	8.21	1.90	58.6	-7.1	0.17	0.136	8.1	0.06	38.4	0.60	4.7
	SE	0.25	0.58	0.34	2.2	7.0	0.04	0.006	0.4	0.23	1.1	0.05	0.5
	H	0.52	9.41	2.57	59.6	5.1	0.25	0.155	6.7	-0.02	38.7	0.60	4.4
	SE	0.33	0.76	0.46	3.0	8.9	0.05	0.008	0.5	0.26	1.2	0.05	0.6
	L	-0.32	6.98	1.89	52.4	5.4	0.24	0.132	7.9	0.58	37.5	0.74	4.2
	SE	0.34	0.79	0.48	3.1	8.9	0.05	0.008	0.5	0.24	1.1	0.05	0.5
9	C	0.19	12.82	3.45	75.0	4.2	0.27	0.168	6.1	0.14	38.9	0.60	8.2
	SE	0.25	0.77	0.29	3.5	0.3	0.01	0.004	0.1	0.32	1.1	0.04	0.7
	H	0.07	12.50	2.99	76.8	4.4	0.24	0.162	6.2	-0.31	37.7	0.55	7.7
	SE	0.33	1.01	0.38	4.7	0.3	0.02	0.005	0.2	0.35	1.3	0.05	0.8
	L	-0.33	11.07	3.16	68.2	3.6	0.29	0.160	6.3	0.26	39.7	0.74	8.0
	SE	0.33	1.04	0.39	4.9	0.3	0.02	0.005	0.2	0.32	1.1	0.05	0.7
10	C	-0.82	13.84	2.08	90.2	6.3	0.15	0.151	6.7	0.04	38.6	0.46	10.7
	SE	0.27	0.85	0.27	4.4	3.3	0.01	0.004	0.2	0.29	1.6	0.06	0.8
	H	-0.27	13.02	0.91	88.1	5.9	0.08	0.148	6.8	-0.39	35.4	0.38	9.9
	SE	0.35	1.11	0.36	5.9	4.1	0.02	0.005	0.2	0.33	1.8	0.06	0.9
	L	0.04	13.07	1.77	81.7	14.5	0.13	0.160	6.3	-0.88	37.7	0.50	11.3
	SE	0.36	1.15	0.37	6.1	4.2	0.02	0.005	0.2	0.30	1.6	0.06	0.8

W	L	NFI	DFI	ADG	MW	FCR	GE	MR	ME	DADG	DMW	PADG	PMW
11	C	-0.35	15.85	2.35	100.9	12.5	0.13	0.155	6.5	-0.73	35.8	0.45	13.2
	SE	0.29	1.08	0.43	5.3	4.5	0.02	0.004	0.1	0.34	1.3	0.10	1.1
	H	1.09	15.63	1.31	93.4	15.6	0.08	0.166	6.1	-0.53	32.3	0.20	11.7
	SE	0.38	1.42	0.58	7.2	5.7	0.03	0.005	0.2	0.38	1.5	0.11	1.2
	L	-0.33	13.19	1.09	87.7	22.1	0.07	0.150	6.7	-0.28	34.8	0.53	14.2
	SE	0.40	1.46	0.60	7.4	5.7	0.03	0.005	0.2	0.35	1.4	0.10	1.1

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